La diagnosi delle malformazioni congenite nei tre trimestri di gravidanza: il ruolo dell’autopsia tra clinica, genetica e diagnostica per immagini

SESSIONE I

Il primo trimestre di gravidanza

Moderatori: Maria D’Armiento (Napoli) - Vincenzo Nardini (Pisa)

EVALUATION OF THE FIRST-TRIMESTER PRODUCTS OF CONCEPTION: SPECIAL TECHNIQUES AND GOALS OF PATHOLOGIC EXAMINATION IN CASE OF MALFORMATIONS

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Introduction

The study of first trimester products of conception is the object of increasing attention and are one of the most common specimens submitted to pathology. Embryopathology concerns the study of pathologic conditions and malformations like early development failure, development alterations and true malformative lesions. It is well known that the most severe genetic abnormalities emerge as an interruption in embryogenesis and that the most complex developmental abnormalities caused by external factors have their onset in the early stages of embryo formation and are often also the cause of pregnancy loss. The goal of pathologic examination include the documentation of an intrauterine pregnancy, the confirmation of the gestational age of products of conception, the definition of the interval of retention after embryonic or fetal death and the documentation of suspected or unsuspected anomalies. In order to achieve these goals early abortion specimens need to be examined using special technique like the dissecting microscope and, for example, acrylic resin embedding technique.

METHODS: Examination of first trimester products of conception is primarily concerned with the identification of decidual tissue, villous tissue and embryonic or fetal tissue. The analysis of embryonic or fetal tissue by stereomicroscope dissection and semi-thin sections embedded in acrylic resin permits the acquisition of better morphological detail even in case of autolysed and poorly preserved samples. Stereomicroscope dissection is used to accurately define the existence of an empty chamber, to reveal appendages, like the yolk sac or the body stalk. It also useful in identifying clinical suspected malformations detected by ultrasonography examinations.

Results

The use of stereomicroscope dissection and the accurate histological studies using resin embedding techniques allowed us to describe first-trimester product of conception and to find out suspected or unsuspected malformation also because they allow to apply the different classifications employed to describe the embryo in first trimester spontaneous abortion. This is very important because the prerequisite for anatomic evaluation and diagnosis of structural abnormalities in the embryonic period is knowledge of the normal embryonic development. The dating of growth arrest is extremely important. In the majority of cases the age of arrest is different from the age of the expulsion of the ovular chamber. distinguishes between the different moments in pregnancy loss: initial phase of embryonic suffering, actual moment of death, retention and expulsion. This difference is important to identify the autolytic processes undergone by the embryo, that can be misleading in the identification of gross morphological abnormalities. In our study we underline the importance of resin embedding for the precise application of the Carnegie classification leading to better diagnostic accuracy. The classification of embryos made by the Carnegie Institute of with the study of the presence or absence of various anatomical structures, helps to identify the precise point at which arrest of development occurred and the presence of structural abnormalities. Early detection of malformation is tremendously improved with improvement in imaging technology so pathologist have to be prepared to identify structural abnormalities in all these specimens. The prerequisite for any examinations of embryos and fetus and diagnosis of structural abnormalities is knowledge of the normal embryonic and fetus development.

CONCLUSIONS: The pathological examination of first trimester product of conception using the aforementioned techniques permit a significant reduction in the number of diagnoses of blighted ova, while increasing the number of diagnoses of autolytic embryo or embryo represented by only appendages. The possibility of examining specimens with more precise technique enable to detect malformation an structural abnormalities (apart from appropriate further testing such as karyotyping). A targeted examination of the anatomy should always be carried out and can be important in the dialogue with the clinicians to confirm the results of ultrasound examinations.

References


In the past 50’ years, prenatal diagnosis (PD) previously based on both imaging tests and laboratory tests has been improved by the introductions of new molecular techniques leading to ameliorate fetus and embryo screening respectively.

In the II trimester period DP is mainly based on noninvasive ultrasonography examination and invasive cytogenetics investigations such as chorionic villus and amniotic fluid analysis and more recently on the screening of circulating fetal DNA (cfDNA).

Nowadays, noninvasive ultrasound examination is considered the first-line diagnostic analysis to survey embryo and fetal pregnancy status and to support invasive analysis in acquiring fetal tissues. Invasive prenatal diagnosis is routinely applied to mainly identify monogenic diseases and aneuploidy with an estimated risk ~ 0.5-1%.

In fetus with ultrasound anomalies, not detected by conventional cytogenetics techniques, further molecular and cytomolecular analysis are recommended in order to detect cryptic rearrangements escaped by prenatal karyotype. Ar-ray comparative genomic hybridization (array-CGH) and Next Generations Sequencings (NGS) investigations provide an important insights into cases with abnormal ultrasound outcome. Although these techniques rapidly increase timing and power of resolution, the diagnostic assessment must be evaluated by a multidisciplinary task that should involve early prenatal consultations with specialists involved in case management and treatment planning. Moreover, the molecular pathology exams, including array-CGH and NGS techniques, on aborted fetuses or in pregnancies with negative outcomes (i.e. IUGR/SGA, fetal or neonatal death and neurological defect at birth) might be supported by autopsy evidences and placental study respectively.

NGS analysis has revolutionized diagnosis in the last decade, allowing non-invasive screening for single gene or chromosomal defects using a single sample of maternal blood from early in pregnancy and may reflect gene expression occurring within the placenta. Through this analysis it may possible to measure circulating DNA to quickly identify genetic anomalies and to avoid the abort high-risk pregnancies. Prospective cohort studies are now required to validate these findings to determine the clinical applicability of measuring circulating DNA to develop novel biomarkers for a wide spectrum of pregnancy complications.

LA DIAGNOSTICA ANATOMOPATOLOGICA DELLE MALFORMAZIONI CONGENITE: RUOLO E METODOLOGIE

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The aim of this presentation is to define the target population, to point out the specificity of the optimal autopsy’s method-ology, to discuss the many problems you can confront with while performing an autopsy and the socio-sanitary goals of the autoptical approach.

The target population is mainly composed by legal termination of pregnancy’s cases in case of certain or suspected malformations/syndrome/congenital disease, that are the most consistent group, in which the autopsy is mandatory to confirm and complete the malformative pattern clinically found/ suspected, and the spontaneous abortion group, the less frequent one, in which you can find a fetus with a malformation not suspected during intrauterine life, and it is useful to have the complete picture of the pathology and to plan the subsequent pregnancy.

During autopsy, one must keep in mind that the malformation pattern is mainly macroscopically highlighted, therefore is crucial to use reproducible, shared, standard and objective protocols to register it permanently. In the protocol approved in the Gazzetta Ufficiale della Repubblica Italiana “Protocolli diagnostici nei casi della morte improvvisa infantile e della morte inaspettata del feto” in 2014, the protocol defines the correct methodology to register correctly and objectively the malformation pattern, how to make photographic report and potential radiographies and how to sample in a reproducible way material for histology, and ancillary techniques. In the autopsy report in critical to cite the reference documentation used to define correctly the different parameters found. It is also fundamental to adapt the evisceration techniques to the single malformation clinically suspected or highlighted (very important are the oriented clinical informations) and to report the not malformative diseases to understand how to manage correctly the subsequent planning of the future pregnancy.

In the view of spending review is useful is the knowledge that the autopsy is an economical and affordable tool, still consid-ered and proved to be the gold standard compared to others imaging techniques (such as Magnetic Resonance), that, even if useful in certain settings, are much more expensive and
frequently less accurate. Speaking of costs, is necessary to keep in mind that the essential levels of care in the autopsy’s field are the achievement of everything is certain and definite for diagnosis, proceeding step by step; only this is affordable and sustained by the Health Service and not the research field. Another critical point of discussion is how to make correctly a diagnostic report, in which the goal is the diagnosis of a syndrome, in collaboration with the professionals involved in the case (such as the genetist, the neonatologist, the obstetrician, the gynecologist), but that is often impossible, so it is important for the scientific societies to define the better way to make a diagnostic report (how to write a understandable and usable list of malformations for the clinician and the family). Some of the important socio-sanitary goals of the autopsy are to have a complete epidemiological picture of the malformations and to make a correct and optimal anatomo-clinical conference with the family, to help them to understand what happened and why in order to allow a correct counseling to the couple and to the family, also limiting the sanitary costs.

IL MALFORMATO MISCONOSCIUTO ALL’INDAGINE ECOGRAFICA DEL I E DEL II TRIMESTRE: È SEMPRE FRUTTO D’UN ERRORE DIAGNOSTICO?
A. M. Marconi

FETAL ENDOUTERINE DEATH AND UNIDENTIFIED CONGENITAL MALFORMATIONS: DIAGNOSTIC METHODOLOGY AND RESULTS
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Fetal autopsy of the third trimester death, or in the first week after delivery, presents some issues and its purpose is to define the death cause, and if it’s possible the pathogenesis. Since placental pathology is considered the principal cause of fetal death, the pathologist could miss, during fetal autopsy, malformations, above all if they are not usual or minor and not reported from ultrasound imaging.

The detection of these “missed” malformations is extremely important because:

- These malformations could be the cause or co-factor of the fetal death
- The presence of three, or more minor malformations, is associated to greater possibility of finding major visceral malformation
- Multiple minor malformations are more related to neuropsychiatric retard
- When it’s possible it’s important to give to parents an adequate explanation of this tragic event, an appropriate counseling above their reproductive risk and to suggest possible genetic exams
- Identify minor malformations and sindromic conditions is important for epidemiologic studying order to plan the sanitary cost.

Ultrasound imaging can miss some malformations, not only the minor.

Moreover mother can choose to not perform US examination, or even in presence of malformations identified by US examination, can decide to keep the baby.

The pathologist has to perform a precise and conscientious examination, following a strict protocol of analysis, produce photographic proofs of pathologic aspects and, if necessary, preserve visceral specimens for further analysis.

Only this method leads good results and allows, if required, a “second opinion” in selected complex cases.

An autopic protocol has been proposed in the ordinary supplement n 89 of Gazzetta Ufficiale Serie Generale n.272 of 2014, November 22 (Decreto 2014, October 7).

The autopic protocol has to be used with wisdom and adjust to the specific situation to analyze placenta and fetal somatic and visceral characteristic. Considering all the possible malformations and syndromes it’s a good tool to not miss all these observations.

At last is essential to know all the clinical informations of pregnancy and any mother’s pathology.

References
Supplemento ordinario n.89 della Gazzetta Ufficiale Serie Generale n.272 del 22-11-2014 (Decreto 7 ottobre 2014)

Mercoledì, 23 novembre 2016

Aula Tramontana – 13:30 - 17:30

**PATOLOGIA ULTRASTRUTTURALE**

Corso formativo di patologia renale

Moderatori: Giovanna Cenacchi (Bologna) - Gianna Mazzucco (Torino)

**ALLESTIMENTO DELLA BIOPSIA RENALE**

V. Papa

**GLOMERULOSCLEROSI FOCALE E SEGMENTARIA**

G. Mazzucco

**INFOLDING PODOCYTES**

S. Pizzolitto

**C3 GLOMERULONEPHRITIS**

A. Barreca

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C3 glomerulonephritis (also known as Glomerulonephritis with dominant C3- C3 GN) are a form of C3 glomerulopathy together with Dense Deposit Disease (DDD)/ glomerulonephritis membranoproliferative (MPGN) type II. It’s a recently described pathological entity characterized by bright C3 staining with absent or scanty immunoglobulin deposition on immunofluorescence (IF) microscopy [1] without the characteristic highly electron-dense transformation observed in DDD [fig. 1]. Most patients typically presents with subnephrotic proteinuria and hematuria and in about 50% of cases also with...
hypertension and decreased glomerular filtration rate. From the laboratory point of view C3 GN obviously show at presentation low C3 and normal C4 serum levels, suggesting a disfunction in the alternative pathway of complement. It’s a rare disease with a typical age of onset of 30 years (range 7-70 years).

The main aspect on light microscopy is a membranoproliferative pattern, other less common histologic appearances include mesangial proliferative, endocapillary proliferative and crescentic ones. Rarely glomeruli may appear normal by light microscopy.

There is some pathologists that supposed a correlation between specific morphological patterns and pathogenesis, as for example in familial C3 GN with mutation in CHFR5 gene, but larger studies are necessary to confirm this date.

The main differential diagnosis are DDD and postinfectious glomerulonephritis (PIGN). In fact cases of PIGN, especially beyond the acute stage, may have deposition of C3 without immunoglobulin. The clinical course is useful because most cases of PIGN has a resolution of disease with normalization of the decreased peripheral C3 level in 8-12 weeks. It’s important to follow the patient clinically and serologically over several months to control C3 serum levels. Besides PIGN show more numerous subepithelial hump-like deposits than mesangial ones.

A few studies reported cases of PIGN progressing to either C3GN or DDD in the same patient and conversely cases of C3 glomerulopathy progressing to PIGN or MPGN type I. C3GN seems to be less aggressive than DDD [3]. C3 GN and DDD are due to a dysregulation of the alternative pathway of the complement system. Therefore a diagnosis of C3 GN requires complement investigations, with genetic screening and complement serological tests. Familiar forms of C3 GN are determined by mutations in the complement regulatory protein H, factor I, complement factor H-related genes (CFHR1-R5) and CD46.

The serological investigations show reduced C3 with increased C3 breakdown product (e.g. C3d) and low C5 with increased soluble C5b-9 and C5a. The main acquired causes of C3 GN are autoantibodies directed versus the alternative pathway C3 convertase (C3 nephritic factors -C3NeF), factor B and factor H. All these complement serological tests should be performed in specialist laboratories. These abnormality favor excessive activation of the alternative complement pathway in the fluid phase, with accumulation of complement debris in the glomerular capillary wall.

Eculizumab, an antibody directed towards C5, may be an effective treatment for C3 glomerulopathy, but so far available data are mainly in the form of case reports. Further prospective studies are needed.

To the end MPGN type I-III represent a general histological pattern that need a reclassification of the disease on the pathogenetic basis into immunoglobulin-mediated pathology (related to infections, autoimmune diseases or paraproteinemias) versus complement-mediated entities. This could permit to achieve better diagnostic and therapeutic approaches.

References

Figure 1. A schematic diagram showing an approach to the classification of disease in a biopsy showing the morphological changes of a glomerulonephritis with dominant C3. [2]
**Mercoledì, 23 novembre 2016**
Aula Scirocco – 13:30 - 17:30

**PATOLOGIA ENDOCRINA**

Novità classificative in patologia endocrina

Moderatori: Fulvio Basolo (Pisa) – Giuseppe Pelosi (Milano)

**TIROIDE: UMP E NIFTP**

G. Tallini

**POLMONE: CHE COSA C’È E CHE COSA POTREBBE ESSERCI**

G. Pelosi

**PANCREAS/GI: DA NEC A MINENS**

S. La Rosa

**SURRENE: PROBLEMATICHE EMERGENTI NELLA CLASSIFICAZIONE**

G. Nesi

Patologia molecolare endocrina: come e quando

Moderatori: Guido Rindi (Roma) – Aldo Scarpa (Verona)

**THYROID: MOLECULAR PATHOLOGY OF THYROID TUMORS**

Ugolini C. 1, Basolo F. 2.1. Anatomia Patologica 3 Univ.- Dipartimento di Medicina di Laboratorio. Azienda Ospedaliero-Universitaria Pisana- Pisa

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The diagnosis of thyroid neoplasms is subjected to continue evolutions. In the last years morphological and molecular studies have modified the diagnostic, clinical and surgical approach to thyroid neoplasms. Particular attention has been pointed on follicular thyroid lesions, due, first of all, to the difficult FNA preoperative differential diagnosis and second, to their favourable prognosis. The most reliable morphological criteria for the FNA preoperative diagnosis of the malignancy are the nuclear features of papillary carcinomas.

The follicular variant (FV) is one of the most common variants of papillary thyroid carcinoma (PTC). The encapsulated forms of FVPTC show an overall good prognosis demonstrating an indolent disease course with recurrence rate less than 1% within the first 15 years. Consequently, these neoplasms have been very recently re-classified as “non-invasive follicular tumour with papillary-like nuclear features/NIFTP”.

The recent publication of an integrated genomic characterization (TGCA) by Giordano et al. of papillary thyroid carcinoma (PTC) can help pathologists to identify the molecular similarities in the different subtypes of malignancies. Nevertheless, the conclusive diagnosis of NIFT-P is feasible only by histology, through the complete study of neoplasm capsule. The real impossibility of a preoperative diagnosis of NIFT-P induced several molecular and genetic studies.

In the last decade, gene expression profile has been analyzed in different thyroid lesion focusing the attention on the differential diagnosis between benign and malignant lesions and indentifying same important deregulated genes. Borup et al in 2010, analyzed the global transcriptome signatures and found a strong correlation between down-regulation of genes involved in growth arrest and apoptosis. Griffith et al. (2006) indicated 12 genes (MET, TFF3, SERPINA1, TIMP1, FN1, TPO, TGFA, QPCT, CRABP1, FCGBP, EPS8, PROS1) important to the develop of a panel of markers for the diagnosis of thyroid tumours. In 2011 Vierlinger et al, by a meta-analysis study on PTCs and nodular goiters, identified a single gene, SERPINA1, as a potent mRNA marker for PTC diagnosis with 99% accuracy.

Up to now, very few data about the gene expression profile of NIFTPs are available. On these basis our group are studying a specific molecular signature of NIFTPs that is distinct from that of follicular adenomas and infiltrative FVPTCs using nanoString mRNA expression analysis and the uncorrelated shrunken centroid (USC) machine-learning algorithm. The results revealed distinct expression profiles of FAs and IFVPTCs that resulted in the separation of cases into two different clusters (C1 and C2), with a few exceptions. The NIFTP samples were almost equally subdivided within the two above-mentioned clusters. Notably, the main part of NIFT-P samples within the C1 cluster were wild type, on the other hand the majority of the samples within the C2 cluster were mutated, carried on point mutations, such as BRAF K601E, HRAS Q61R and NRAS Q61R.

By a pragmatic point of view these results could be applied in clinical practice through the creation of a classifier. In particular in presurgical FNA. It is widely accepted that NIFTPs cannot be recognized with certainty by cytology because their nuclear features are overlapping to those of infiltrative encapsulated FVPTC.

Consequently, we could suggest that our classifier, in addition of a wide genotyping analysis, could be tested on nodules with indeterminate diagnoses by cytology to obtain additional data that might be useful in the diagnostic and prognostic characterization of these tumours. In fact, because of the apparently benign clinical behaviour of NIFTPs, the preoperative recognition of these neoplasms would have important implications for the therapeutic and surgical strategies employed to avoid overtreatment of the patients.
Next-generation sequencing (NGS) was applied to 148 lung neuroendocrine tumours (LNET) comprising the 4 WHO classification categories: 53 typical carcinoid (TC), 35 atypical carcinoid (AC), 27 large cell neuroendocrine carcinoma (LCNEC), and 33 small cell lung carcinoma (SCLC). A discovery screen was conducted on 46 samples using whole-exome sequencing and high-coverage targeted sequencing of 418 genes. Eighty-eight recurrently mutated genes from both the discovery screen and current literature were verified in the 46 cases of the discovery screen and validated on additional 102 LNET by targeted NGS, and their prevalence was evaluated on the whole series. Thirteen of these 88 genes were also evaluated for copy number alterations (CNA). Carcinoids and carcinomas shared most of the altered genes but with different prevalence. Combining mutations and copy number changes, MEN1 alterations were almost exclusive of carcinoids, while alterations of TP53 and RB1 cell cycle regulation genes and PI3K/Akt/mTOR pathway genes were significantly enriched in carcinomas. Conversely, mutations in chromatin-remodelling genes, including histone modifiers and members of SWI/SNF complexes, were found at similar rates in carcinoids (45.5%) and carcinomas (55.0%), suggesting a major role in LNET pathogenesis. One AC and one TC showed a hypermutated profile associated with POLQ damaging mutation. CNAs were lower in carcinoids than carcinomas, however ACs showed a hybrid pattern where gains of TERT, SDHA, RICTOR, PIK3CA, MYCL and SRC genes were found at rates similar to carcinomas, while MEN1 loss rate mirrored that of TCs. Multivariate survival analysis revealed RB1 mutation (p=0.0005) and TERT copy gain (p=0.016) as independent predictors of poorer prognosis. MEN1 mutation was associated with poor prognosis in AC (p=0.0045), while KMT2D mutation correlated with longer survival in SCLC (p=0.0022). In conclusion, molecular profiling may complement histology for a better diagnostic definition and prognostic stratification of LNET.

MOLECULAR ENDOCRINE PATHOLOGY, HOW AND WHEN: MOLECULAR PATHOLOGY OF ADRENAL TUMORS

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Pheochromocytoma and paraganglioma

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are neuroendocrine tumours arising from chromaffin cells of the adrenal medulla or of paraganglia in the head and neck region or along the sympathetic trunk. PCC and PGL can be either familial or sporadic. Germline mutations in the SDHA, SDHB, SDHC, SDHD, SDHAF2 (together SDHX), VHL, RET, NF1, TMEM127, MAX, KIF1B, PHD2, FH or the most recently identified HIF2A occur in about 40% of PCC/PGL patients [1]. Indeed, recent data claim that overall germline mutations are identified in 28.7% of PGLs and 4.5% of PCCs cases that lack any clinical sign of suspect for being inherited (single location, non syndromic, no familial history) [2]. Therefore, the major role for molecular pathology in PCC/PGL disease is related to the screening even in apparently sporadic cases of germline mutations, which may be feasible using next generation sequencing [3]. In sporadic forms, somatic mutations in RET, VHL, MAX and HIF2A genes are also reported in 17% of tumors. Moreover, recent reports identified somatic NF1 and H-RAF mutations in 22-26% and 5-7% of sporadic PCCs/PGLs, respectively [4,5]. Although the disease is the perfect example of genetic heterogeneity, two main transcriptomic signatures have been evidences. The first one, named cluster 1, is enriched with VHL-, SDHX- and FH-mutated tumors, and shares a pseudohypoxic profile. The second one, named cluster 2, groups tumours related to mutations in RET, NF1, TMEM127 and MAX, and involves a kinase pathway [1]. The
first integrative genomic study, recently published, demonstrated the crucial role of predisposing mutations as being the main drivers of PCC/PGLs [6]. However, there is no role for molecular markers in patients’ prognostic stratification or in the definition of therapeutic strategies, to date, except for the adverse prognostic impact of the presence of SDHB mutations in paragangliomas.

**Adrenocortical carcinoma**

Adrenocortical carcinoma (ACC) is a rare malignancy and despite recent advances on a better definition of the standard of care, in terms of surgical procedures, adjuvant treatment and chemotherapeutic protocols for advanced disease, its prognostic remains dismal, with a 20-40% 5-year survival. Although ACC has generally been considered a single entity by pathologists and clinicians, remarkable heterogeneity is observed from the morphological standpoint (with several histological variants identified), the clinical (with variable and poorly predictable outcome) and the genetic perspectives. Pediatric ACCs are frequently associated to the Li–Fraumeni syndrome (TP53 mutations) and the Beckwith–Wiedemann syndrome (alterations of the insulin-like growth factor/IGF2) [7], whereas TP53 germline mutations (p.R337H) are associated to the onset of pediatric ACC in Southern Brazil [8]. Recently, it has been recognized that ACC even in the adult population can be associated with the Lynch syndrome [9]. At the somatic level, few genes driving ACC onset and progression have been identified so far, being most of the molecular data available on ACC restricted to gene expression, array-CGH or methylome profiles in comparison to the benign counterpart of adrenocortical adenoma. Mutations in TP53 gene are detectable as somatic events in about one-third of adult cases. Mutations in CTNNB1 gene, coding for beta-catenin protein, are detectable in a subset of ACC (about 40%) but are also present in a comparable percentage of adrenocortical adenomas, and their role in the pathogenesis and progression of ACC is still on debate [10]. Recent mutations of ZNRF3 (Zinc and ring finger protein 3) gene have been also found to be of high prevalence in ACC [11]. However, none of those genomic alterations above demonstrated a significant impact in the prognostic stratification of patients or as predictive markers of response to treatment. Many other mutations have been described by whole genome sequencing but are of very low prevalence, thus confirming the heterogeneous molecular background of ACC but still needing adequate investigation of their potential role as biomarkers in the clinical setting.

**References**

grading system was first adopted by the International Union Against Cancer (UICC), subsequently endorsed by both the American joint Cancer Committee (AJCC) and the WHO. The current WHO 2010 grading system was designed to provide clinicians with an effective tool for patient stratification and clinical management. A large set of data proved its efficacy in the clinical ground. Open questions entail the definition of specific cut-offs between G1 and G2 for NETs at different anatomical places. Indeed, it appears that in some sites, i.e. pancreas, the higher cut off of 5% may be more informative. On the same line within G3 high grade NECs, data support different behavior according to some NET-like morphology and Ki67 values at the lower end of this category. In specific NEN with non-poorly differentiated morphology (by convention defined as well differentiated) may well associate with Ki67 cut-off higher than 20% but usually below 55%. Patients with such G3 NENs fares better survival, display frequent somatostatin subtype 2A receptor expression, overall being closer to patients with G2 NENs. This was proven especially in the pancreas. Overall, in retrospective and prospective series, the application of the current WHO 2010 grading system stratified the G3 fraction in about 10% of investigated cases depending on the anatomical site. According to the current data a fraction of about 20% of such rare cases would fit this new NEN G3 entity. Such aspects will be critically revised and discussed.

**SURRENE: DIAGNOSI DIFFERENZIALE IN PATOLOGIA SURRENALICA**

A. Fassina

**Mercoledì, 23 novembre 2016**

Aula Libeccio – 13:30 - 17:30

**IMMUNOISTOCHIMICA**

Nuovi marcatori, nuove prospettive, nuove sfide

Moderatori: Marco Chilosi (Verona) – Claudio Doglioni (Milano)

**IN SITU ANALYSIS OF THE MELANOMA MICROENVIRONMENT**

Francesca Maria Bosisio 1,2, Giorgio Cattoretti 2, Joost van den Oord 1

1 Laboratory of Translational Cell and Tissue Research, KUL, Leuven, Belgium; 2 Università degli studi di Milano-Bicocca, Milan, Italy

Melanoma is one of the most antigenic tumors, but it also has an enhanced ability to escape the immune response. In spite of this high antigenicity, the phenomena of melanoma regression is frequently just partial (23-58%), while complete regression is rare (12.4%), suggesting the onset of mechanisms of immune escape that allow the tumor to resist the immune attack. Two of the major mechanisms of immune escape are immune editing and immune modulation. Immune editing is based on the recognition of highly antigens tumor proteins by T cells favoring the selection of less antigenic clones that can escape the immune attack. Immune modulation is the ability of melanoma cells to influence the inflammatory response through several mechanisms. Immune modulation is a dynamic process that varies during melanoma progression, in part due to immune editing as well. Therefore, even in the presence of inflammatory cells in the tumor environment, their functional status can be already altered by the immune modulation exerted by melanoma cells in order to shut the inflammation down and lead to a melanoma devoid of inflammation with its progression. The quickest way to assess the presence of an immune response against the tumor is the evaluation of Tumor-Infiltrating Lymphocytes (TILs) on a simple hematoxylin-eosin staining. In melanoma, not only the presence of TILs is evaluated, but also the pattern that TILs form around the tumor. The classical patterns of TILs are: a brisk infiltrate, when TILs are present diffusely all over the tumor area (diffuse variant) or all along the periphery of the tumor (peripheral variant); a non brisk infiltrate, when tumoral areas with associated TILs are present as well as tumoral areas without TILs infiltration; an absent infiltrate, when the TILs can be totally absent or present in the peritumoral area, but they don’t get in contact with the melanoma cells. It is known since more than 20 years that in melanoma this patterns are an independent prognostic factor both in the primary melanoma and in the metastases, and it is as well known that the entity of the TILs infiltration is also an independent predictor of the sentinel lymph node status. The introduction of immunotherapy has renewed the interest in TILs and has coined the question whether the TILs pattern and/or the composition of the tumor-associated inflammatory infiltrate could be an additional piece of information to predict which patient would show response to immunotherapy. Even though the morphologic evaluation of the pattern of lymphocytes infiltrating the melanoma (“TILs”) is considered a prognostic factor, a lot of controversial study exist on its real impact on prognosis. This could be due to a lack of examination of their functional status in patient’s tissue. This evaluation could become mandatory to define the subset of patients that could benefit from immunotherapies. According to the functional status of the intratumoral TILs, two groups could be identified, one with high expression of activation markers and low expression of inhibition markers, and the other with low expression of activation markers and high expression of inhibition markers. Surprisingly, cases with prevalently exhausted T cells were present both in the brisk and non brisk groups, further dividing these categories in “mostly active” and “mostly exhausted” subgroups. These evidences point out the fact that a functional evaluation of the TILs is needed, in order to go beyond the less informative morphological classification in patterns. Finally, while the role of T lymphocytes in tumor immunity has been extensively studied in melanoma, less is known about the importance of B lymphocytes. Recently, we observed that melanomas rich in plasma cells occurred at an older age and were thicker, more often ulcerated and more mitotically active. Plasma cells were oligoclonal, suggesting an antigen-driven response, and they often expressed IgA in addition to IgG, both in the primary melanoma and in the associated lymphnodes. Melanomas with sheets/clusters of plasma cells were associated with worse prognosis.
The great majority of neuroendocrine neoplasms (NENs) arise in the gastro-entero-pancreatic area and the bronchial tree. The main applications of immunohistochemistry on these tumors may be summarized as follows: 1. demonstration of neuroendocrine (NE) differentiation; 2. grading; 3. evaluation of production of peptide hormones and bioamines; 4. determination of the primary site of metastatic NENs; 5. prognostic markers and 6. treatment-related biomarkers.

1. Markers of neuroendocrine differentiation are chromogranin A, synaptophysin, CD56/NCAM (Neural Cell Adhesion Molecule), Protein Gene Product 9.5 (PGP 9.5), and Neuron Specific Enolase (NSE). The chromogranin A is the most specific and in high grade neuroendocrine carcinomas its expression tends to be lost, becoming focal in neoplastic tissue. Such stainings are mandatory.

2. Grading of neuroendocrine neoplasms according to WHO 2010 classification of digestive NENs is based on morphology, mitotic count and Ki-67 index: three grades with incremental ranges of mitosis on 10 HPF and Ki-67 expression percentage. Ki-67 expression in NENs is usually heterogeneous, thus hotspots should be selected and counted. On biopsies the neoplastic mass is randomly sampled potentially influencing accurate grading assessment. Nonetheless retrospective and prospective studies supported the prognostic efficacy of Ki-67 on biopsies. Encouraging results are also emerging also on cytology samples. As for lung NENs, the actual classification (WHO 2015) doesn’t include Ki-67 as diagnostic classification tool, while morphology and mitotic count only are considered. The assessment of Ki-67 is recommended by WHO 2015 for small biopsy to differentiated carcinoid and poorly differentiated NE carcinoma. In the current literature Ki-67 has been proposed as a prognostic factor in lung NENs.

3. Numerous antibodies are available for the evaluation of hormonal production in NENs of the digestive tract (gastrin, insulin, glucagon, etc.) and extra-digestive site (ACTH, calcitonin, etc.). The routine use of such staining is not recommended, since it doesn’t provide useful prognostic information. However, they may be relevant in NENs with clinical syndrome related to hormonal overproduction. In addition also hormonal product assessment may be of help to identify the tumor origin.

4. The sample analyzed may represent a metastatic lesion of unknown primary. Antibodies to gene transcription factors CDX2 and TTF1 are most used for this purpose, suggesting respectively a digestive and a pulmonary origin. Islet1 and PAX8 are also suggested for the assessment of a potential pancreatic origin. The use of transcription factor immunohistochemistry is limited by the their potentially promiscuous expression intrinsic to their function. This is exemplified by TTF1 expression in high grade NE carcinomas (small cell carcinoma in particular) at many anatomical sites outside the lung.

5. In the current literature some prognostic markers are described for NENs such as p27, CK19, CD117, p53, CD99 etc. but to date except Ki67 none of these antibodies has a well-defined role in the diagnostic routine workup.

6. Treatment-related biomarkers such as somatostatin receptors, in particular type 2A (SSTR2A), mammalian target of rapamycin (mTOR) pathway, MGMT and vascular endothelial growth factors (VEGF) have been investigated. Such studies represent a pre-clinical rationale for the introduction of drugs such as somatostatin analogue, inhibitors of pathway mTOR, alkylating agents, and anti-VEGF monoclonal anti-body for the NENs’ therapy. However, to date and with the exception of SSTR2A, none of these markers is routinely used in the choice of specific target-therapy.

PD-L1: UNO SCENARIO COMPLESSO

M. Callea

The field of cancer immunotherapy evolves rapidly and consistently and new biomarkers need to be investigated and integrated in this dynamic context in order to fight cancer with new target therapies. Recently several studies focused on the crosstalk between tumor cells and immune-microenvironment cells and, in this scenario, a special focus was placed on Programmed cell death 1 (PD-1) and its ligand Programmed cell death ligand 1 (PD-L1/B7-H1/CD274) because of the involvement of this pathway in down-regulating intensity and duration of T-cells immune responses. To that, many studies explored, by immunohistochemistry, the PD-L1 expression in different tumors and it has been well demonstrated how PD-L1 membranous expression on tumor cells and/or infiltrating immune cells is correlated to a better chance of response to anti PD1 drugs. Despite this, it is still very difficult to identify patients who really get benefits from an anti-PD1/PD-L1 therapy and this is due to many issues revolving around the "PD-L1" framework. These problems mainly concern PD-L1 detection methods, materials and results evaluation. Firstly, there are different antibodies targeting different PD-L1 epitopes and that alone creates trickiness among pathologists. Reproducible criteria should be reach in order to facilitate patients selection for anti-PD1 therapy and oncologists-pathologists “interplay”.

To date, two PD-1 inhibitors (Nivolumab and Pembrolizumab) are already in place in clinical practice and approved by the US Food and Drug Administration for unresectable or metastatic melanoma and for squamous non-small cell lung cancer (NSCLC) treatment. FDA extended indications to the use of Nivolumab in second line treatment for patients with metastatic Renal cell Carcinoma. Nevertheless, there is no a uniform “PD-L1 report” and the approach to this topic is still confusing.

LA MESSA A PUNTO ATTRAVERSO TESTAGGIO ANTICORPALE DI UN “AB DIFFICILE”: BAP 1 NEL MELANOMA UVEALE.(AICT)

E. Anelli

MUTATION SPECIFIC ANTIBODIES IN HEMATOMATOLOGY

A. Parisi

Hematopathology today is currently an integration of histology and immunohistochemistry. The classification of lymphomas and myeloid neoplasms often requires additional information about translocations and mutations, sometimes available in advance or discovered in parallel by molecular biology analysis, fish and cytogenetics exams performed on peripheral blood or bone marrow
aspirate; there is however increasing interest in immunohis-
tochemical markers that can be used in the diagnostic routine
on formalin fixed and paraffin embedded material, to find the
presence of these mutations.

The BRAF V600E mutation is present in many malignant epit-
ithelial tumors and it occurs either in hematological setting, es-
pecially in hairy cell leukemia and in the sometimes not easy
chapter of “histiocytosis”. There is a monoclonal antibody
that recognizes the mutated protein product of BRAF V600E
(VE1), showing cytoplasmic staining, which is applicable also
to paraffin-embedded decalcified bone marrow material.

Calreticulin gene mutations are present in a proportion
of myeloproliferative neoplasms (especially in cases of essential
thrombocytopenia and primary myelofibrosis without JAK2
mutation); the antibody CAL2, recently introduced, allows us
to identify the presence of mutations, with high sensibility and
specificity, showing a diffuse cytoplasmic staining in mega-
karyocytes of bone marrow biopsies performed to confirm the
clinical suspicion.

MARCATORI INGINECOPATOLOGIA: UPDATE

E. D’Angelo

Mercoledì, 23 novembre 2016

Aula Maestrale – 13:30 - 17:30

Qualità e sicurezza

Moderatori: Paolo Dalla Palma (Trento) - Giuseppe Santeusanio
(Roma)

IL PANORAMA DEGLI ERRORI IN ANATOMIA
PATOLOGICA

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Sperimentale e Chirurgia, Università di Roma “Tor Vergata”

Introduzione

L’Anatomia Patologica è un servizio clinico - diagnostico con
forte componente d’interpretazione soggettiva, rapportabile
ad un processo industriale ad alta complessità che riceve, pro-
cessa e diagnosticata migliaia di campioni ogni anno che ha in-
trinsenamente molteplici possibilità di rischi ed errori in molti
passaggi dell’intero percorso diagnostico - assistenziale, che
comprende tre fasi (pre-analitica, analitica e post-analitica). Il
percorso diagnostico - assistenziale viene avviato dalla richiesta
del medico curante al fine della formulazione della diagnosi
e firmato (referto). Il percorso diagnostico termina quando il
repero viene inviato al servizio clinico e consegnato al clinico
che ha richiesto l’esame che, pesandone ogni parola ai fini
della migliore gestione diagnostica - terapeutica, comunicherà
al paziente la diagnosi conclusiva (fase post-analitica).

La complessità dell’intero percorso diagnostico - assisten-
ziale offre ampia opportunità di errori che possono verificarsi
(a) nel servizio clinico prima che i campioni biologici ar-
rivino nel servizio di anatomia patologica, (b) nel servizio di
Anatomia Patologica durante la fase di esame macroscopic,
campionamento / riduzione, di allestimento tecnico, della
valutazione diagnostica ed emissione della del referto, e (c)
nel servizio clinico dopo che il referto ha lasciato il servizio di
Anatomia Patologica ed è stato consegnato al reparto / clinico
richiedente. Si tratta di errori che possono essere secondari a
componenti tecnici e / o cognitivi, attivi e latenti, dell’intero
percorso diagnostico, di pertinenza sia del servizio clinico sia
del servizio di anatomia patologica.

Possibilità di errori nel servizio clinico (fase pre-analitica)

Gli errori che si possono verificare nel servizio clinico sono
spesso commessi dai medici che procurano i campioni o dal
personale incaricato di consegnare i campioni al servizio di
Anatomia Patologica. Errori come mettere in contenitore con un
fissativo non idoneo o senza fissativo sono facilmente individuabili. Inviare campioni ottenuti con
procedura sbagliata (biopsia incisionale invece di una biopsia
cesionale), chiedere un esame intraoperatorio quando non è
necessario, etichettare in maniera errata o illeggibile un
campionamento / prelievo da parte di un medico
clinico (ambulatorio, day hospital, sala operatoria, ecc.) con
l’asportazione di un campione biologico (prelievo biotico o
asportazione parziale o totale di un organo conservati in modo
tuttavia in un contenitore), la compilazione della richiesta
di esame da parte del clinico e l’invio al servizio di Anato-
mia Patologica (fase pre-analitica). Continua nel servizio di
Anatomia Patologica dove il campione biologico e la richi-
esta di esame vengono registrati (accettazione) dalla equipe
tecnica con l’assegnazione di un numero identificativo (fase
pre-analitica). Il campione viene esaminato macroscopicame-
mente con un campionamento / prelievo da parte di un medico
anatomopatologo (fase analitica) e produzione di biocassetta
(contenenti il materiale campionato); le biocassette vengono
prese in carico dalla equipe tecnica (fase pre-analitica) per la
processazione, inclusione in paraffina, taglio al microtorno
e colorazione (di routine o speciale) delle sezioni al paziente
sul trasporto del medico curante come referto (chirurgo, radiologo, endoscopista,
ecc.) per ritornare allo stesso medico curante come referto
posta oggetto. I vetrini allestisiti, unitamente alla
rispondente richiesta di esame, vengono consegnati al
patologo (fase analitica) per la visione al microscopio e la
emissione di una diagnosi che si conclude con un atto scritto
firmato (referto). Il percorso diagnostico termina quando il
repero viene inviato al servizio clinico e consegnato al clinico
che ha richiesto l’esame che, pesandone ogni parola ai fini
del migliorare diagnostica - terapeutica, comunicherà
al paziente la diagnosi conclusiva (fase post-analitica).
Possibilità di errori nel servizio di anatomia patologica (fase pre-analitica e fase analitica)

Gli errori che si possono verificare nel servizio di Anatomia Patologica comprendono: (i) errori che si possono verificare quando il materiale biologico e la richiesta di esame giungono al servizio di Anatomia Patologica e vengono registrati con un numero identificativo dalla equipe tecnica, (ii) errori che si possono verificare nel laboratorio di istologia, e (iii) errori che si possono verificare durante l’esame macroscopico, campionamento / prelievo e l’osservazione al microscopio per la formulazione e trascrizione della diagnosi e la generazione del referto.

Errori di accettazione e registrazione nel laboratorio di istologia rappresentano un problema importante e comprendono lo scambio di campioni, l’identificazione errata del paziente, dei campioni, della sede di origine, della lateralità e del clinico che deve ricevere il referto istopatologico. Simile all’errore del servizio clinico, questi errori possono causare conseguenze dannose nelle fasi analitiche e post-analitiche.

Gli errori che si possono verificare nel laboratorio di istologia sono sotto il controllo della equipe tecnica. Alcuni di questi errori sono relativamente facili da individuare, mentre altri possono essere quasi impossibili da scoprire. Lo scambio di vetrini è l’evento più importante ed è praticamente impossibile di rilevare soprattutto per i lavoratori che lavorano esenzialmente un solo tipo di campione (ad esempio, solo biopsie prostatiche). Altre possibilità di errore nel laboratorio di istologia si possono avere nelle fasi di processazione, inclusione, taglio, colorazione delle sezioni e riunione dei vetrini con le corrispondenti richieste di esame. Difetti della processazione possono provocare gravi danni alla morfologia del tessuto e impedire la corretta valutazione morfologica delle sezioni allestite e colorate con tecniche speciali (i.e. valutazione dei fattori prognostici nel carcinoma della mammella). Difetti di inclusione possono provocare danni importanti nelle fasi successive del taglio e colorazione (es. una lesione pigmentata che viene posizionata in modo errato nel castello metallico e non viene scoperta o addirittura eliminata dal taglio). Anche il taglio di sezioni (tropo spesso, troppo sottili, incomplete rispetto al materiale incluso e sezioni che non intercettano la lesione) può provocare problemi alla corretta interpretazione morfologica dei vetrini allestiti. L’introduzione di contaminanti, durante la fase della inclusione in paraffina, del taglio di sezioni al microtomo e della colorazione con ematossilina - eosina possono creare importanti e gravi problemi per la corretta interpretazione del quadro morfologico e per l’assegnazione di una diagnosi al paziente corretto.

Errori che si possono verificare durante l’esame macroscopico, riduzione / prelievo del campione biopitico o operatorio e durante l’osservazione al microscopio e la formulazione della diagnosi e la generazione del referto sono sotto il controllo diretto del patologo. Questi errori comprendono lo scambio di campioni, contaminazione del campione con materiale estraneo, assenza o incompleto campionamento della lesione, misure assenti, inesatte o errate del campione e della lesione, mancanza di campionamento di aree pertinenti necessarie per una corretta caratterizzazione o stadiazione della lesione, errata o incompleta trascrizione del numero identificativo del campione sulle biocassette.

Gli errori che possono verificarsi durante l’osservazione al microscopio e la generazione del referto comprendono lo scambio dei vetrini, scarsa formulazione cognitiva, scarsa capacità di giudizio, creazione di un referto incomprensibile, mal formulato e mal organizzato, l’assegnazione di un referto al paziente sbagliato o al clinico sbagliato ed errori di tipografia. Gli errori di tipografia, in particolare, possono essere difficili da individuare, soprattutto quando si controllano numerosi referti nella stessa seduta. Anche i più piccoli errori tipografici possono alterare profondamente il significato di un referto (ad esempio “non è presente neoplasia” in vece di “è presente neoplasia”). Paradossalmente, l’incidenza di questi tipi di errori è inferiore rispetto agli errori delle fasi pre-analitica e post-analitica e anche la gravità di questi tipi di errore può essere sostanzialmente inferiore a quella degli errori nelle altre due fasi.

Anche se fuori dal controllo diretto del patologo, molti di errori che si possono verificare nella fase pre-analitica possono contribuire a generare errori da parte del patologo, errori che possono causare gravi danni al paziente e per i quali il patologo potrà essere ritenuto responsabile.

Possibilità di errori nel servizio clinico (fase post-analitica)

La consegna tempestiva di un referto al reparto / clinico richiedente direttamente responsabile della cura del paziente è un obiettivo del servizio di anatomia patologica. Errori nel raggiungimento di questo obiettivo spesso si verificano per errori che si generano nella fase pre-analitica e analitica del percorso diagnostico-assistenziale. Gravi errori possono verificarsi per la consegna tardiva di diagnosi critiche, per la consegna di referti al servizio / clinico sbagliato ed a seguito dell’interpretazione del referto da parte del clinico richiedente.

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1. Danno minimo (senza morbilità): per ritardo (senza morbilità); per ritardo o trascurato.

Categorie, impatto clinico e incidenza degli errori

Gli errori in Anatomia Patologica sono stati raggruppati in quattro categorie: campione (perdita, dimensione (grandezza / volume), misure (assenti / errate), tessuto estraneo, difetti di campionamento, di processazione / inclusione / taglio / colorazione, procedure non eseguite), identificazione [paziente, campione, localizzazione anatomica, lateralità (destro vs sinistro)], interpretazione [diagnosi false positive / false negative; errori di classificazione (assenza di classificazione, classificazione non corretta, grado, stadio, margine; ecc.)] e referto (insufficiente o inadeguato per informazioni errate / assenti / non diagnostiche; errori di dettatura o di trascrizione che non alterano la diagnosi; errori di trasmissione / consegna) (2).

Lo stesso Autore (2) classifica i gradi di gravità dell’errore in base alle conseguenze cliniche. Nessun impatto sulla cura: nessun danno per referto errato non trasmesso o non ricevuto e mancato incidente per referto errato ricevuto ma ignorato o trascurato. Danno minimo (senza morbilità): per ritardo
For several years an increase of litigation for damages related to alleged cyto-histopathological diagnostic errors has been stressed. Schematically, at the base of diagnostic errors of this type, may take place adverse events within the pre-analytical phase, from arrival of the sample up to the vision of slides, within the analytical phase (and in this case it is usually a misinterpretation) or, within the post-analytical phase, in relation to the wording of the report, to its interpretation or to its possible “communicative ambiguity.” However, not infrequently, elements products in more than one of the above phases contribute to erroneous result. Errors actually subject of legal disputes, collected over a period of 7 years, broadly confirm the above general theoretical approach, although, obviously, the judicial dynamics often give to evolution of individual cases, significantly different connotations than parameters with which commonly, by professionals, severity of a diagnostic error is perceived. The judicial evaluation of a diagnostic error is, in fact, judged according to the damage that leads to the patient and not only to the “incorrect” diagnosis. It follows that an error based on a complex differential diagnosis, widely reported in the medical literature, between two variants of lymphoma or sarcoma, which seems minor, if not marginal, becomes clinically relevant if completely different therapies should be provided. Conversely a seemingly major mistake which diagnose a malignant lesion as benign, may be of no importance if the ‘right diagnosis’ would not make any significant change to the course of the disease.

In the series collection of diagnostic “errors” or “alleged errors” of judicial significance, an important place is given to pigmented lesions, in which, in some cases additional cutout from cell blocks, after many years, significantly helped to highlight initially dubious or even absent deep infiltration. Several cases considered “errors” can be attributed to the so-called Vanishing carcinoma phenomenon, that is the absence of infiltrating tumor on surgical sample after a diagnosis of malignancy on biopsy. This phenomenon, known in medical literature especially for prostate cancer, was found in the collection series, in cases involving larynx, uterus and stomach. The failure to search for specific biological markers in a well-defined tumor histotypes such as an extraintestinal GISTs, to make a personalized cancer therapy, can make claim as erroneous a correct but generic diagnosis of high-grade pleomorphic sarcoma. It should, however, highlighted the sharp decline of the proceedings under criminal law against those of civil type that result, however, in substantial growth.
GESTIONE DELLA SICUREZZA IN ANATOMIA PATOLOGICA: LA TRACCIABILITÀ PERCHÉ E COME ORGANIZZARLA

G. Fabbretti

GESTIONE DELLA QUALITÀ IN ANATOMIA PATOLOGICA: FASE DIAGNOSTICA (ANALITICA). APPROPRIATEZZA DEL REFERTO: I REQUISITI MINIMI E LO STANDARD DI REFERTAZIONE

F. Crivelli

GESTIONE DELLA QUALITÀ IN ANATOMIA PATOLOGICA: FASE POST-DIAGNOSTICA (POSTANALITICA). SECOND OPINION: COME UTILIZZARLA?

G. L. Taddei

ETICA E GESTIONE DELLE CONSULENZE ANATOMOPATOLOGICHE

A. Fabiano

VALIDAZIONE E CONTROLLI DI QUALITÀ IN IMMUNOSTOCHIMICA

M. Chilosi

RIORGANIZZAZIONE TERRITORIALE DEI LABORATORI DI ANATOMIA PATOLOGICA: PRO E CONTRO

R. Giardini

TAVOLA ROTONDA

Moderatori: Domenico Ientile (Palermo) – Roberto Mencarelli (Rovigo)

ARCHIVES AND STORAGE OF BIOLOGICAL MATERIAL

F.M. Vecchio

A clear discipline of the terms of storage of biological material is not properly defined.

The main sources on the subject are constituted by:

Circular n. 61 (December 19th, 1986) of Ministry of Health, General Directorate of Hospitals, having as a topic the retention period of the health records at the public health institutions and private nursing homes

Statement of the third section of the Italian Superior Council of Health concerning the definition of “remaining documentation diagnostics” (October 14th, 1987).


The most critical issues are briefly presented with a view to discussion.

TRACCIABILITÀ, CONTROLLI DI QUALITÀ, SICUREZZA

G. Fabbretti

Mercoledì, 23 novembre 2016

Aula Marin – 13:30 - 17:30

SEMINARIO BIOBANCHE

La ricerca clinica e le terapie biologiche: ruolo e responsabilità del patologo

Moderatori: Mattia Barbareschi (Trento) – Giorgio Stanta (Trieste) – Aldo Scarpa (Verona)

RICERCA CLINICA E ATTIVITÀ CLINICA: UN UNICO ELEMENTO PER IL TRATTAMENTO OTTIMALE DEI PAZIENTI

G. Stanta

Research which is directly performed on patients’ liquid and solid tissues is today a cornerstone of clinical activities. As it was shown by a Scandinavian study, hospitals where clinical research is carried out can provide better outcomes in patients’ treatment. This also includes the development of precision medicine, if we consider the great importance of N-1 trials today and in the future. Patients must also be followed up by performing appropriate biological sampling, which can define specific treatments by evaluating “Actionable Mutations”. Pathologists are in the frontline in this activity (if they wish), and they must learn to work together in wide networks with specialists in other disciplines, including oncologists, epidemiologists, molecular biologists, and above all the patients themselves. Thus all our archives become very important sources for the development of medicine. The only obstacle is reproducibility of results, which must be pursued by preanalytical conditions, standardization of methods, suitable controls, an accurate microdissection to assess heterogeneity aspects.

BIOBANCHE E ARCHIVI: LE NUOVE PROSPETTIVE DI RICERCA E DIAGNOSTICA

R. Lawlor - A. Scarpa
The need to regulate derives from the lack of any applicable law and by the ontological and teleological differences between the material from the archive with respect to medical records and other medical documentation. The obligation for the conservation of the material from the archive, imposed on health structure, becomes increasingly more expensive and difficult to implement over time and with the progressive increase of the number of samples that the same structure is required to retain. The drawback of obligation, including economic issue, stands out further if correlated with the peculiar method of preservation and archiving of samples. It seems thus appropriate, on this specific topic, to explore also the possibility of an externalisation of the obligation of preservation in favour of structures that satisfy the requirements for the conservation of the above-mentioned material.

Having stated the need to put a minimum period of duration of the obligation of storage, indicating the need for regulatory action on the point, it is believed that this period can be, congruently, determined in 10 years. The term of 10 years is suitable to adequately protect the patient as regards the diagnostic needs underlying the conservation of the material and to safeguard his good health. This term is also corroborated by guidelines of the CAP. The term of ten years is a minimum time limit after which the structure no longer has the obligation to keep the material. In any case, if legal proceedings, civil or criminal, are in course, the healthcare facility, heard the interested doctor, is required to evaluate the opportunity for the conservation of material, even beyond the period of ten years. Samples fixed and embedded in paraffin are perishable material if not kept in suitable spaces. They must also be compliant with all regulations on tracking and traceability as reported in paragraph 3.2 and subsequent 3.2.1 and 3.2.2 of the guideline. With regard to the method of preservation of the material in the archive it must be considered that the slides can also be stored in digital form, using techniques that allow to maintain the characteristics of the material and related medical and legal diagnostic needs which is stretched in archiving. The problem for many healthcare institutions of storage environments large enough to contain thousands of catalogued specimens requires to reconcile both the essential requirements of the protection of the probative value and balance sheet of the data contained within a histological preparation and the undeniable need for budget (economic and structural) of health care organizations calls to ensure the preservation of histological data. Temperature (30 °C < 27 °C), parasites flooding and humidity controlled (>30% and < 70 %), must be guarantee for paraffin tissue blocks. For the slides preservation in digital form or material may be understood as an alternative mode, left to the discretion of the structure.

**GESTIONE DEGLI ARCHIVI DI ANATOMIA PATOLOGICA**

A. Sapino¹, R. Tuccillo², S. Moriconi³, R Siliquini⁴

¹Istituto di Candiolo – Fondazione del Piemonte per l’Oncologia (FPO), IRCCS: Università di Torino, Dip. di Scienze Mediche; ²Judge Legal Secretary of the Regional Administrative Court of the Calabria, Center of Catanzaro; ³Medical manager, Higher Health Council, Secretary of Section I, Ministry of Health; ⁴Department of Public Health Sciences and Pediatrics, University of Turin

The Italian Ministry of Health through a working group of the “Higher Health Council” has produced a document of recommendation “Guidelines Tracking, Collection, Transport, Preservation and Storage of cells and tissues for diagnostic investigations of PATHOLOGICAL ANATOMY” available at http://www.salute.gov.it/imgs/C_17_pubblicazioni_2504_allegato.pdf, which specifically considers the problem of preservation and archiving of sample in pathological anatomy.

Different legal issues are discussed and specific legislative documents are taken into consideration, thus an in-depth reading of the whole document is recommended. However the specific purpose of this abstract is to summarize the main points related to tissue archiving.

**Minimum periods of storage and archival of tissue and methods of archiving.**

There is a distinction between the material processed for sampling and the unprocessed material, the so-called leftover material not sampled. While for the first category of material, there is a problem of long-term archival, for the second category, the needs underlying the relative preservation are limited and substantiated temporally and functionally.

**A. Not sampled tissue (so called reserve or leftover)**

The need for conservation is limited in time and has to match with the function performed by the same material, i.e. by the need to integrate with additional samples that should be taken, in the case of a lack or unfitness for the diagnosis. The leftover material, moreover, is bulky and perishable. The costs for a conservation, according to rules may be, therefore, large and the maintenance of suitable premises is somewhat complex. In this case the preservation of the material must be guaranteed up to the formulation of the diagnosis, date from which the same has no more a particular diagnostic utility or legal value. It follows that for the conservation of the reserve not sampled, it seems appropriate and consistent with the diagnostic and medico-legal purpose, the provision of a requirement for the conservation of 15 days, following (i.e. starting from) the date of validation of the diagnostic report.

The conservation of the leftover tissue should take place in appropriate environments and with systems to guarantee the security, tracking and conservation suitable to ensure a possible use for further investigation. It is suggested, in particular, the preservation of material with vacuum systems.

**B. Sampled material paraffin blocks and slides**

With regards to blocks in paraffin and slides the provision of a minimum term of preservation is more complicated and requires examining a plurality of competing factors. Needs of legal certainty and factual situations require setting a final date to the period of bonds. In view of this, fixing a time limit of minimum duration implies that at the end of its term there is no obligation for destruction or disposal of the material, but only the extinction of the obligation of the conservation of the material. The health care facility may, therefore, continue to hold the material for a longer period than indicated. The need to regulate derives by the lack of any applicable law and by the ontological and teleological differences between the material from the archive with respect to medical records and other medical documentation. The obligation for the conservation of the material from the archive, imposed on health structure, becomes increasingly more expensive and difficult to implement over time and with the progressive increase of the number of samples that the same structure is required to retain. The drawback of obligation, including economic issue, stands out further if correlated with the peculiar method of preservation and archiving of samples. It seems thus appropriate, on this specific topic, to explore also the possibility of an externalisation of the obligation of preservation in favour of structures that satisfy the requirements for the conservation of the above-mentioned material.

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**FISSAZIONE OTTIMALE DEI TESSUTI: FORMALINA IN VARIE MODALITÀ E OLTRE**

C. Marchiò

**ALTERNATIVE FIXATION TO FORMALIN**

C. Ghimenton

Anatomia Patologica: Azienda Ospedaliera Universitaria Verona

Alternative fixative to the most commonly used formalin exist from may years, but their use hasn’t become widespread. They can be broadly subdivided in two large groups: the first concerning glyoxal (a dialdehyde), the latter ethanol.
Glyoxal allows a morphology similar to that obtained using formalin, with the exception of a complete RBC lysis. Furthermore, has an acute very hazardous effect in case of ingestion, in case of skin contact (irritant), of eye contact (irritant), of inhalation. No data are yet available about carcinogenic or teratogenic effect. The second group is composed by fixatives mainly based on ethanol. This chemical is highly flammable and toxic by ingestion. A group of chemicals are usually added to prevent tissue shrinkage.

Preanalytical phase is extremely important to allow a perfect penetration of the various chemical, as is in daily practice with formalin. The use of heat, vacuum or a cold fixation could improve the morphology. Immunocytochemical reactions must be tailored to the chemical used, usually with good results. Molecular analysis performed on tissue fixed with formalin alternatives showed a good nucleic acids recovery, especially in the group of ethanol based.

In conclusion, formalin alternatives can be used in daily practice, paying attention to preanalytical phase and being aware of some morphological differences (usually not dramatic).

ADOPT IL PRIMO USE CASE DI BBMRI-ERIC: 10.000 CASI DI CARCINOMI DEL COLON

M. Lavitrano

ARCHIVE TISSUE NETWORK (NIPAB) IN THE CLINICAL RESEARCH

V. Canzonieri

Pathology Unit, CRO Aviano - National Cancer Institute - IRCCS

From the pathologist point of view, archived tissue slides and paraffin blocks retain their clinical usefulness long beyond initial diagnosis and treatment. Ensuring the availability of original tissue slides and paraffin blocks to pathologists is necessary for several reasons: extramural review, comparison with subsequent specimens, later ancillary studies, quality control and quality assurance activities conducted in pathology departments with access to tissue archives, including diagnostic reports, slides, and blocks; in medical centers with training programs, an archive of slides has a fundamental role in education, both in faculty-based teaching and in self-directed learning and the need to preserve tissue for medicolegal purposes. Such cases may be filed years after the slides were made and for unanticipated reasons. On the other hand, to support their research, investigators justifiably need high-quality, well-characterized, and clinically annotated human tissues. In recent years, the focus on translational studies and personalized medicine has resulted in ever increasing requests for archived clinical material for use in institutionally approved research. Therefore, unquestionably, archived diagnostic tissue from humans represents a rich resource for research. Why retrospective survival studies? Clinical research today can and should become an indistinguishable part of applied medicine strictly related to medical excellence. Moreover, a large part of these studies can be easily performed with a retrospective type design and reproducibility of clinical research can be increased by better organization and standardization of retrospective clinical studies that can accelerate the clinical use of important biomarkers. Finally, these studies can also be preparative to obtain better oriented prospective studies. Why archive tissues? They are available for almost every patient for clinical research and diagnostics and represent the major collection of human tissues with the entire clinical heterogeneity and with available clinical and follow-up information. Today we can perform most kinds of molecular analyses in those tissues and the studies can be conducted at a lower cost. The time period to conclude the research project is shorter because the tissues have already been collected and after carrying out an experiment, the results can be validated immediately and used for routine patient care shortly after. At the end of 2015 the secretariat of NIPAB, Italian Network of Pathology Archive biobanks, which is the Italian network of Pathology for paraffin archive was officially established at the CRO of Aviano (Pathology and Scientific Directorate).

Dr. Vincenzo Canzonieri and Dr Mattia Barbareschi are the secretary and the coordinator of this network. Dr. Mauro Trunini (next President of SIAPEC, Italian Society of Pathology and Cytology Anatomy Diagnostics) Prof. Giorgio Stanta for international relations (BBMRI, OECl, Impactnetwork, European Society of Pathology) and Prof. Marialuisa Lavitrano coordinator BBMRI Italy) are the founders and members of the steering committee of NIPAB. Unfortunately, during this year, some organization problems have determined a delay in launching the operative phase of NIPAB activities. It will be soon available a registration form for Italian Pathology departments which want actively participate in the network program. The form can be downloaded from the websites of the CRO (www.cro.it) of SIAPEC (www.siapec.it) and BBMRI Italy (www.bbmri.it), and when completed, should be sent to Dr. Vincenzo Canzonieri (vcanzonieri@cro.it - tel. 0434-659618, 0434-659292 or to anatomiapatologica@cro.it, Pathological Anatomy Secretary, ref. Mrs Anna Canoletta or dirscienti@cro.it 0434-659282 Scientific Directorate, ref. Mrs. Elettra Gislon). You can also send it by fax to 0434659370, Pathology CRO. Participation is voluntary and will result in the expression of interest in cases where NIPAB transmits to the network the opportunity to participate, for funding, to national and international research programs on paraffin material. The Directors of the Pathology departments (or delegates), if interested must, depending on the type of research project, communicate the availability (number of cases and other information contained in the tissue in paraffin application form) relating to the object of the research project. All participants will be full partners with the possibility of having access to a portion of the loan (if the project is approved) and to be involved as “authorship” in publications. Of course it will assess the extent of the contribution (number of cases made available and speed in answering the “call”) to confirm the membership to individual project and define the “benefits” related to these contributions.

I TESSUTI NELLA RICERCA SPERIMENTALE

M. Daidone

I TESSUTI E IL LORO USO NELLA RICERCA FOR-PROFIT

M. Macilotti
Mercoledì, 23 novembre 2016
Aula Maestrale – 18:00 - 20:00

SESSIONE PLENARIA
Quale futuro per la nostra disciplina?
Moderatore: Mauro Truini (Genova)

NOI GIOVANI PATOLOGI, SIAPEC IAP E LA NOSTRA PROFESSIONE IN CONTINUA EVOLUZIONE
M. Basciu

UNDERSTANDING THE PRESENT TO NAVIGATE THE FUTURE
F. Visinoni

In anatomic pathology, the current state encompassing the pre-analytic processes of tissue collection, handling, examination, preparation, processing and storage are largely uncontrolled, inconsistently performed and/or not standardized according to sound scientific data. Pre-analytic defects result in nearly three-quarters of the problems in laboratory diagnostics. This is evident in quality surveys from well-respected institutions that document high miss rates in the required basics of information related to patient and tissue identity, let alone parameters documenting quality aspects related to the surgical specimen and its preservation.

This talk will describe the historical approach to tissue processing and identify gaps from world-wide observations in current laboratory practices. It will also offer potential methodological and technological solutions and process improvements that laboratories may consider in serving the ultimate users of pathology information: the clinician and the patient. It illustrates the need for scientifically validated specimen guidelines and a performance based, standardized and documented “chain of custody” of the pre-analytical steps from the patient’s body through fixation. For thought leaders and professional standard setters, opportunities for optimizing molecular studies exist in specimen collection, transfer, grossing, fixation and decalcification protocols. Finally it will also outline the guidelines for the laboratory of the future, a laboratory “patient safety centered”.

In this evolving era of molecular profiling and personalized therapeutic decision-making, a well-reasoned and coordinated focus on pre-analytic processes that optimizes specimens for subsequent testing will result in:

* Improved specimen quality for molecular testing
* Improved accuracy of diagnostic and molecular test results
* Enhanced satisfaction of clinicians and patients

L’ANATOMIA PATOLOGICA, LA RIORGANIZZAZIONE PER PROCESSI E LA CONSEGUENTE GESTIONE DEL RISCHIO: UNA SFIDA PER I PROSSIMI ANNI
S. M. Mezzopera

TARGET MOLECOLARI ED INNOVAZIONE IN ONCOLOGIA
C. Pinto

THE FUTURE OF PATHOLOGY: BIG DATA
A. Lagostena

Researches on large shared medical datasets provide greater opportunities for improving health systems as well as individual care. Big data and Open data are closely related but they’re not the same. Open data is accessible data that can be used to analyse patterns and trends, make data-driven decisions, and solve complex problems.

Galliera is one N.I.San partners. The Italian Health Network for sharing of standard costs, indicators and outcomes founded in 2009 as a network for the exchange of information on standard costs of health activities who operate in sharing the results based on the determination of the average cost per hospitalization episode, used as a benchmark for all N.I.San members. Genetics: next-generation sequencing enables researchers to study biological systems at a level never before possible. Next-generation sequencing allows us to make DNA into digital, the challenge ahead is the proper interpretation of the data: the ability to understand the biological significance of them.

Digital imaging represents next evolution in pathology. Digital pathology, today mainly used for telepathology and training, will also become tool for making diagnoses.

Giovedì, 24 novembre 2016
Aula Levante – 08:30 - 12:30

DERMATOPATOLOGIA
Pitfalls in dermatopatologia
Moderatori: Claudio Clemente (Milano) - Giovanni Angeli (Novara) - Marco Santucci (Firenze)

PITFALLS NEL CAMPIONAMENTO DELLA PATOLOGIA CUTANEA
T. Zanin
PITFALLS IN MELANOCYTIC TUMORS

C. Clemente
Servizio di Anatomia Patologica e Citopatologia; IRCCS Policlinico San Donato, San Donato (Milano)

The histopathologic diagnosis of benign and malignant melanocytic tumors requires a suitable biopsy, assessment of many histologic criteria, awareness of potential pitfalls, relevant experience, and, in difficult cases, second opinion. The pitfalls may be many and relate to benign melanocytic tumors simulating melanomas and melanomas simulating benign melanocytic tumors or non-melanocytic neoplasms. An example of difficult histopathological diagnosis is the deep penetrating nevus in which some basic diagnostic parameters such as lack of maturation in depth, cytological atypia, diffuse and strong immunohistochemical expression of HMB45 in the deep portion of the tumor, may lead to misinterpretations. The immunohistochemistry helps in the differential diagnosis although an ideal and specific marker has not been described yet. Melanocytic cells can be epithelioid, spindle, clear, rhabdoid, signet ring-shaped and can be arranged in numerous architectural growth patter (e.g. trabecular, nested, glandular, sheets, papillary etc.) mimic primary or metastatic different tumors like carcinomas, sarcomas, plasmacytoma and high grade malignant lymphomas. The melanoma can undergo heterologous differentiation, such cartilaginous, osteoid, rhabdoid, fibroblastic and myxoid and suggest diagnostic misinterpretation. Following the rare melanomas that can mimic other tumors:

- Melanomas with rare cytologic features
- Signet-ring melanoma
- Rhabdoid melanoma
- Balloon cell melanoma
- Clear cell melanoma
- Spindle cell melanoma
- Small cell melanoma
- Melanoma with paradoxical maturation
- Giant cell (monster cell) melanoma
- Oncocytic metaplasia in melanoma
- Melanoma with psammomatous bodies
- Amelanotic melanoma
- Melanomas with rare stromal/matrix features
- Desmoplastic melanoma
- Myxoid melanoma
- Metaplastic osteo-cartilaginous melanoma
- Melanomas with divergent differentiation
- Neurotropic melanoma
- Melanoma with ganglioneuroid differentiation
- Melanoma with neuroendocrine differentiation
- Melanomas with rare architectural pattern
- Polypoid melanoma
- Verrucous melanoma
- Follicular melanoma
- Melanomas mimic other tumors
- Melanoma with neuroid differentiation
- Plamacytoid melanoma
- Melanoma «pseudo-lymphoma like»
- Melanoma with pseudo-achantholytic or pseudo-glandular pattern
- Pseudo-sebaceous melanoma
- Angiotropic, angiomatoid, pseudovascular melanoma
- Melanoma like carcinoid, paraganglioma, hemangiopericytoma
- Amelanotic like fibro-histiocytic melanoma
- Melanoma with composite immunophenotypic expression
- Melanocarcinoma

Recognition of these histologic unusual variant is important to avoid misdiagnoses.

MELANOCYTIC TUMORS WITH BIALLELIC MUTATIONS OF THE BAP1 GENE (BAPOMAS)

G. Ferrara
Department of Oncology, Anatomic Pathology Unit, Gaetano Rummo General Hospital, Benevento, Italy

Biallelic inactivating mutations of the BAP1 tumor suppressor gene on 3p21.1 and loss of nuclear immunoreactivity for the BAP1 protein are characteristic of BAPOmas (1) which represent about 0.005% of all excised melanocytic tumors. BAPOmas encompass a morphological spectrum ranging from completely benign (Wiesner nevi,(2)) through melanocytic borderline atypical intradermal tumors (MBAITs) (3), to overt malignancies. A BRAFV600E mutation, detectable with the VE1 immunostain, is present in about 70% of BAPOmas.(3).

Histopathologically, Wiesner nevus is dermal-based, with no involvement and no hyperplastic response of the epidermis. It is composed of ‘monotonously atypical’ epithelioid cells with ‘inclusion-like’ cytoplasms intermingled with lymphocytes. The lack of epidermal response, the ‘inclusion-like’ quality of the cytoplasms of tumor cells, and the lymphocytic infiltration are the main differential features of Wiesner nevus from epithelioid Spitz nevus. In addition, Wiesner nevus is more often seen in a ‘combined’ setting with a ‘congenital’ or ‘congenital-like’ nevus.

If diagnosed according to strict histopathological criteria, a Wiesner nevus requires no further action after excision. The patient, however, should be checked for multiple fibroma-like nevi: such nevi are the hallmark of the rare familial tumor predisposition syndrome (TPDS; OMIM 614327) due to germline BAP1 inactivating mutation, which is associated with atypical Spitz tumors/MBAITs, cutaneous and uveal melanoma, and internal neoplasms (mesothelioma, renal clear cell carcinoma, lung adenocarcinoma, head-neck squamous cell carcinoma, breast carcinoma, myelodysplasia, medulloblastoma, meningioma).(1,3)

Some preliminary data (Dr. Thomas Wiesner; personal communication) suggest that loss of heterozygosis at the BAP1 locus is associated with good prognosis even in the presence of clear-cut morphological features of malignancy. In our opinion, such data must be confirmed on larger numbers and with longer follow up periods; thus, while waiting for more solid prognostic information, we cautiously suggest to manage BAP1-negative melanocytic tumors with overtly malignant histopathological features as per conventional melanoma; morphologically borderline BAP1-negative tumors (MBAITs) should undergo a conservative re-excision followed by echotomographic onitining of the regional nodes.

References

Pustular dermatoses constitute a spectrum of conditions defined by the finding of non-infectious neutrophilic intraepidermal microabscesses. The clinical spectrum ranges from localized involvement to generalized disease with associated acute systemic inflammation and multi-organ involvement. By definition a pustule is a vesicle filled with neutrophils. The recruitment mechanism and the predisposition of neutrophils to be recruited are the main variables of this cutaneous inflammatory patter. Many molecular pathways and genetic mutation are now better defined.

Neutrophils derive from bone marrow precursors and are first involved in innate immune responses. Despite for a long time neutrophils had been labelled as mere phagocytes, in the last years other new functions and properties have been better defined. They have a unique way of dying called NETosis which stands for neutrophil extracellular traps (NETs). Stimulated neutrophils can extrude intracellular organelles together with nuclear proteins, mainly histones, to form a net that entraps extracellular microbes. This mechanism is designed at removing microorganisms but the exposition on endogenous antigens and the concentration of inflammatory lymphokines in the “NETs” can trigger and self-sustain autoimmune or autoinflammatory processes. Among neutrophils recruiters keratinocytes play a relevant role in psoriasis and other pustular dermatoses. Synthesis of IL-17E, a cytokine with a distinctive role, is greatly increased in lesional keratinocytes. Pustular psoriasis may be considered the prototype of pustular dermatoses. It can present as localized or generalized variants. Generalized pustular psoriasis is rare, acute form of dermatosis characterized by the widespread eruption of numerous sterile pustules on an erythematous base. The histologic diagnostic clue is the presence of intraepidermal collection of neutrophils forming the so called spongiform pustules of Kogoj. Variable epidermal psoriasiform changes accompany neutrophil migration.

The main differential diagnosis of generalized pustular psoriasis is with AGEP (acute generalized exanthematus pustulosis). Overlapping histological features make it difficult to diagnose this entity in the absence of clinical information. Smaller intraepidermal pustules and underlying vasculitic changes are more characteristic of AGEP. Clinical history of recent drug intake may be diriment.

Rare forms of Sweet Syndrome can be exceptionally characterized by the presence of intraepidermal pustules. More typical dermal neutrophilic infiltration and edema associated with fever and systemic symptoms are of help for the correct diagnosis.

Subcorneal pustular dermatosis (Sneddon-Wilkinson disease) is a rare, chronic, relapsing, vesciculopustular dermatosis. Trunk, intertriginous areas, and the flexor aspect of the limbs are typically involved. Placcid pustules may coalesce to form serpiginous lesions mimicking those of annular variant of psoriasis. A wide subcorneal pustule is a diagnostic clue although the differential diagnosis with psoriasis may be difficult if not impossible. The presence of intercellular deposits of IgA in some cases allows to better classify this entity as IgA pemphigus. Pyoderma gangrenosum may be associated with subcorneal pustular dermatosis and other diseases in which TNF-α plays a role.

The main localised variants of pustular psoriasis include: acrodermatitis continua of Hallopeau and palmoplantar pustulosis. Acrodermatitis continua of Hallopeau is a chronic, sterile, pustular eruption that predominantly affects the fingertips with nail involvement. Much debate has been done in the past whether to consider Acrodermatitis continua of Hallopeau a distinct entity. Recently, the same recessively inherited mutations in the IL36RN gene, which encodes interleukin-36 receptor antagonist (IL-36Ra), have been demonstrated in both generalized pustular psoriasis and acrodermatitis continua of Hallopeau in a familiar setting.

The relationship between palmoplantar pustulosis and pustular psoriasis has long been the subject of debate. In about 20% of cases both diseases can coexist. However palmoplantar pustulosis has distinctive characteristics: pustules involve primarily acrosyringia and clinically a strong association with smoke has been established. The explanation may be given by the fact that nicotine is excreted in the eccrine palmar ducts and furthermore 40% of patients with palmoplantar pustulosis have antibodies to nicotinic acetylcholine receptors that have immunoregulatory functions. Nicotine also induces neutrophils to release NETs in a dose-dependent manner.

Differential diagnoses of pustular dermatoses include also: contact dermatitis, pachyonychia, dermatophyte infection, drugs. A correct diagnosis may be very difficult in some cases. Careful identification of all histological clues together with the evaluation of the clinical picture are essential.

References

Yebabe M. Mengesha and Michelle L. Bennett - Pustular Skin Disorders; Am J Clin Dermatol 2002; 3 (6): 389-400

PITFALLS NELLE NEOPLASIE MESENCHIMALI I

M. Montesco

PITFALLS NELLE NEOPLASIE MESENCHIMALI II

A. Parafioriti

PITFALLS IN INFLAMMATORY DISEASES: PUSTULAR DERMATOSES

A. Cassisa

Servizio di Anatomia Patologica; ASST-Mantova Ospedale Carlo Poma

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Neutrophils derive from bone marrow precursors and are first involved in innate immune responses. Despite for a long time neutrophils had been labelled as mere phagocytes, in the last years other new functions and properties have been better defined. They have a unique way of dying called NETosis which stands for neutrophil extracellular traps (NETs). Stimulated neutrophils can extrude intracellular organelles together with nuclear proteins, mainly histones, to form a net that entraps extracellular microbes. This mechanism is designed at removing microorganisms but the exposition on endogenous antigens and the concentration of inflammatory lymphokines in the “NETs” can trigger and self-sustain autoimmune or autoinflammatory processes. Among neutrophils recruiters keratinocytes play a relevant role in psoriasis and other pustular dermatoses. Synthesis of IL-17E, a cytokine with a distinctive role, is greatly increased in lesional keratinocytes. Pustular psoriasis may be considered the prototype of pustular dermatoses. It can present as localized or generalized variants. Generalized pustular psoriasis is rare, acute form of dermatosis characterized by the widespread eruption of numerous sterile pustules on an erythematous base. The histologic diagnostic clue is the presence of intraepidermal collection of neutrophils forming the so called spongiform pustules of Kogoj. Variable epidermal psoriasiform changes accompany neutrophil migration.

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Differential diagnoses of pustular dermatoses include also: contact dermatitis, pachyonychia, dermatophyte infection, drugs. A correct diagnosis may be very difficult in some cases. Careful identification of all histological clues together with the evaluation of the clinical picture are essential.
Follicular lymphoma (FL) represent 32% of all lymphomas in adults. It derives from germinal centre cells B-cells with which it shares many morphologic, phenotypic and genetic features. The recent update of the WHO classification of Lymphoid Tumours (1) revises this category, adding to the so called “usual type” FL, other variants which have proved in the literature to be provided with peculiar immuno-morphologic and genetic features. FL usual type is usually positive for the translocation (14;18) with overexpression of the Bcl2 protein, although 10-15% of cases may turn out negative for the protein. Among the latter some are true Bcl2 negative cases since they do not bear the t(14;18) while others maybe regarded as “pseudonegative” FL since usage of alternative anti-Bcl2 clones (such as E17 or SP66) to the most widely used one (clone 124) lets the expression be identified. This phenomenon has been shown to be caused by mutations in the translocated BCL2 gene locus which affect the protein structure in the clone 124 binding site (2). Translocation positive and negative FLU are reported by some authors to moderately differentiate RS-like cells in a NHL from HRS cells in a CHL. Immunohistochemical aspects that could be useful in differentiating RS-like cells can be rarely observed in non-Hodgkin lymphomas and could represent an important diagnostic challenge. With this report we present a case of Peripheral B-cell Lymphoma, centrofollicular type of Grade IIIa, with CD30+ Reed Sternberg-like cells and summarize histological and immunohistochemical aspects that could be useful in differentiating RS-like cells in a NHL from HRS cells in a CHL.

Methods and materials
A 54-year-old woman with a “silent anamnesis”, presented with an inguinal lymphadenopathy of 2 cm about two months-lasting, in absence of B symptoms, systemic and cutaneous signs.

Results
On the microscopic examination there was a nodal structure completely effaced by a follicular proliferation with a mixture of centrocytes and centroblasts (>15/high-power field in most follicles), consistent with a grade 3A follicular lymphoma (Fig.1). Some centroblasts showed a classic Reed-Sternberg (RS) and Hodgkin cell (mononuclear variant)-like morphology (Fig.2). Immunohistochemical studies revealed that centrocytes and “typical centroblasts” were positive for CD20, CD10, BCL6 and negative for BCL2 (consistent with a diagnosis of follicular lymphoma at inguinal site) whereas the RS-like cells showed a “bizarre phenotype”, halfway between the "bizarre phenotype", halfway between
a centre germinal B-cell and a typical HRS cell: CD30+, CD15-, CD20+, PAX5+, IRF4/MUM1+ and CD45+ (Fig.3). LMP1 and EBER-ISH were negative. The histological and immunohistochemical findings were consistent with a diagnosis of “Peripheral B-cell Lymphoma, centrofollicular type of Grade IIIa, with CD30+ Reed Sternberg-like cells”.

**Conclusion**

RS-like cells can be observed in non-Hodgkin lymphomas and could represent an important diagnostic pitfall. Immunohistochemical aspects that could be useful in differentiating these cells from typical HRS cells of a CHL were the co-expression of typical Hodgkin markers (CD30) and B-cell markers (CD20 and PAX5 §, strong and homogeneous), positivity for CD45 and co-expression of CG-markers (CD10 and Bcl6, almost never co-expressed in HRS cells of a CHL). Curiously, we observed a “crescendo pattern” for IRF4/MUM1 passing by centrocytes and centroblasts to RS-like cells (an aspect already described in LNH-B enriched with RS-like cells), whereas follicular lymphomas are typically negative for IRF4/MUM1. The expression of IRF4/MUM1 seems related to a molecular signature/imprinting by CD30 positivity (CHL, PTCL-NOS CD30+ and ALCL ALK+/ALK-).

**References**

follicular T-cell lymphoma and other nodal lymphomas of TFH derivation. The latter two subtypes were listed among PTCLs/not otherwise specified (NOS) in the 2008 edition of the Classification (1). The rationale for the creation of this new category is represented by the common derivation of all these neoplasms from TFH elements as well as by the fact that they share similar clinical, cytological, phenotypic and molecular features. Clinically, they show for instance high stage, Coombs test positivity, anemia, and polyclonal hypergammaglobulinemia. On cytological ground, they consist of small-medium sized elements, provided with a clear rim of cytoplasm and a slightly irregular nuclear profile. At times, these elements represent a minority of the whole examined population. In all instances, they are intermingled with a variable amount of EBV-infected B-blasts that can give rise to a secondary diffuse large B-cell lymphoma. At immunophenotyping, lymphomatous cells express at least two, possibly three of the following markers: PD-1, CXCL13, ICOS, SAP, BCL6, CD10, and CCR5. The gene signature is close to the one of normal TFH cells and distinct from that of the remaining T- and NK-cell lymphomas. NGS studies reveal recurrent mutations of the following genes: TET2, IDH2, RHOA, FYN, and CD28: ITK/SIK and CTLA4-CD28 fusions have also been reported. Some of these lesions may represent the rationale for targeted therapies. The distinction among the three subtypes is based on architectural features. ATLL is characterized by partial preservation of the marginal sinus and hyperplasia of high endothelial venules (HEV) and follicular dendritic cells (FDC). Follicular T-cell lymphoma resembles follicular B-cell lymphoma at low power but is formed, at higher magnification, by the above described clear elements with TFH profile instead of centrocytes and centroblasts. Finally, the third subtype displays a diffuse growth pattern without hyperplasia of HEV and FDC. Transition from one subtype to the other can be observed in serial biopsies from the same patient.

Revision of PTCL/ NOS
Except for Lennert’s lymphoma (lympho-epithelioid T-cell lymphoma) that brings cytotoxic, non-activated phenotype, this remains a very heterogeneous category both on morphologic and phenotypic grounds. However, gene expression profiling (GEP) reveals a signature that differs from the one of all remaining T-cell neoplasms and allows further differentiation in three subgroups related to Th1 (GATA3), Th2 (TBX-21), and cytotoxic T-lymphocytes (1). Such distinction is provided with prognostic relevance, GAT3 and cytotoxic T-lymphocytes being provided with a significantly more aggressive clinical course. In addition, GEP studies have confirmed that tumors strongly expressing CD30 (in more than 75-80% neoplastic cells) have a worse response to chemotherapy than ALK-negative ALCL. NGS has so far been applied to a limited number of these neoplasms. Recently, VAV1 lesions have been reported as characteristically occurring in about 15% of PTCLs/NOS.

Promotion of ALK-negative ALCL from provisional to accepted entity
Several independent GEP studies have underlined that ALK-negative ALCL has a gene signature that is close to the one of the ALK-positive form, the differences being related to the occurrence of the t (2;5) and variants in the latter, which causes overexpression of the ALK gene and protein (1). This can explain the morphologic and phenotypic identity between ALK+ and ALK-negative ALCLs. A further element is the recent discovery of JAK1/STAT3 mutations in the setting of ALK-negative ALCL, producing downstream the same effects as t (2;5) and variants. Molecular studies have also allowed the distinction of prognostically different subtypes of ALK-negative ALCL, characterized by lesions of DUSP22 and TP63 or TP53. While the former herald a good response to therapy (and are also found in 50% of primary cutaneous ALCLs), the latter have a deleterious impact on the clinical outcome. Finally, aberrant levels of ERBB4 transcripts have been reported, possibly playing a pathogenetic role.

In the setting of ALK-negative ALCL, one should remind the recently added provisional entity of breast-implant-related ALCL. A further element is the occurrence of the t (2;5) and variants in the latter, which cause overexpression of the ALK gene and protein (1). This has so far been applied to a limited number of ALCL. NGS has so far been applied to a limited number of these neoplasms. Recently, VAV1 lesions have been reported as characteristically occurring in about 15% of PTCLs/NOS.

THE 2016 REVISION OF THE WHO CLASSIFICATION: CUTANEOUS LYMPHOMA

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Within the 2016 revision of the World Health Organization classification of Haematopoietic and Lymphoid Tissues [1], the subject of primary cutaneous lymphomas will be focused with particular attention to the refinement of both clinico-pathologic and prognostic features, with the aim to guide the best therapeutic strategy and improve the knowledge of such a specific topic.

As to T-cell lymphomas, mycosis fungoides (MF) will be confirmed to represent a variegated disease with well defined clinico-pathologic variants with prognostic value. The need of a comprehensive staging will be stressed and phenotypic determination of CD30 will be encouraged, to allow the identification of the progressing forms [2]. The chapter of primary cutaneous CD30+ T-cell lymphoproliferative disorders will comprise two main entities - primary cutaneous anaplastic large cell lymphoma (PC-ALCL) and lymphomatoid papulosis (LyP) - which will experience several updates concerning their biologic and histologic features [3]. The identification of a specific rearrangement, involving DUSP22 locus on chromosome 6p25.3, in up to 30% of PC-ALCL cases underscores the biologic similarity with systemic ALCL, together with providing a further tool in the differential diagnosis with other cutaneous lymphoma subtypes. Moreover, the occurrence of sporadic cases displaying ALK rearrangement and/or expression is reported, particularly in the pediatric setting, an indication which requires further characterization. As to LyP, three new variants will be formalized (namely, LyP type D, LyP type E and LyP with 6p25.3 rearrangement): the recognition of these different types is important to avoid...
misdiagnosis of other often more aggressive types of cutaneous lymphoma. The chapter of rare subtypes will be enriched by the entry the provisional entity of primary cutaneous acral CD8+ T-cell lymphoma, an indolent form benign behavior, requiring a careful distinction from aggressive forms [4]. The provisional entity of primary cutaneous CD4 positive small/medium T-cell lymphoma will be downgraded to the status of lymphoproliferative disorder, to stress the indolent nature of the disease and to avoid unnecessary overtreatment, and its diagnostic features will be better characterized [5]. As to gamma-delta lymphoma, a particular attention will be paid to excluding the less aggressive forms (i.e. MF, LyP) which may present a γ/δ phenotype [6].

As to the chapter of B-cell lymphomas, the major updates will be devoted to their molecular landscape. In particular, it has been better defined the incidence of BCL2 rearrangement in the setting of primary cutaneous follicle center cell lymphoma, roughly occurring in 20% of cases [7]. As to primary cutaneous diffuse large B-cell lymphoma, leg type, several imbalances in different components of the B-cell receptor signalling pathway have been underlined, with particular occurrence of mutations in MYD88 L265P, which show an association with an inferior survival [8]. However, several points remain to be clarified, which have been partially addressed by the recent literature [7].

In conclusion, the incoming revision will represent a valuable tool to improve the daily management of the lymphoma-affected patients and to allow prospective collection of more precise biologic and clinical data, to maximize the therapeutic strategies.

References

PRESENTAZIONE DI DUE ESEMPI DIAGNOSTICI-PATOLOGICI E DISCUSSIONE: I LINFOMI CUTANEE

A. Cimadamore

We detail a difficult and rare case of cutaneous T-cell lymphoma. The patient is a 92-year-old man with two year-history of dermatosis localized to both hands, clinically interpreted as chronic eczema. His past-history was also positive for psoriasis and eczema.

The first biopsy specimen was small with artifacts, nevertheless considered by the pathologist strongly suggestive for a T-cell lymphoma, not otherwise specified lacking the clinical correlation; however, without a definitive classification of the lesions, his dermatologist started treatment with phototherapy (PUVA) obtaining a complete resolution of lesions at the hands. After few months, however, the patient suffered for the rapid occurrence of multiple plaque and nodular lesions covering almost the 80% of the total skin surface and necrotizing; after failure to respond to radiotherapy, the dermatologist referred the patient to our University hospital for reconsidering the diagnosis and therapy at the Clinic of Hematology. We received a second skin biopsy specimen, showing a lymphoid infiltration, mainly localized in the dermis with diffuse infiltration of the epidermis and adnexa and involvement of the subcutaneous tissue. The elements were medium-sized lymphoid cells, with reactivity for T-cell antigens, cytotoxic molecules, TCRγ-M1 and CD56 (partial) and negativity for CD4, CD8, EBV, CD30 and TCRβ-F1. Proliferative activity was high. PCR showed monoclonal rearrangement for T-cell receptor gamma gene. We had the opportunity to review and study again the original histologic material of the first biopsy, which showed a neoplastic T-cell infiltrate with the same phenotype. We herein present and discuss the spectrum of differential diagnoses among primary γ/δ T-cell lymphomas and other aggressive T-cell lymphomas in the skin.

Considering the age and comorbidities, the patient received steroids and low doses of methotrexate, without obtaining a significant improvement. He is still alive with stable disease after five months from diagnosis. Up to now, we have not found any extra-cutaneous involvement.

In conclusions, our case outlines the diagnostic difficulties of such rare cases, the need of a detailed phenotyping on formalin-fixed and paraffinembedded material and, finally, the possibility that some primary cutaneous γ/δ lymphoma may show a relative prolonged indolent and localised phase. In such cases, the distinction from Mycosis Fungoides with γ/δ phenotype is particularly difficult without any clinical and pathologic correlation.

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: UPDATE OF CLINICO-PATHOLOGICAL AND GENOMIC ANALYSIS WITH PRIMARY CUTANEOUS INVOLVEMENT

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Introduction

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, aggressive haematologic disease characterized by skin lesions and a simultaneous or subsequent involvement of peripheral blood, bone marrow and lymph nodes. It typically affects elderly patients. Histologically, tumour cells constitute a uniform population of blastic or more pleomorphic medium-size elements with a CD4/CD56 co-expression as well as positivity for dendritic cell (PDC) markers such as CD123, BDCA2, TCL1 and CD2AP. Multigain chemotherapy, is considered the frontline therapy, but allogeneic stem cell transplantation (allo-HSCT) offers a durable disease control.
Few studies investigated copy number changes by array-based comparative genomic hybridization (array-CGH). This is an update of our previous study performed in order to provide new insights on BPDCN and identify new oncogenetic pathways.

**Methods**

We collected 27 cases and analyzed clinico-pathological data. Molecular analysis were performed using array-CGH and SNP6 array.

**Results**

In our cohort, median age was 60 years (range 9-83) with an overall-survival (OS) of 20 months: 17 patients died from the disease and 3 patients died from the therapy. Fifteen patients were treated by multi-agent chemotherapy (mostly with acute leukemia-type regimen) and 3 of them consolidated the remission with allo-SCT. Two elderly patients presented a single skin lesion without any systemic involvement and were treated with local radiotherapy without evidence of relapses or progression. One of them died from cardiovascular disease, the second is still alive without evidence of disease after 64 months of follow-up. All cases had typical PDC immunophenotype. CD4 and/or CD56 were lost in 3 of them and aberrant expression of CD2 and/or CD7 were seen in 9/26. Genomic analysis showed losses on chromosomes 9 (73% of cases), 13 (62%), 12 (50%), 7 (23%). In particular, loss of CDKN2A locus (9p21.3) occurred in 19/26 patients. Six patients of them were characterized by biallelic loss of 9p21.3, confirmed by FISH and, compared to hemizygous loss, had reduced survival. Furthermore, loss of RB1 (13q13.1-q14.3), CDKN1B and ETV6 (12p13.2-p13.1) locus were observed in 16 and 13 cases respectively and the loss of 7p12 in 6 of our patients (IKZF1 gene). SNP6 analysis confirmed the same genomic aberrations found using aCGH.

**T-CELL LYMPHOBLASTIC LYMPHOMA AND LANGERHANS CELL PROLIFERATION OCCURRING IN THE SAME LYMPH NODE: A CASE DISCUSSION**

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We herein discuss the case of a 65 year old woman presenting with palpable laterocervical lymph nodes, in absence of systemic sign and symptoms with the sole exception of neutropenia. Histologic examination of an excised lymph node revealed an effacement of the architecture upon the presence of nodules of proliferation of lymphocytes mimicking follicles, surrounded by an expanded paracortical zone, filled with large cells. The population within the nodules displayed a blastoid morphology and a T-cell (CD2-, CD3+, CD5-/+ , CD7+), “precursor” (TdT+/- , CD34-/+ , CD1a-, CD117-, CD56-) phenotype, thus consistent with a diagnosis of acute lymphoblastic lymphoma / leukemia. The larger cells in the paracortical area displayed the cytologic and phenotypic (S100+, CD1a+, langerin+) features of Langerhans cells, with focal, aberrant expression of CD56, suggesting the possibility of a concomitant Langerhans cell histiocytosis (LCH). Whole tissue analysis of T-cell clonality displayed a TCRβ clonal rearrangement. The bone marrow biopsy resulted negative for disease.

The interplay between lymphoid populations, both of T and B phenotype, and Langerhans cell is well recognized, as about 30% of cases of Langerhans cell LCH has detectable clonal immunoglobulin od T-cell receptor rearrangement, including some cases with both T- and B-cell gene rearrangements [1]. As well, sporadic reports have documented the synchronous or sequential occurrence of a lymphoid or myeloid neoplasm in patients affected by LCH [2,3]. These observations may be potentially related to the lineage plasticity of a common haematopoietic precursor, or, in alternative, may suggest the possibility of a transdifferentiation of two different neoplasms.

**References**


**SESSIONE II**

**Patologia osteomidollare: neoplasie mieloidi**

Moderatori: Ada Maria Florena (Palermo) – Maurilio Ponzoni (Milano)

**MYELOPROLIFERATIVE NEOPLASM: UPDATES**

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According to the updated WHO classification of the tumours of the haematopoietic and lymphoid tissue the diagnosis of Philadelphia chromosome negative myeloproliferative neoplasms (Ph-MPN) requires the integration of clinical, morphological and molecular data.

Specifically, so called “driver” mutations have been identified in MPN and comprise mutation of Janus kinase 2 gene (JAK2 V617F and exon 12 mutations) which can be identified in about all the cases of polycythaemia vera (PV) and in about 5060% of those with essential thrombocythaemia (ET) and primary myelofibrosis (PMF). Moreover, mutations in the gene encoding the thrombopoietin receptor (MPL) can be documented in about 58% of the patients with PMF and 35% with ET. Finally, the last discovered mutations in the gene encoding the endoplasmatic reticulum protein calreticulin (CALR) have been reported in about 20% of PMF and ET patients, accounting for about 80% of the JAK2-negative cases. Identification of these mutations is of paramount utility in the differential diagnosis between reactive and neoplastic conditions associated with leukocytosis, thrombocytosis or polyclaglia. On the contrary, due to their lack of specificity, they cannot be helpful to differentiate among the Ph-MPN and their evaluation must be integrated with the clinical and morphological features of the bone marrow.

Focusing on PV the updated WHO classification proposed to reduce the level of Haemoglobin required for a diagnosis of
Polycythemia vera at a level of 16.5 g/dL in men and 16.0 g/dL in women, in order to collect most of the “masked” cases. In this contest, the analysis of the bone marrow morphology became a major criterion for the diagnosis of polycythemia vera.

In the context of PMF, a great effort has been made to improve the morphological and clinical features helpful for the differential diagnosis between ET and pre-fibrotic PMF. Finally, a new and more detailed grading system for the evaluation of the stromal changes has been proposed, considering separately the grade of fibrosis and that of collagen deposition and osteomyelosclerosis. This new system seems to be reproducible and could probably be helpful in the evaluation also of minor post-therapy modifications.

MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASMS (MDS/MPN)

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Myelodysplastic / myeloproliferative neoplasms (MDS/MPN) are myeloid malignancies with clinical, laboratory and morphological features that at onset overlap between myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). In this group of diseases, the integrated approach of different techniques (cytology, histology with immunohistochemistry, flow cytometry, cytogenetics and molecular genetics), coupled with clinical data, is of paramount importance for diagnosis. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia comprehends in this group chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia, BCR-ABL1-negative (a-CML), juvenile myelomonocytic leukemia (JMML), MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) and MDS/MPN unclassifiable. The new classification scheme in particular takes into account the new clinical, cytogenetic and molecular genetic evidences that are useful to correctly classify these cases both by a diagnostically and a prognostic point of view. In particular, CMML now needs for diagnosis not only the presence of persistent peripheral blood (PB) monocytosis (≥1x10^9/L), but also that monocytes account for ≥10% of the white blood cell count; moreover, prognostic stratification takes into account 3 blast-based groups: CMML-0 (<2% blasts in PB and ≤5% blasts in bone marrow – BM), CMML-1 (2-4% blasts in PB and 5-9% blasts in BM), CMML-2 (5-19% blasts in PB and 10-19% blasts in BM and/or presence of Auer rods), A-CML now is known to be associated in 1/3 of cases with SETBP1 and/ or ETNK1 mutations, while CSF3R abnormalities are rarer, contributing to better differentiate this entity from chronic neutrophilic leukemia. JMML has been refined in molecular diagnostic parameters, since 90% of cases carry somatic or germ-line mutations of PTPN11, KRAS, NRAS, CBL or NF1, which are usually mutually exclusive and activate the RAS-MAPK pathway. MDS/MPN-RS-T, previously known as refractory anemia with ring sideroblasts and thrombocytosis, is now recognized as a full entity, because of its peculiar molecular signature, which clearly indicates the hybrid – MDS and MPN- nature of the disease: mutations of the spliceosome gene SF3B1, related to the presence of ring sideroblasts, are frequently associated with MPN-driver mutations of JAK2, CALR or MPN.
and skills in diagnostic molecular biology. To obtain the certificate of specialist in pathology the resident must have performed a minimum of: 40 autopsies, 1200 gross descriptions, 4000 histological diagnosis, 800 cytological diagnosis, 200 frozen sections diagnosis and 50 molecular diagnosis. The Italian Schools of Pathology are certified by a National Committee (Osservatorio Nazionale). Certification is based on demonstration of appropriate facilities, diagnostic facilities, and diagnostic activity. Universities can increase the volume of diagnostic activity by establishing a Teaching Network (Rete Formativa) with other qualified Hospitals of the Region.

**IL MODELLO ITALIANO: L’ESPERIENZA DI 4 SCUOLE ITALIANE**

M. Basciu, F. Sarocchi, I. Montagnani, G. Mallet

**GIORDANO FELLOWSHIPS, ESCOP COURSES E EDUCATIONAL PORTAL DELLA ESP**

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**SURVEY ABOUT RESIDENTS’ TRAINING THROUGHOUT EUROPE, BY THE ESP TRAINEE SUBCOMMITTEE**

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The aim of the survey was to record information about the general training and working conditions and the preferences of pathology residents throughout Europe with special attention to the Italian responses, and to correlate them with the type of institution and workload of the departments. 293 pathology residents from 33 countries participated and 285 gave permission to use their responses. 189/285 were female and 95/285 male and 189/285 worked in a University Hospital. The residents were accepted to start the training program through exams, interview or CV submission. 54,4% follow a structured educational program with rotation among the pathology fields. 93,7% participate in frozen sections. 83,5% have an educational supervisor irrespective of the type of their institution, whereas the efficiency of the educational supervisor showed statistically significant correlation to the type of institution (P=0,002). The number of performed autopsies, the role of the resident in the microscopic examination of the cases, the adequacy of time for the microscopic examination of the cases as judged by the residents and the examined by the residents cases per month showed statistically significant correlation to the type of institution (P=0,017, P=0,016, 0,033 and P=0,043, respectively). Totally, the most popular field is Digestive pathology. Overall, there are major differences between the European countries regarding the duration and the structure of each country’s training program, the ways of earning the Certificate of Pathology Specialty and the general working conditions of the residents.

**PAM50 MOLECULAR SUBTYPING AND RISK OF RELAPSE IN ESTROGEN RECEPTOR-POSITIVE/HER2-NEGATIVE BREAST CANCER: MULTICENTRIC STUDY**

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**Goal**

The PAM50/Prosigna gene expression-based assay has reached level 1b evidence for the identification of post-menopausal patients with ER+/HER2-negative early breast cancer who do not need chemotherapy since their risk of distant relapse at 10-years is below 10% with endocrine therapy-only. At the same time, immunohistochemical detection of Ki67 is being proposed by International Consensus Guidelines as a biomarker to help identify low risk outcome patients despite the lack of level 1 evidence to support the use of this biomarker for this purpose. Here, we aimed to compare the ability of Ki67 to identify those patients at a low risk of recurrence as defined by the clinically and analytically validated commercial version of the PAM50 assay, Prosigna, through multicentric study.

**Methods and materials**

Prosigna and IHC data were evaluated from 3 independent cohorts (GEICAM 2012-09 clinical trial, Vall d’Hebron Institute of Oncology [VHIO] Central Lab and Campus Bio-Medico University of Rome - Molecular Diagnostic Lab) for a total of 324 consecutive postmenopausal women with early breast cancer, ER+/HER2-negative and node-negative disease. Prosigna™ diagnostic test is based on NanoString nCounter technology used for gene expression profile analysis. This is a robust and highly reproducible method for detecting the expression of 50 genes in a single reaction with high sensitivity. The methodology serves to bridge the gap between genome-wide (microarrays) and targeted (real-time quantitative PCR) expression profiling allowing analysis and validation of a large number of mRNAs and does not require the conversion of mRNA to cDNA by reverse transcription or the amplification of the resulting cDNA by PCR. Immunohistochemistry data were obtained by pathological report.

**Results**

Intrinsic subtype distribution (by Prosigna assay) was 63.0% Luminal A, 34.6% Luminal B, 1.2% HER2- Enriched and 1.2% Basal-like. ROR (risk of recurrence) group distribution was Basal-like. ROR (risk of recurrence) group distribution was 48.1% ROR-low, 32.4% ROR-intermediate and 19.5% ROR-
high. No significant differences in subtype, or ROR distribution, were observed across the 3 cohorts. The concordance rate between IHC subtype (low Ki67 vs. high Ki67) and Prosigna subtype (i.e. Luminal A vs. others) was 72.5% (kappa score = 0.43; moderate agreement) and 73.4% (kappa score = 0.39; moderate agreement) when Ki67 cutoffs of 14% and 20% were evaluated, respectively. The percentages of Luminal A tumors across Ki67 0-10%, 10-20%, 20-30% and >30% groups were 81.3%, 56.2%, 38.6% and 20.0%, respectively. Similar results were obtained when Ki67 was compared with ROR. The optimal Ki67 cutoffs for identifying ROR-low samples within tumor sizes of ≤2 cm and >2 cm were 14.5% and 14.0%, respectively.

Conclusion
This is the first report to compare risk prediction using Prosigna data with Ki67 data in the same sample set. Our results highlight the important discrepancy between both biomarkers, suggesting that gene-expression analysis is need in patients with HR+/HER2-negative breast cancer. Moreover, our study shows that 14% is the optimal cutoff for identifying low risk outcome patients who can be spared adjuvant chemotherapy when gene expression-based assays are not available.

THE USE OF A MOLECULAR TEST FOR BREAST CANCER PROGNOSIS: CLINICAL-PATHOLOGICAL CORRELATIONS AND THERAPEUTIC IMPLICATIONS ON A SELECTED COHORT OF PATIENTS.

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Goal
Over the last decade, gene signatures of prognosis are emerging for tailoring personalized treatment strategies based on the risk profile of individual patients (1) the prognostic value of current multigene tests in these cancers is limited. First-generation prognostic signatures (Onctype DX, MammaPrint, Genomic Grade Index. These molecular tests are particularly useful for patients who are affected by estrogen receptor-positive breast cancers of indefinite prognosis (2-4). We focused on Endopredict® (Myriad Genetics), a multi-gene test, which gives both a pure molecular fingerprint of the tumors (the “EP score”) and a score obtained by combining the EP score with the tumor size and number of metastatic lymph nodes (the “EPclin score”) (5). EPclin score discriminates between patients having “low risk” or “high risk” of relapse within 5 years. The attitude to use gene signatures in routine clinical work still varies in different countries. We decided to test the influence of the EPclin score on the therapeutic decision [adjuvant hormonal therapy (HT) alone or HT + chemotherapy (CT)] related to breast cancer with not univocal prognosis based on standard parameters.

Methods and materials
From July 2014, we prospectively collected 56 cases of breast cancer for which the therapeutic indication was debated at multidisciplinary meeting. The criteria of case selection were: tumor size < 2 cm; lymph node status negative or from 1 to 3 positive lymph nodes; high expression of hormonal receptors (>50% of cancer cells); intermediate expression of Ki67 (15-30%); HER2 negativity. For each case, clinical and pathological data were recorded [age, tumor size, nodal status, grade according with Elston-Ellis, Estrogen (ER) and Progesterone (PgR) receptors expression rates, Ki67 proliferation rate]. EndoPredict® was performed on each case and EP score and EPclin score were recorded. We then correlated both scores with clinical and pathological data. To investigate whether the molecular test results would have facilitate the agreement on the therapeutic protocols, the data base was submitted to 26 oncologists, who were asked to indicate the therapeutic option (HT versus HT + CT) before and after the Endopredict® results.

Results
EP score was significantly related with lymph nodes status (p = 0.008), tumor grade (p < 0.001) and PgR expression (p = 0.007); EPclin score was related with tumor grade (p < 0.001), PgR expression (p = 0.033) and Ki67 proliferation rate (p = 0.009). In 11 cases the risk was assessed as “high” according with EP score and “low” with EPclin score, while in 3 cases the risk was “low” by EP score and “high” by EPclin score. Treatment agreement was low (Cohen’s K: 26%; Z: 26.47) when oncologists were blind to Endopredict® results and improved following the results of the molecular test (Cohen’s K: 58%; Z: 24.32). The therapeutic indication changed from HT to HT + CT for 9 patients and from HT + CT to HT alone for 6 patients.

Conclusions
Both EP score and EPclin score correlate with grade and PgR expression. EPclin score gives a more comprehensive estimation of the risk of relapse and improves the agreement between oncologists in the subgroup of patients for whom the therapeutic decision is not univocal.

References

MICROCYSTIC ELONGATED AND FRAGMENTED (MEF) PATTERN OF MYOINVASION IN LOW GRADE ENDOMETRIAL CANCER AS PREDICTOR OF LYMPH NODE METASTASIS: A CLINICOPATHOLOGIC ANALYSIS OF 27 CASES FOCUSING ON PATTERN OF MYOMETRIAL INVASION

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Goal
To investigate the association of the microcystic, elongated
and fragmented (MELF) pattern of myometrial invasion, lymphovascular invasion (LVI) and lymph node metastasis, we reviewed a series of low grade endometrioid endometrial adenocarcinomas and correlated the presence of MELF pattern invasion and LVI with lymph node metastasis.

**Methods and Materials**
A total of 76 cases of total abdominal hysterectomy and bilateral salpingo-oophorectomy for low grade endometrioid endometrial adenocarcinoma has been collected between January 2015 and July 2016. Of these cases, we identified and reviewed 27 cases with concurrent lymph node dissection. For each case, we evaluated and recorded: tumor grade, MELF pattern invasion, LVI, depth of myometrial invasion, FIGO stage and lymph node metastasis. Immunohistochemical and genotypic evaluation of the three DNA mismatch repair (MMR) proteins hMLH1, hMSH2, hMSH6, along with microsatellite instability (MSI) analysis were performed in 15 of 27 cases.

Fisher’s exact test was used to discriminate significant differences between categorical variables (p < 0.05).

**Results**
Lymph nodes metastasis were identified in 7 of 27 cases (2.5%): 6 of 12 MELF-positive cases (50%) and 1 of 15 MELF-negative cases (6.6%). Of the 6 MELF-positive tumors with lymph node metastasis, all cases exhibited LVI. At univariate analysis, lymph node metastasis correlated with MELF pattern invasion (p = 0.02) and LVI (p = 0.02). Moreover, MELF-pattern invasion correlated with LVI (p = 0.0003), but not with depth of myometrial invasion and tumor grade. No significant association between lymph node metastasis and depth of myometrial invasion or tumor grade was found. Abnormalities in at least one of the three MMR genes were identified in 9 of 15 cases (60%). A high microsatellite instability (MSI-H) was found in 6 of 8 MELF-negative tumors (75%) and in 3 of 7 MELF-positive tumors (43%), all of which (100%) with lymph node metastasis. At univariate analysis, MMR deficiency/MSI-H phenotype and MELF pattern correlated with lymph node metastasis (p = 0.04).

**Conclusion**
MELF pattern invasion was found to be statistically related to LVI and lymph node metastasis suggesting that this growth pattern may be associated with tumors having more aggressive behavior. Interestingly, 3 out of 6 (50%) MELF positive tumors with lymph node metastasis were related to MMR deficiency and MSI-H phenotype status. These data underline the importance of a better approach to primary diagnosis, with emphasis on MELF pattern of invasion, LVI and MMR status, and may help to identify a subset of low grade/low stage endometrial cancer with high risk of lymph node metastasis, selecting a subgroup of patients who may benefit of lymphadenectomy or adjuvant therapy.

**MUTATIONAL ANALYSIS OF BRCA1 AND BRCA2 GENES IN HIGH GRADE SEROUS OVARIAN CARCINOMA WITH NEXT GENERATION SEQUENCING (NGS): CORRELATION BETWEEN SOMATIC AND GERM LINE MUTATIONS**

Michele Paudice1, Anna Garuti2, Viviana Gismondi3, Liliana Varesco1, Ezio Fulcheri1,4, Simone Ferrero5, Gabriele Zoppolii5, Valerio Gaetano Vellone1

**DISC-Anatomic Pathology, University of Genoa; DIMI-Oncological Internal Medicine, University of Genoa; Hereditary Cancer Center, IRCCS**

**San Martino-IST, Genoa; Fetal and Placental Pathology Unit, IRCCS Instituto Giannina Gaslini, Genoa; DiNOGMI- Obstetrics and Gynecology, University of Genoa**

**Goal(s)**
To analyze the prevalence of germline and somatic mutations in BRCA1/2 genes and the frequency of promoter methylation of BRCA1 gene in patients affected by High Grade Serous Ovarian Cancer (HGSC); to determine how much these genes are involved in the natural history of ovarian cancer and to evaluate the concordance between BRCA germline genetic test on peripheral blood and BRCA somatic on tumor tissue.

**Methods and Materials**
Patients with the following features were considered eligible for the purpose of the study: surgery for ovarian cancer performed at the IRCCS San Martino-IST, histopathologically confirmed diagnosis of HGSC, absence of neoadjuvant chemotherapy, genetic test on peripheral blood for germ line mutations performed at S.S. Hereditary Cancer Center. For each patient formalin-fixed-paraffin-embedded (FFPE) specimens were available at the archive of Anatomic Pathology Unit. Each case was reviewed by a pathologist expert in gynecological pathology. One paraffin block for each patient was chosen with the following features: optimal fixation and storage, high representativeness of the entire neoplasia, high tumor cellularity (>75%), low percentage of stroma, fibrosis and necrosis. From each selected sample, micro sections (20μm) were obtained and transferred to the Laboratory of Molecular Biology in Eppendorf tubes to search somatic mutations by Next Generation Sequencing (NGS). The main steps for the DNA sequencing were: DNA extraction from tumor tissue, determination of BRCA1/2 gene libraries, enrichment and emulsion PCR, sequencing and data analysis through a dedicated software. Also promoter methylation of BRCA1 gene were investigated with MLPA.

**Results**
A total of 38 patients fulfilled the inclusion criteria and somatic mutations of BRCA1/2 were analyzed; also BRCA1 promoter methylation were available. In 10 cases (26%) germline mutations were found. In 4 cases (11%) only somatic mutations were detected. Promoter methylation of BRCA1 gene was found in 8 cases (21%), in 2 cases with a concomitant somatic mutation. However, in 18 cases (47%) no genetic alterations in BRCA1 genes were found. In only one case, germ line mutation on peripheral blood was not confirmed on tumor tissue, probably for a deficiency of bioinformatic filters.

**Conclusions:** This study has shown how BRCA genes are crucial in HGSC and pathogenic alterations of the BRCA genes have been detected in more than half of the cases. Searching for mutations directly on the tumor tissue has yielded positive results: in all but one case, germline mutations, found on the peripheral blood were also confirmed on the tumor tissue. The success of this method depends on both the quality of the paraffin sample and the bioinformatic skills, which are essential for a proper data management. Clinically, sequencing of tumor DNA may allow patients with platinum-sensitive HGSC and who carry only BRCA somatic mutations to benefit from gene therapy (PARP inhibitors) as maintenance after first disease relapse.
MOLECULAR ANALYSIS FOR EGFR AND ALK MUTATIONS ON ENDOSCOPIC GUIDED BRONCHIAL BIOPSIES PROCESSED BY LIQUID-BASED CYTOLOGY

Arciullo D.1, Magnini D.2, Mulè A.1, Brunelli C.1, Fusco L.2, Varone F.2, Martini M1, Fadda G.1
1. Division of Anatomic Pathology and Histology, 2. Pulmonology and Bronchoscopy Unit - Catholic University, Foundation “A. Gemelli” University Hospital

Aim
Molecular classification entered in routine pathology practice, and latest classifications require an immunohistochemical (IHC) and molecular evaluation for administering new targeted EGFR mutation and ALK-translocation treatments for advanced lung adenocarcinoma. EBUS-TBNA is recommended over surgical staging as a first line test for lung cancer diagnosis and staging. The aim of this retrospective study is to evaluate the adequacy of cytological samples obtained by EBUS-TBNA and processed with a liquid based technique to evaluate the molecular characteristics of adenocarcinomas (ADC).

Materials and Methods
We retrospectively examined all patients who underwent EBUS-TBNA from October 2012 to October 2015 with diagnosis of hilar and/or mediastinal nodal metastasis from lung ADC. EBUS-TBNA samples were fixed in a methanol-based solution (CytolitTM) and promptly delivered to the pathology laboratory to be processed in accordance with manufacturer’s protocol (ThinPrep5000, Hologic Co., Marlborough, MA). Techniques used in this study were: LBC with immunohistochemistry (for the diagnosis of ADC) and DNA extraction (for EGFR mutation and ALK translocation analysis).

Results
From October 2012 to October 2015, 408 EBUS-TBNA cytological samples have been collected from lymph nodes of 313 patients. Two hundred and twenty-three lymph nodes had a diagnosis of malignancies and the specific subtypes were: adenocarcinoma (39,5%), non small cell lung cancer - non otherwise specified (14,4%), small cell carcinoma (11,2%), squamous cell carcinoma (7,6%), neuroendocrine tumor (including carcinoid) (4,5%), large cell carcinoma (1,3%), tumor cells - non otherwise specified (10,3%), extra-thoracic tumor metastases (7,6%) and lymphoproliferative disorder (3,6%).

Conclusions
Our retrospective analysis confirmed that EBUS-TBNA samples processed with a liquid based cytology technique are adequate for EGFR mutation and ALK translocation analysis in a high percentage of cases of primary lung adenocarcinoma (86,6% success rate). In fact liquid based cytology (LBC) enables the evaluation of the cell component from samples uniformly processed and the collection of material from limited samples. In selected cases a cell block can be obtained by the material stored in the PreservCyt solution. Our mutational analysis failure rate (11/82, 13.4%) can be explained by considering that EGFR and ALK molecular analysis has been activated during the first three years of use of EBUS-TBNA technique at our center, so the ability to provide adequate samples has gradually improved. The results of our study shows that pathology diagnosis and EGFR mutation and ALK translocation analysis can be satisfactorily performed on EBUS-TBNA samples processed by liquid based cytology, thus allowing the clinicians to select the most appropriate treatment for advanced lung adenocarcinoma.

References

METHODODOLOGICAL APPROACH TO METROS TRIAL: A PHASE II STUDY IN ROS1 RERARRANGED OR MET DEREGLATED NON-SMALL-CELL LUNG CANCER (NSCLC)

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1 Azienda Ospedaliero Universitaria Pisana, Pisa; 2 Dipartimento di Patologia Chirurgica, Medica e Molecolare e dell’Area Critica, Università di Perugia; 3 AUSL della Romagna, Ravenna.

Goal
The METROS trial is a multicenter prospective phase II study designed to assess the efficacy and safety and tolerability of Crizotinib in pretreated metastatic NSCLC with MET amplification or MET exon 14 mutation or ROS1 rearrangement. Crizotinib is an orally active inhibitor of receptor tyrosine kinases effective in NSCLC with ALK rearrangement, recently came up as effective in patients with ROS1 rearrangement and at least in some patients with MET deregulation, particularly individuals with exon 14 skipping mutations or with high levels of MET amplification. Our goal was to validate the molecular techniques applied for this trial.

Material and Methods
At the time of the present analysis, 288 patients were enrolled. All of them were analyzed for ROS1 translocation and MET amplification by FISH analysis. 154 patient were also evaluated for MET mutations. Formalin-fixed and paraffin-embedded tissues were used for molecular studies (biopitic, surgical and cell block samples). ROS1 status was investigated by a 6q22 ROS1 Break Apart FISH probe Ruo Kit that is composed of a 6q22 ROS1 (Tel) SpectrumOrange probe, approximately 317 kb in size and positioned telomeric of the ROS1 gene, and a 6q22 ROS1 (Cen) SpectrumGreen probe, approximately 557 kb in size and positioned centromeric of the ROS1 gene. MET amplification was analyzed by a MET Probe Mix consisting of MET SpectrumOrange probe, that spans the 456 kb region encompassing the MET gene on
The polymerase chain reaction and direct sequencing analysis of MET exon 13 to 15 region were performed to investigate the presence of exon 14 and its splice site mutations.

Results

29 patients were found to be ROS1 rearranged (12%), with 41.7 mean value of translocated nuclei. Thirty patients were MET amplified (5%), with a mean MET/CEP7 ratio of 3.47. Fifty-nine samples out of 288 (20%) were not adequate for WTS, excluding the presence of driver chromosome translocations in the two cases analyzed. WTS showed enrichment of the following pathways: Apoptosis signaling pathway (P00060), Ubiquitin Proteasome pathway (P00060) and Parkinson disease (P00049) in both cases. By WTS seven proteins with genes among the most abundantly expressed (top 10%) were selected and tested, by immunohistochemistry, on cases and controls. Follicular dendritic cell secreted protein (FDCSP) and Serglycin (SRGN) proved to be novel markers of follicular dendritic cells. They showed better specificity and sensitivity values than well known markers in FDCS differential diagnosis with other mesenchymal tumors (specificity of 97.78%, and 100%; sensitivity of 71% and 65%, respectively).

In this setting, we calculated that the best markers for FDC-S diagnosis were CXCL13, CD21, CD35, FDCSP and SRGN. This combination could discriminate 97.92% of FDC-S cases. By WGS we identified and confirmed deleterious mutations of genes belonging to known pathways: sonic hedgehog, VEGF/CREB, RAS signaling, FOS/JUN and c-MYB and others belonging to unknown pathways. Additionally, duplication and inversion were detected in chromosome 16 ad 4, respectively.

Conclusions

FDC-S is an enigmatic entity with unpredictable behavior, additionally, it is an uncommon disease with high incidence of misdiagnosis. Thanks to an international effort, by the use of high throughput techniques, we identified novel FDC-S markers and interesting deleterious mutations affecting pivotal pathways making little steps forward better diagnosis and better cures for the next generations of patients.

AUTOIMMUNE ENTEROPATHY: CLINICOPATHOLOGICAL FEATURES OF A SERIES OF 40 PATIENTS

M. Salemme, V. Villanacci, F. Facchetti

Goal

Autoimmune enteropathy (AIE) is an uncommon disorder characterized by protracted diarrhea, small intestinal villous atrophy, lack of response to dietary exclusion, and evidence of autoimmunity (presence of circulating gut epithelial cell antibodies and/or associated autoimmune conditions)1,2. It was described for the first time in children with intractable diarrhea, especially within the first 6 months of life3; however this condition is increasingly recognized in adults4. The pathogen-
eres of the disease is based on a dysregulation of gut humoral and immune function, resulting in an autoimmune response elicited by several autoantigens that alters the intestinal permeability. In addition to sporadic forms, AIE may be part of the IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) syndrome, caused by genetic mutations leading to T-cell overactivity. Furthermore, the disease is often diagnosed in patients with underlying immunodeficiencies. A prompt recognition of the disease, especially the syndromic form, is critical for an early and effective treatment (based mainly on both nutritional support and immunosuppressive therapies). In this view, histological evaluation of small intestinal biopsies is crucial in the diagnosis; morphological features reported in the literature are variable and not specific of the disease, mimicking other conditions, such as celiac disease, refractory sprue and acute graft-versus-host disease (GvHD). Masia and Brown recently reported the largest series of AIE, including 25 patients, both pediatric and adult; to better understand the histopathological features of the disease they classified duodenal morphological findings in four patterns, i.e. the “active chronic duodenitis” pattern (characterized by villous blunting, mononuclear inflammation of the lamina propria and neutrophilic cryptitis), the “celiac disease-like” pattern (characterized by villous blunting and marked increase in intraepithelial T lymphocytes), the “acute GvHD-like” pattern (characterized by villous blunting and increased apoptosis in crypt epithelium) and finally the “mixed” pattern (admixture of 2 or more of above 3 patterns). The most common detected pattern of injury in their cohort was the “active chronic duodenitis” pattern, followed by the “celiac disease-like” pattern. In addition histological analysis of other sites of the gastrointestinal (GI) tract (esophagus, stomach and colon) showed a spectrum of morphological abnormalities in all cases. Aim of our study was to report our experience in this field analyzing the clinicopathological findings of AIE cases seen at our institution over a period of 15 years.

**Methods and Materials**

We retrospectively collected 40 cases with a diagnosis of AIE seen at our institution between January 2000 and December 2015. For each case we evaluated clinical features including presence of underlying genetic syndromes, immunodeficiencies or other autoimmune conditions, signs/symptoms at presentation, abnormalities at laboratory tests and, where available, treatment after diagnosis and subsequent follow-up. From the histological point of view, duodenal biopsies of all cases were reviewed and classified in one of the four patterns proposed by Masia and Brown. Besides, all available endoscopic biopsies of other sites of the GI tract (esophagus, stomach and colon) were analyzed.

**Results.** Our series included 23 children and 17 adults with female predominance (53%). The age at the time of diagnosis ranged from 2 months to 73 years. One patient was carrier of IPEX syndrome, while 22 cases showed underlying immunodeficiencies. All patients presented with severe and protracted diarrhea. Extrapancreatic associated autoimmune conditions (diabetes mellitus, thyroid disease, uveitis, pancreatic, renal and hepatic injury) were observed in 13 patients, while the presence of circulating gut epithelial cell antibodies was identified in only 4 cases. After the diagnosis patients were treated with different types of therapies (including nutritional support, steroids, immunosuppressive agents); follow-up data (available in 30 cases) revealed a complete clinical remission in 27 patients while 3 patients died. Histological analysis of duodenal mucosa showed the presence of the “celiac disease-like” pattern in the majority of cases (50%) followed by the “mixed” pattern (“active chronic duodenitis” pattern and “acute GvHD-like” pattern). Morphological abnormalities in other sites of the GI tract were observed in most cases; in particular esophageal biopsies (available in 18 cases) showed presence of esophagitis in 13 cases, while the gastric mucosa (available in 32 cases) revealed chronic gastritis in 16 cases and active inflammation in 13 cases. Examination of colonic biopsies of 27 patients demonstrated acute inflammation of the lamina propria in 20 cases, while chronic inflammatory infiltrate was observed in 5 cases. Finally, additional findings such as apoptotic bodies in crypt epithelium, nodular lymphoid infiltrates or an increase number of eosinophils in the lamina propria were commonly detected, either inside and outside small intestine.

**Conclusion**

We present the largest series of AIE reported to date, including both pediatric and adult patients, and both syndromic and nonsyndromic cases. Our study confirms the clinical variability of this condition and stresses the crucial role of histological analysis to achieve an early and accurate diagnosis. The morphological heterogeneity of this disease has been highlighted, with protein histological abnormalities observed both in small intestine and in the other sites of the GI tract. The classification of duodenal morphological features in four patterns allows a better categorization of this condition, helping the pathologist to standardize the histological report. Moreover, awareness of the high frequency of morphological abnormalities outside the small intestine may facilitate the recognition of this uncommon disease, that lacks truly specific histopathological findings.

**References**

Introduction

Kidney transplantation is the best treatment option for patients with end-stage renal disease. The success of this procedure is largely based on the donor nephron mass (NM), as the nephron represents the functional unit of the kidney. However, the NM shows an extraordinary inter- and intra-organ heterogeneity, with subsequent challenges in its clinical and histologic quantification. Furthermore, after transplantation the number of nephrons can be reduced by cold ischemia time, transplant trauma, and the potential nephrotoxicity of immunosuppressive therapy, that may result in kidney failure. To date, histopathological evaluation of core biopsies from donor kidney before transplant is the gold standard procedure to predict recipient’s outcome. Given the heterogeneous distribution of the nephrons across the kidney, there are not standard protocols to define the NM, either on core biopsies and on surgical samples. Therefore, the role of this parameter as a biomarker in renal transplant patients is unknown.

Goal

Here we set out to define whether digital pathology can assist in the clinical workup of kidney transplant. To address this aim, we sought (i) to evaluate the NM by means of digital histomorphometric analysis and (ii) to evaluate the homogeneity of the nephron density across the entire organ.

Materials and methods

Five kidneys removed from brain-dead donors but found not to be amenable for subsequent implant (female n=2, male n=3, mean age of 62.5, range 21- to 83-year-old) were prospectively collected. For each case, 6 tissue wedges were sampled, encompassing distinct topographic areas of the kidney. Representative 4-μm-thick sections were cut from formalin-fixed paraffin-embedded blocks of all samples and stained with hematoxylin and eosin using standard protocols. In order to minimize human-related biases, each stained slide was digitalized and blindly analyzed by three pathologists using a dedicated navigation software. Subsequently, a customized counting grid was employed to evaluate the glomerular density of the tissue slide. Using a specific add-on module, both the cortex thickness and glomeruli size were measured. Finally, the total volume of the renal cortex was calculated using bioinformatic Cartesian-based algorithms.

Results

Table 1. Histomorphometric parameters of the cases included in the study

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney volume (cm³)</td>
<td>178.1</td>
<td>88.2</td>
<td>66.1</td>
<td>111.7</td>
<td>145.2</td>
</tr>
<tr>
<td>Renal cortex thickness (mm)</td>
<td>6.7</td>
<td>4.4</td>
<td>5.1</td>
<td>4.9</td>
<td>8.0</td>
</tr>
<tr>
<td>Mean glomerulus size (μm)</td>
<td>223.86</td>
<td>174.96</td>
<td>185.50</td>
<td>205.46</td>
<td>174.26</td>
</tr>
<tr>
<td>Glomerular density (%)</td>
<td>8.10</td>
<td>8.30</td>
<td>7.40</td>
<td>8.74</td>
<td>9.19</td>
</tr>
<tr>
<td>Nephron mass</td>
<td>497,156</td>
<td>389,450</td>
<td>301,369</td>
<td>353,734</td>
<td>855,279</td>
</tr>
</tbody>
</table>

Table 2. Histomorphometric parameters across distinct anatomical sites of the study group.

<table>
<thead>
<tr>
<th></th>
<th>Superior segment</th>
<th>Middle segment</th>
<th>Inferior segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cortex thickness (mm)</td>
<td>5.40</td>
<td>5.24</td>
<td>5.12</td>
</tr>
<tr>
<td>Mean glomerulus size (μm)</td>
<td>197.89</td>
<td>195.46</td>
<td>191.63</td>
</tr>
<tr>
<td>Glomerular density (%)</td>
<td>8.41</td>
<td>9.06</td>
<td>7.00</td>
</tr>
</tbody>
</table>

Conclusions

The present study is the first to investigate the histomorphometry of pre-transplantation kidneys by means of digital image analysis. Our findings suggest that a single preimplantation biopsy could be representative of the overall donor NM if performed in the superior kidney segment. The integration of traditional pathology with cutting-edge digital technologies and bioinformatic tools, could be extremely beneficial in the implementation of reproducible diagnostic schemes for renal transplantation.

References

Giovedì, 24 novembre 2016
Aula Scirocco – 08:30 - 12:30

PATOLOGIA MAMMARIA
SESSIONE I
L’infiltrato linfocitario tumorale nel carcinoma mammario

Moderatori: Patrizia Querzoli (Ferrara) - Elita Giardina (Bari)

IL RUOLO DEL PATOLOGO
A. Vingiani

IL RUOLO DELL’ONCOLOGO
M. V. Dieci

LINFONODO SENTINELLA: NUOVE PROSPETTIVE
G. Viale

SESSIONE II
Highlights from...

Moderatori: Antonio Rizzo (Castelfranco Veneto) - Mattia Barbareschi (Trento)

HIGHLIGHTS FROM BREAST CANCER CONFERENCE AND ESP

M. P. Foschini, L. Morandi
Section of Anatomic Pathology at Bellaria Hospital, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy.

The European Breast Cancer Conference (EBCC) is a multidisciplinary congress focused on breast cancer (BC). The tenth edition was held in Amsterdam in March 2016. Among the many interesting sessions, I would like to propose to your attention the lecture given by Prof. S.R. Lakhani on BRCA1 and BRCA2 related BC. BRCA1 and 2, genes encode proteins important to maintain genomic integrity due to their role in DNA repair. Cells lacking BRCA1 or 2 proteins cannot repair the double strand DNA breaks. Women with germline mutations in BRCA1 or 2 carry a high risk of developing breast and ovarian cancers. BC arising women with germline mutations in BRCA1, are more often high grade, highly proliferative and lack expression of ER, PR and Her2 (triple negative - TNBC). These cancers often express ‘basal’, myoepithelial cell type cytokeratins CK14, 5/6 and 17, that means that they show a ‘basal-like’ phenotype.

Sporadic BC do not present BRCA1 mutations, while they can present methylation of the BRCA1 gene promoter in up to 14% of cases, leading to complete loss of BRCA1 protein expression. Methylation is often accompanied by LOH affecting the other allele, leading to BRCA1 inactivation. Breast cancers with BRCA1 methylation show a similar morphology to BRCA1 germline tumours and sporadic basal like BC. Therefore it is suggested that BRCA1 or its pathway (DNA
institutions have implemented policies requiring them. Many
established part of pathology practice, and many healthcare
and on treatment choices. Obtaining second opinions is an
cer should obtain second opinion on both diagnosis/pathology
recommended that every patient with diagnosis of breast can-
an accurate diagnosis of breast lesions in order to ensure the
cancer and highlighted the importance of
dance in breast pathology and

diagnostic disagreement than malignant lesions, to assure that
cancer is not misdiagnosed and assess patient risk for develop-
ment of breast cancer to guide surveillance and risk reduction
measures. Even if a second opinion can `improve diagnostic
accuracy and therefore the quality of patient care, as well as
providing educational opportunity to the pathologists, the
rules for obtaining second opinions are not well established.
In this session a multidisciplinary panel of breast cancer ex-
erts discussed importance and the clinical impact of second
opinions in the management of patients with breast cancer.
In particular the breast cancer experts panel discussed the
clinical importance of prognostic and predictive factors in the
classification of breast cancer and they tried to understand the
technical issues that may cause diagnostic difficulty. Besides,
the identification of borderline histopathology entities and
subjective evaluations were discussed with special emphasis
on causes of diagnostic discrepancies among different pat-
thologists and their clinical consequences.

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CARATTERIZZAZIONE DEI PRECURSORI NON
OBBLIGATI DI CARCINOMA MAMMARIO
N. Fusco

PERCORSO DIAGNOSTICO PREOPERATORIO CON
PARTICOLARE RIFERIMENTO AL MANAGEMENT DEI
B3
S. Bianchi

CONTROLLO DI QUALITÀ HER2 SU VETRINO
VIRTUAL E
F. Pietribiasi

UNA SOLUZIONE PER I CARCINOMI HER2 EQUIVOCI?
C. Marchiò

RIPRODUCIBILITÀ DIAGNOSTICA E CONTROLLI DI
QUALITÀ
S. Asioli

Department of Biomedical and Neuromotor Sciences, Surgical Pathology
Section, Alma Mater Studiorum, University of Bologna, Italy.

The companion meeting of International Society of Breast Path-
ology, it was held on March 13rd 2016, in Seattle, during the
Annual Congress of the Society’ North American Pathology
(USCAP), focused on second opinions and diagnostic concor-
dance in breast pathology and highlighted the importance of
an accurate diagnosis of breast lesions in order to ensure the
most effective treatment. Many Breast Cancer Groups have
recommended that every patient with diagnosis of breast can-
cer should obtain second opinion on both diagnosis/pathology
and on treatment choices. Obtaining second opinions is an
established part of pathology practice, and many healthcare
institutions have implemented policies requiring them. Many
hospitals in the United States mandate a second review of pa-
thology slides from outside laboratories before surgical inter-
ventions or for all cancer diagnoses before treatment planning
Second opinions may also be important for high-risk non-
malignant borderline breast lesions, which have greater di-
agnostic disagreement than malignant lesions, to assure that
cancer is not misdiagnosed and assess patient risk for develop-
ment of breast cancer to guide surveillance and risk reduction
strategies. Even if a second opinion can `improve diagnostic
accuracy and therefore the quality of patient care, as well as
providing educational opportunity to the pathologists, the
rules for obtaining second opinions are not well established.
In this session a multidisciplinary panel of breast cancer ex-
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In particular the breast cancer experts panel discussed the
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subjective evaluations were discussed with special emphasis
on causes of diagnostic discrepancies among different pat-
thologists and their clinical consequences.

Giovedì, 24 novembre 2016
Aula Libeccio – 08:30 - 12:30

PATOLOGIA TESTA COLLO
Lezioni dalla diagnostica
Moderatori: Eugenio Maiorano (Bari) – Stefania Staibano
(Napoli)

IMPORTANZA DEL PDTA NELLE NEOPLASIE TESTA-
COLLO
P. Morbini
In head and neck area, oral squamous cell carcinoma (OSCC) is the commonest malignancy accounting for 95% of oral malignant lesions in the developing countries. Despite great progress in chemotherapy, radiotherapy, and targeted therapy in the last three decades, the prognosis of OSCC remains poor due to aggressive local invasion and metastasis, leading to recurrence. Camisasca et al. (1) have reported that the 5-year survival rate was 92% in OSCC patients without recurrence and 30% in patients with recurrence (P < 0.001, log-rank test). The median survival was 76.8 months in patients without recurrence and 42.5 months in patients with recurrence (P < 0.001, log-rank test). Postoperative tumor recurrence leads to poor prognosis and identifying related factors is an emerging issue in clinic. In clinical practice, a second neoplastic manifestation may be related to neo-carcinogenesis (Second Primary Tumor, SPT) or Local Recurrence (LR). Recently Braakhuis et al. proposed a new entity based on molecular analyses namely Second Field Tumor (SFT)(2). It comes from the same genetically altered mucosal field as the primary OSCC. In this case a cluster of premalignant cells develops from one aberrant cell clone leading to a relatively large field. Within this field, two independent processes develop into two OSCCs sharing some, but not all, genetic alterations.LR, SPT and SFT may have a different clinical behavior and a correct identification of those entities, together with the detection of actionable mutations and epigenetic modifications can be a key issue in defining the prognosis, stratifying patient outcome, and influencing the choice of appropriate treatment. The impact of next-generation sequencing techniques in this field is going to revolutionize our approach to manage these lesions. In this context Montebugnoli et al. (3) and Morandi et al. (4) described a new approach to assess clonality in OSCC evaluating mutations found in mtDNA, TP53 and NOTCH1. Their purpose was to differentiate secondary neoplastic lesions into LR, SPT or SFT following the Braakhuis et al. (2) classification with more consistent genetic information respect to clinical criteria suggested by Hong et al. (5).Moreover the same group developed a new method based on bisulfite next-generation sequencing (NGS) from oral brushings to early detect oral premalignant lesions (OPML) and OSCC (6). This approach has been applied even in patients surgically treated for an OSCC in the site of surgical resection. Knowing of an altered DNA methylation pattern in this area similar to that seen in primary OSCC may be related to poor outcome and high risk of LR or SPT.

References
transcripts of the E6 and E7 viral oncoproteins. However, there is still a lack of agreement over the optimal combination of tests useful in the pathology setting. Moreover, new screening modalities that can detect HPV-associated disease in biological.

**VALORI E LIMITI DEL LINFONODO SENTINELLA NELLE NEOPLASIE ORALI**

E. M. Silini

**NEOPLASIE ODONTOGENE EPITELIALI: ASPETTI DIAGNOSTICI**

C. Rubini

**NEOPLASIE DEL MESENCHIMA ODONTOGENO: ASPETTI DIAGNOSTICI**

E. Maiorano

**OSTEOSARCOMAS OF THE JAWS: SIMILARITIES AND DIFFERENCES FROM THEIR SKELETAL COUNTERPARTS**

A. Righi

_SSD Anatomia Patologica, Istituto Ortopedico Rizzoli, Bologna_

Osteosarcomas of the jaws is a relatively rare neoplasm accounting between 2% and 13% of all cases of osteosarcoma published in literature in the different series. Although morphologically and radiologically identical to their peripheral counterparts, osteosarcomas of the jaws are distinct in several crucial aspects. Firstly, patients affected by osteosarcomas of the jaws are one to two decades older with an average age of 33–39 years. About predominant differentiation pattern, at difference to their peripheral counterparts where the osteoblastic histotype is the most frequent, 50% of osteosarcomas of the jaws are predominantly chondroblastic according to reported series. Secondly, hematogenous spread seems to be much more infrequent and to occur later in the course of the disease. Patients with peripheral osteosarcoma present with primary metastases in approximately 25% of cases and up to 90% develop lung metastases subsequently when treated by surgery only. Consequently, peripheral osteosarcoma is considered a systemic disease at the time of diagnosis that can be cured only by (neo-)adjuvant chemotherapy in addition to radical surgery in the vast majority of cases. In osteosarcomas of the jaws, on the other hand, hematogenous metastases are reported to affect only 6–18% of patients after an average time of 17–23 months. Wide resection is therefore widely accepted as the mainstay of treatment with 5 year survival rates reaching up to 75% without additional (neo-)adjuvant therapy. Regarding adjuvant treatment in osteosarcomas of the jaws conflicting results have been reported because of the rarity of osteosarcomas of the jaws that has precluded prospective studies which is reflected by the mainly descriptive reports with small cohorts of patients and inconsistent treatment modalities. According to the multivariate analysis relative to the series of osteosarcomas of the jaws reported in literature, the wide margin of surgical resection is the only independent prognostic and predictive parameter in these rare neoplasms.

**NUT CARCINOMA: FATTI E MISTERI**

A. Franchi

**NEW PROPOSAL FOR TERMINOLOGY IN SALIVARY GLAND CYTOLOGY**

E. D. Rossi

_MD PhD MIAC-Division of Anatomic Pathology and Histology-Catholic University of Sacred Heart-Rome-Italy_

Salivary gland tumors (SGTs) are uncommon, accounting for approximately 3-10% of neoplasms of the head and neck region. SGT’s are often clinically asymptomatic, growing slowly and only being noticed by the patient or during clinical exam after growing to a palpable size or involving adjacent structures such as nerves, ducts, or muscles. However, with increasing use of screening modalities such as ultrasound and high resolution computed tomography (CT) there has been an increase in the detection of non-palpable as well as incidental salivary gland nodules. Fine-needle aspiration (FNA) has become widely accepted for the evaluation of salivary gland masses. It can differentiate between neoplastic and non-neoplastic salivary gland lesions, and in cases of a neoplasm, FNA can distinguish many benign tumors and can usually differentiate between low and high grade carcinomas. The latter is useful in determining the extent of surgery including preservation of the facial nerve in case of parotid tumors and indications for neck dissection.

Based upon the cytologic interpretation, all malignant tumors and many benign tumors are typically treated by surgical excision. In a subset of benign cases such as certain pleomorphic adenomas and Warthin tumors, there is also the option for managing the lesions non-surgically by clinical follow-up and imaging depending upon patient wishes and health status. Inherent in the cytologic interpretation of any salivary gland FNA, one must keep in mind that the rate of malignancy varies depending upon the size of the mass and the location - The ROM increases from 20-25% in the parotid gland, to 40-50% in the submandibular gland, and to 50-81% in the sublingual and minor salivary glands. Salivary gland FNA shows a range of sensitivities and specificities depending upon a variety of factors including: FNA technique, cytologic preparation, experience, lesion heterogeneity, and cystic component. FNA performs best when applied to the sampling of palpable parotid tumors where a majority of pleomorphic adenomas and Warthin tumors are accurately detected and classified. However, for many of the uncommon to rare low-grade salivary gland neoplasms, FNA lacks specificity in being able to precisely classify the tumor as a specific subtype. Nonetheless, for management purposes, FNA is useful for distinguishing low grade from high grade neoplasms.

The above-mentioned issues are further intensified due to the lack of a tiered diagnostic framework by which salivary gland FNA specimens are reported. The aim of this classification scheme will be to provide a practical, uniform reporting system whose diagnostic categories will be linked with risks of malignancy and correlated with clinical management strategies. This classification system will describe a novel and uniform international approach for classifying and reporting salivary gland FNA samples. The new reporting system is evidence-
based using data from the literature as well as upon the experience of a multi-disciplinary group of leading experts involved in the field of salivary gland cytopathology.

Each diagnostic category of this novel salivary gland reporting system will include detailed descriptions of the cytologic criteria as well as a comprehensive set of photomicrographs demonstrating all of the key microscopic features along with annotated descriptions for each image. The classification system thereby will function as an atlas for pathologists and cytotechnologists involved in diagnostic cytopathology, and it will be streamlined and concise enough to be useful for cytology professionals at all levels of their education.

THE MULTIFACETED ORAL SQUAMOUS CELL CARCINOMA: SIMILAR MORPHOLOGY, BUT DIFFERENT BEHAVIOR.

Department of Advanced Biomedical Sciences, School of Medicine, Pathology section, University of Naples “Federico II”, Naples

Oral Squamous Cell Carcinoma (OSCC) accounts for more than 90% of Head and Neck Cancers (HNCs), and represents one of the most common human malignancies, worldwide. The incidence of this kind of cancer has registered a continuous increase, during the last decades; moreover, OSCCs are frequently diagnosed at an advanced stage of progression, resulting also resistant to radio-chemotherapy treatments and showing an overall poor prognosis. In the last 30 years there has been a noticeable change in the epidemiology of HNCs, due to the rapid expansion of a subset of tumors relates to high-risk Human papilloma virus (HPV) persistent infection. Currently, oral cancers include at least two major sub-groups: the “classic” type, alcohol and smoking-related and the HPV-related SCC, selectively restricted to the oropharynx (HPV+/OPSCCs). HPV+/OPSCCs show either a more favourable biological behavior and a better response to conventional therapeutic treatments. Here We present two paradigmatic OSCC and OPSCC cases.

CASE # 1 On November 2015, a 54-year-old female with a history of diabetes and hypertension, non-smoker and non-drinker, went to her dentist for a laterocervical swelling on the left, fixed, not painful, developed few months earlier. Past medical history reported the persistence of a pharyngeal-tonsillar pain, attributed to repeated episodes of cooling. On physical examination, it is not found macroscopic lesions in the oral cavity. The patient was then submitted to a FNAB for further evaluation of the palpable mass that showed a lymphnode metastases from squamous cell carcinoma. Deep manual inspection revealed a lesion at the left-base of the tongue, not ulcetered. The MRI examination confirmed the presence of the neoplasm. Histological examination of the excision showed an invasive squamous cell carcinoma, extended to local-regional level, positive for p16 protein and the presence of HPV-DNA (genotype 16). At present, is disease-free.

CASE # 2 On February 2013, a 63-year-old male with a history of allergic rhinitis, non-smoker and non-drinker, was admitted to our Department for the development of edema in the left periorbital region with the left eye proptosis and diplopia. The patient dates the onset of the disease on few years earlier with the appearance of obstruction of the nasal and paranasal cavity and, on May 2012, complete and permanent obstruction of the left nasal cavity. The MRI exam performed documented a solid lesion, which committed the nasal cavity, ethmoid region, sinus cavity, the maxillary and medial portion of the left orbital cavity. The rhinoscopy examination confirmed the complete absence of air flow throughout the area affected by neoplasia. No enlarged lateral cervical lymph nodes were seen. Histological examination revealed a squamous cell carcinoma, positive for p16 protein and for HPV-p16 DNA (InnoLipa), but negative for HPV-RNA (RNAscope method). These findings indicate that the virus was present in tumor, but not transcriptionally active. In literature, transcriptionally active HPVs have been identified in <50.0% of SCC of the head and neck region. The patient died for disease one year after diagnosis.

Giovedì, 24 novembre 2016
Aula Maestrale – 08:30 - 12:30

PATOLOGIA MOLECOLARE
SESSIONE I

Eterogeneità tumorale, resistenza, monitoring e outcome: solido, liquido o semiliquido?
Moderatori: Antonio Marchetti (Chieti) – Massimo Barberis (Milano)

LA REBIOPSIA E IL DNA TUMORALE CIRCOLANTE: POLI CONTRAPPONI O COMPLEMENTARI?
S. Veronese

CIRCULATING TUMOR CELLS: DIRECT EXPERIENCE
R. Zamarchi
IOV-IRCCS, Padova (Italy)

The ever-greater access to screening, the greatest sensitivity and specificity of imaging and the growing number of new molecules have been changing the fate of cancer patients. We can hope now that by taking advantages of treatments tailored to the tumor of individual patient, we will definitively fight cancer. However, just because of the growing number of successful treatments, the number of long-term surviving patients has been increasing, and consequently it has been raising the need of new tools for their follow-up. To address this issue, we should identify a tumor-specific marker that (a) is expressed constantly throughout the disease course; (b) is associated with disease outcome; (c) can reflect “just in time” tumor evolution during its natural history or under any treatments’ pressure and (d) is minimally invasive.

The Circulating Tumor Cells (CTCs) meet all these criteria. Indeed, CTCs have been revealed in almost all disease stages and their levels have been reported both prognostic, and predictive of treatment efficacy. Consistently with clinical validity recently confirmed in metastatic breast cancer, the quanti-
tative evaluation of CTCs has been entered in the ASCO and NCCN guidelines (2015) as prognostic biomarker. However, despite CTC level promises to be an appealing tool for reevaluating disease conditions throughout the continuum of the care, there is some limiting an extensive use of CTC assay in clinical that we can summarize as below:

- **CONSENSUS**: So far we are lacking a consensus about requirements necessary and sufficient to define an event as CTC;
- **“EMT AFFAIR”**: The available technologies are mostly EpCAM-dependent, so that others cells (more aggressive? undifferentiated? EMT cells?) can not be yet quantified;
- **SENSIBILITY**: We can detect CTCs in no more than 50% of metastatic patients;
- **“MOLECULAR AFFAIR”**: As we are lacking a comprehensive genomic characterization of circulating compartment, we don’t know its heterogeneity levels.

Main pitfalls and work in progress will be critically discussed.

**CYTOLOGICAL EVALUATION OF LIQUID BIOPSY: A NOVEL APPROACH**

C. Mignogna and N. Malara

*Department of Health Science. University “Magna Graecia” of Catanzaro*

Despite rapid advances in the cancer stem cell field, there is a limited ability to morphological evaluation of migrating cancer cell in vivo. Many experimental studies were reported in literature to isolate and select the circulating tumor cells (CTCs)

1, using immunomagnetic beads2 or with sophisticated imaging system. Nevertheless, it is clear that CTC detection due to rarity of CTC population and their underestimation requires the development of new technologies that operate focusing on the morphological cellular preservation. The malignant cells from solid tumour begin to circulate at the earliest stages in cancer formation. On the other hand, the presence of epithelial tumour cells was proven in peripheral blood of patients undergoing to high dose of chemotherapy. Moreover, Pedrazzoli described the possibility to use culturing system to detect CTC. In the present work, we optimized a protocol to make circulating cancer cells viable for cytological valuations. In particular, we started from the consideration that CTCs release happens in two ways, (i) spontaneously due to higher growing tumor profile and (ii) by active extravasations process due to metastatic phenotype acquisition. Taking into account the heterotypical composition of the tumor tissue generating circulating cancer progeny, we examined the cellular composition of peripheral blood samples. The most used and established methodology to separate different hematological cellular populations, within a blood sample, is the density gradient centrifugation. We performed a density gradient centrifugation as previously reported. Moreover, considering that the hallmark of cancer transformation is the higher proliferation rate, we tested CTCs cellular growth efficiency in vitro. Subsequently, the CTCs were recovered on chamber-slides for a short-time culture. CTCs temporary expansion makes these rare cancer cells viable for further morphological and molecular characterization. Our experience over 50 cases, to date, suggest that our protocol, preserving heterogeneity and without aggressive selective procedures, guarantee the viability of the rare CTCs population for cytological evaluation. The cytology on liquid biopsy can be considered useful in clinic management of oncologic patient who avoids conventional and often invasive procedures of the tissue biopsy.

**References**


6 Natalia Malara, Maria Laura Coluccio, Tania Limongi, Monica Asande, Valentina Trunzo, Gheorge Cojoc, Cinzia Raso, Patrizio Candeloro, Gerardo Perozziello, Raffaella Raimondo, Stefania De Vitis, Laura Roveda, Maria Renne, Ulbaldo Prati, Vincenzo Mollace, Enzo Di Fabrizio: Folic Acid Functionalized Surface Highlights 5-Methylcytosine-Genomic Content within Circulating Tumor Cells. Small 07/2014; 10(21). DOI:10.1002/smll.201400498.


PIASTRINE ED ESOSOMI: SPAZZINI E MESSAGGERI
L. B. Felicioni

SESSIONE II
Marcatori molecolari dell’immunoterapia (stato dell’arte)

Moderatore: Lucio Crinò (Perugia)

IL PROBLEMA CLINICO
L. Crinò

GLI APPROCCI IMMUNOISTOCHEMICI ALL’ANALISI DI PD-L1
R. Franco¹, I. Panarese¹
Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Second University Naples Italy.

PD-L1 is expressed in several solid tumors and frequently in NSCLC, highlighting the strong immunogenicity of lung cancer (1). Activation of PD-1/PD-L1 pathway is considered a prognostic factor in NSCLC, particularly those with high expression of PD-L1 have worse prognosis when associated with an increase of stromal citotoxic T cells (CD8+) (2). PD-L1 was frequently associated with EGFR mutations, being PD-L1 expression induced by costitutive EGFR pathway activation, facilitating evasion of the host anti-tumour immune response. This role of EGFR signalling was independent of its effects on cell proliferation and survival, suggesting an active role for the EGFR oncogene in remodelling the immune microenvironment. Moreover, pharmacological blockade of the PD-1/PDL-1 pathway using EGFR-TKIs reduced PD-L1 expression, leading to decrease of tumor proliferation, with a positive improvement on overall survival. Finally combination of PD-1 blockade with EGFR TKIs may be a promising therapeutic strategy. Patients harbouring EGFR mutations with high PD-L1 expression resulted more sensitive to gefitinib or erlotinib probably because of PD-L1 downregulation induced by the EGFR inhibition (3).

Tumor PD-L1 protein expression is related to improved benefits and better outcomes in patients with advanced NSCLC treated with anti-PD-1/PD-L1 monoclonal antibodies. However, the efficacy of these agents among PD-L1 negative patients is also clinically relevant and not inferior to the standard chemotherapy. Thus, more studies are necessary to enhance the credibility, robustness and reproducibility of predictive biomarkers for immune checkpoint inhibitors (4).

Currently several clinical trials on NSCLC treatment with PD1/PDL1 blockade are in progress, other are closed allowing the use of PD1/PDL1 blockade in clinical practise. The use of this target therapies is strictly dependendet on the demonstration of PDL-1 in cancer tissue. Thus four clones are currently used for clinical application through immunohistochemistry detection of PD-L1: clones 22C3 and 28.8 (Dako) and SP263 and SP142 (Ventana). Particularly for each of these clones exists a specific therapeutic agent, pembrolizumab for clone 22C3, nivolumab for clone 28.8, atezolizumab for clone SP142 and durvalumab for clone SP263. Moreover, different cut-off are proposed to evaluate the PD-L1 positivity, indeed for NSCLCs, for example, for treatment with Nivolumab, cases with positive cancer tumor cells >1%, clone 28.8 DAKO®, could be considered; for the treatment with Pembrolizumab, cases with a positivity of more than 50% of cancer cells, clone 22C3 DAKO could be enrolled; for the treatment with Durvalumab, cases with positivity of 25% cancer cells, SP263 VENTANA® could be included and finally for Atezolizumab cancer (CC) and immune cells (IC) should be considered and the treatment seems to be applicable for cases with CC and/or IC >10%.

References

PATHOLOGISTS AND PD-L1 EXPRESSION
M.C.P. Barberis
Unit of Histopathology and Molecular Diagnostics. European Institute of Oncology. Milano

The binding of programmed death ligand-1 and ligand-2 (PD-L1 and PD-L2) to PD-1 blocks T-cell–mediated immune response to cancer cells (1,2). Monoclonal Antibodies that target either PD-1 or PD-L1 can inhibit the ligand-receptor binding and consequently the T cells can increase antitumor immune response and possibly T-mediated cytolysis. The effect of these antibodies has been studied in different clinical trials on squamous and non squamous NSCLCs (3,4,5,6). Recently two anti PD-1 monoclonal antibodies, nivolumab (Opdivo, Bristol Myers Squibb) and pembrolizumab (Keytruda, merck Sharpe & Dohme ) were approved by the US Food and Drug Administration and by the European Medicine Agency. Other similar drugs, atezolizumab ( Roche-Genentech), avelumab ( Pfizer-Merck Serono) and durvalumab (Astra Zeneca) will probably obtain the approval from the National Health Agencies in the next future. When new drugs are introduced the research of a robust predictive biomarkers begins. The historical lesson of the immunocytotoxic HER 2 expression in breast cancer patients candidate to trastuzumab generated the hope of obtaining a rapid, cost-effective assay to predict response to anti PD-1/PD-L1 agents. A positive immunocytotoxic test for PD-L1 has been shown to be predictive of better response, even if a percentage of patients with negative test responded to the drug . Recently it was showed that in patients with advanced NSCLC and PD-L1 expression on at least 50% of tumor cells, pembrolizumab was associated with significantly longer progression-free and overall survival and with fewer adverse events than was platinum-based chemotherapy – first line of treatment- (7). Different companion tests based on different clones, developed with different revelation systems
with different scores (percentage of tumor cells stained with the antibody) to define the positivity of the assay different expression during time and obvious tumor heterogeneity created some confusion and raised skepticism in the scientific community. In other terms PD-L1 IHC assays were developed according to a “one assay, one drug”. To date, only pembrolizumab has been approved by the FDA in association with a companion IHC test, the Dako 22C3 pharmDx, for therapy in NSCLC. Nivolumab use for non-squamous NSCLC was associated with the PD-L1 IHC 28-8 pharmDx test, but it was not defined as a companion biomarker. Both the tests are provided by Dako and target an epitope in the same domain of the same protein, however the threshold for positivity is different (8). The assay developed with the anti-PD-L1 agent atezolizumab (Ventana SP142) is associated with response to therapy when the signal is present in tumor and/or stromal cells. For Pathologists to run a specific test for a specific drug is impossible. Different immunostains are not present in all the Pathology Labs; moreover it can be impossible to know what kind of checkpoint inhibitor the oncologist uses in a specific case to evaluate the impact of the proposed biomarkers further research and comparative studies need. Two multi-institutional efforts led by the National Comprehensive Cancer Network (NCCN/BMS PD-L1 partnership) and the International Association for the Study of Lung Cancer (IASLC; the Blueprint study) assessed the comparability and performance of different PD-L1 IHC assays in lung cancer.

The Blueprint project was the product of the work shop. Its goal was: “To agree and deliver, via cross industry collaboration, a package of information/data upon which analytic comparison of the various diagnostic assays may be conducted, potentially paving the way for post-market standardization and/or practice guideline development, as appropriate.” The results of this effort were that three assays demonstrated similar analytical performance with respect to percentages of tumor cells. There is a general agreement between observers when assessing tumor cells than when assessing infiltrating immune cells. The conclusion was that this preliminary study should not alter current guidelines as indicated for each therapeutic-diagnostic validated combination pair. Similar results were obtained in a ring study produced in Germany (Deutscher Ringversuch)(9)

With these recent advances this diagnostic procedure seems destined to use. Probably other immunocytochemical or molecular markers representative of the tumoral immune microenvironment could offer more precise predictive information, but at the moment IHC test for PD-L1 remains the only validated procedure.

The pathologists must be trained in a specific program of formation before embarking in routine use of the test to candidate or not the patients with NSCLC to checkpoint inhibitors

**References**

9. Scheel AH et al Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas

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**SESSIONE III**

**Nell’era della NGS – la tecnologia e le linee guida**

Moderatori: Giovanni Tallini (Bologna) – Giancarlo Troncone (Napoli)

**NEXT GENERATION SEQUENCING. NOZIONI DI BASE. LE DIVERSE TECNOLOGIE DISPONIBILI**

G. Filice
Centro di Medicina Molecolare e Predittiva Ce.S.I. - Chieti

Massive parallel sequencing, also known as next generation sequencing (NGS), is a DNA sequencing technology which has revolutionised genomic research. using NGS, It is now possible to sequence an entire human genome in a single day. This technology plays an increasingly dominant role, in fact there are many Centers, even in Italy, which adopt this approach for sequencing DNA or RNA to detect SNV/indels, CNV or gene rearrangements from cancer patients.

Most human tumors are highly heterogeneous and the possibility to test a cancer panel that include the most frequent altered genes in different pathologies allows the full integration of NGS in the diagnostics routine with a considerable saving of time and money.

There are various commercially available NGS platforms with different strategies but all these have common features such as the sequencing of millions of small fragments of DNA in parallel, library preparation with specific adapters and data analysis through dedicated algorithms.

However, there are many challenges to the robust detection of alterations present in only a few percentage of cells, including sequencing errors, and sample preparation artifacts. DNA extraction and formalin-fixed paraffin embedded (FFPE) samples preparation might affect the fidelity of downstream mutation calling. New techniques have been developed to reduce artifacts such as the Duplex Sequencing or the CAPP-sequencing.

Our direct experience is focused in testing different DNA/ RNA cancer panels in lung cancer patients with very interesting results. In fact we studied in deph the tumor heterogeneity and through comprehensive cancer panels we were able to define new genetic rearrangements.

In conclusion there are many future perspectives such as the possibility of using NGS in plasma to monitor patients and quantify the presence of mutated alleles for early prediction of response to targeted therapy.
PERCHÉ LA NGS IN DIAGNOSTICA: APPLICAZIONI CLINICHE (DALL’ULTRADEEP AL MULTIMARKER ESTREMO)

A. Scarpa

LINEE GUIDA SULL’UTILIZZO DELLA NGS IN DIAGNOSTICA E ASPETTI ECONOMICI

G. Tallini

SESSIONE IV
Nell’era della NGS – esperienze nella pratica clinica

Moderatori: Giorgio Stanta (Trieste) – Stefania Uccini (Roma)

NGS NELLA DIAGNOSTICA DEI TUMORI DEL TRATTO DIGERENTE E DEL MELANOMA

G. Troncone

L’ANALISI DEI GENI COMPLESSI: IL MODELLO BRCA NEI TUMORI OVARIICI

F. Buttitta

IL MALDI-TOF COME ALTERNATIVA PER LA DIAGNOSTICA DI MARCATORI MULTIPLI

G. Fontanini

Biosicurezza e formalina

Moderatori: Daniela Massi (Firenze) – Mauro Truini (Genova)

LA GESTIONE DEL RISCHIO IN ANATOMIA PATOLOGICA STRUMENTI E METODI PER UN PERCORSO VERSO L’ECCELLENZA

S. M. Mezzopera

LA GESTIONE DELLA FORMALDEIDE IN UN REPARTO DI ANATOMIA PATOLOGICA E NEL CONTESTO DI UN FORMALIN-FREE HOSPITAL (PROIEZIONE VIDEO)

R. Fiocca - D. Massi

IL RISCHIO FORMALDEIDE NELLE STRUTTURE SANITARIE: LA GESTIONE E LE CRITICITÀ

F. Pugliese

IL RISCHIO FORMALDEIDE NELLE STRUTTURE SANITARIE: LE SOLUZIONI POSSIBILI ED UNA PROPOSTA DI LINEE GUIDA

D. M. Cavallo

Prof. of Occupational Health Dep. of Science and High Technology - University of Insubria – Como

EU Regulation No 895/2014 amending Annex XIV of Regulation (EU) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Formaldehyde has been classified as carcinogenic category 1B [may cause cancer]. Even in a scenario in which the different international institutions (IARC International Agency for Research on Cancer; all’ACGIH American Conference of Governmental Industrial Hygienists; the US-EPA US Environmental Protection Agency; the NIOSH National Institute for Occupational Safety and Health; to ‘OSHA occupational Safety and Health Administration) have adopted a non-uniform classification of carcinogenicity of this substance, the Scientific Committee on Occupational Exposure Limits (SCOEL), deputy to express opinions to the European Commission on the toxicological evaluation of chemical agents occupational use in reference to their potential effects on human health and to issue recommendations of environmental and/or biological exposure limits for workers, considers formaldehyde a carcinogen agent with genotoxic modes of action based on threshold. This threshold (Occupational Exposure Limits - TWA OEL), currently proposed as a reference value, is equal to 0.369 mg/m³, limit value is not exceeded, designated and deemed effective for all purposes, including carcinogens. The formaldehyde concentrations in ambient air (outdoor) range from 0.001 mg/m³ in remote areas to 0.02 mg/m³ in urban areas. The indoor levels, in a typical home environment, ranging from 0.002 mg/m³ and 0.06 mg/m³, cigarette smoking contributes to 10-25% of the exposure indoors. The new classification starting

Giovedì, 24 novembre 2016

Aula Maestrale – 14:30 - 18:00

SESSIONE PLENARIA
Il riassetto dei servizi di A.P. ed il risultato del questionario

Moderatore: Gaetano De Rosa (Napoli)

ANALISI RISULTATI QUESTIONARIO CONOSCITIVO SERVIZI DI ANATOMIA PATOLOGICA

G. Mazzoleni

MODELLO TOSCANO DI INTEGRAZIONE DI ANATOMIA PATOLOGICA

A. Cavazzana
from 01.01.2016; this has trigged the need to consider the carcinogenic risk for the management of health and safety of workers, namely the applicability, for work that involves the use of formaldehyde, the standard for protection from carcinogens and mutagens (D. 81/08, Title IX, Chapter II). The main uses of formaldehyde comprise the preservation of samples in histopathology and surgical wards in Hospitals.

The proposal of the Lombardy Region: given the request made by the preventive operators and the stakeholders, according with the representative social bodies, specific guidelines for the management of risk by exposure to formaldehyde have been developed in order to manage the carcinogenic risk - in case of impossibility to replace it with less hazardous agents, and without prejudice the duty to always contain the exposure values as low as technically possible, by implementing preventive measures, technical and organizational improvement preventive actions. These are expressed in: perform environmental monitoring, according to UNI EN 689/97 (Appendix F) that indicate the criterion that schedule periodic measurements as an investigative tool in support of preventive actions: insert the above findings into the relevant environmental data registry that is an integral part of the chemical risk assessment, as envisaged in the document of risk assessment (Art. 236 Legislative Decree 81/08); establish that the reference values for the activation of the register are identified as: 0.369 mg/m³ as limit value to not exceeded; 0.184 mg/m³ action level, identified as ½ of the OEL-TWA SCOEL; 0.1 mg/m³: the reference value, the limit value of indoor and outdoor air quality proposed by the WHO. It has been proposed that: where the worker is exposed to concentrations above the action value during at least two consecutive measurements of exposure coordinated by the Head of the Prevention and Protection on behalf of the employer service, the physician in assessing whether to propose to the employer the establishment of the exposure register and, if necessary, care for him the seal (art. 243 of Legislative Decree no. 81/08); to limit violation, the setting up of the exposure register is mandatory, as the immediate activation of prompt interventions for risk reduction and control.

ANNO 2016: LA GESTIONE DEL PROBLEMA FORMALDEIDE, GLI ASPETTI TECNICI ED ORGANIZZATIVI

T. Zanin
E-O- Ospedali Galliera - Genova - Italy

According to Commission Regulation (EU) 2015/491 of the 23rd of March 2015 amending Commission Regulation (EU) No 605/2014 of the 5th of June 2014, any facility must apply new or amended rules for classification, labelling and packaging of substances and mixtures Text with EEA relevance starting from the 1st of January 2016. Such Regulation brought into play formaldehyde, that was re-classified as a carcinogenic (category 1B) and mutagenic (category 2) substance by EU Commission Implementing Decision 2014/895.

Therefore European and Italian regulations impose to take into consideration the carcinogenic risk in order to protect health and safety of workers exposed to formaldehyde (D.Lgs. 9 April 2008 N.81, Protection from carcinogenic and mutagenic substances). Formaldehyde must be used only in places provided with all necessary environmental and personal protective equipment and banned from all sites where bioptic and not-bioptic samples are taken (e.g surgery rooms and dermatology, gastroenterology or urology clinics).

This new scenario brought radical changes both in organization and management but also in supplies offered from companies operating in the field. As a matter of fact in the last months the market allows to choose the most suitable technology or method to fix/preserve surgical and/or bioptic samples, ranging from solutions that keep fresh tissue samples vacuum sealed to options that employ formaldehyde but make use of expedients to avoid its dangerousness.

One should also take into consideration organizational and management aspects that the chosen solution implies, since some of them could lead to radical changes in working methodologies.

An important point, come out during the developing of specific guidelines, is to reach the best possible tissue conservation state in order to preserve integrity of DNA and RNA,
The system confirms the previous 5-tiered reporting scheme, but with a reliable tool for their daily practice. To date for preservation and transport of biopsic samples, different containers are available: some implies the transfer of formaline after the insertion of the sample, while others already contain formaline but have protection barriers made of isoparaffins/gels, etc. Even after the introduction of new technologies the use of formaldehyde continues in Anatomic Pathology facilities, where, according to present regulation, all expected procedures to guarantee worker safety must be in place. These safety measures must, first of all, take into consideration the work environment with the introduction of all necessary environmental protection equipment. Moreover supervisors are required to make sure the rules and compulsorily wear the required personal protection equipment during operations with formaldehyde. In the end it has become increasingly important personnel education and training, both to the use of new methodologies with their connected management changes and to the knowledge and respect of safety regulations.

**Elements always more important for tumor mutation studies through molecular biology techniques.** Before EU Regulation emission, industrial companies made available equipment to preserve fresh biological samples vacuum sealed. Afterwards the offer has increased with other kind of supplies, each with its technology peculiarity. To guarantee worker safety must be in place. These safety measures must, first of all, take into consideration the work environment with the introduction of all necessary environmental protection equipment. Moreover supervisors are required to make sure the rules and compulsorily wear the required personal protection equipment during operations with formaldehyde.

In the end it has become increasingly important personnel education and training, both to the use of new methodologies with their connected management changes and to the knowledge and respect of safety regulations.

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**Venerdì, 25 novembre 2016**

Aula Levante – 08:30 - 13:30

**CITOPATOLOGIA**

Le raccomandazioni della Siapec-IAP per la citopatologia diagnostica

Moderatori: Ambrogio Fassina (Padova) – Leonardo Resta (Bari)

**ITALIAN REPORTING SYSTEM FOR THYROID CYTOLOGY**

Guido Fadda

M.D. Catholic University, Foundation “Agostino Gemelli” University Hospital, Rome

The management of patients with thyroid nodules is based on the correct identification of those who are candidate for the surgical removal of the lesion. This decision primarily relies on the assessment of morphological criteria of the cells obtained by fine-needle aspiration (FNA). The final decision is complemented by clinical and imaging findings and, in selected cases, by molecular analysis. Partially unsettled issues concern the definition of the “indeterminate” cytology and the consequent non negligible rate of diagnostic surgery. The Italian reporting system (IRS) published in 2014, which updated the 2007 Italian Consensus Statement for the reporting and classification of thyroid nodule cytology, is meant to provide the Italian cytopathologists and clinicians with a reliable tool for their daily practice. The system confirms the previous 5-tiered reporting scheme, but includes a sub-classification of the “non diagnostic” category for cystic lesions (TIR 1C) and the separation of the “indeterminate” category into two subclasses with expected different risks of malignancy (low-risk indeterminate lesion- LRIL, TIR 3A, and high-risk indeterminate lesion - HRIL, TIR 3B). Thus, there are the following categories: TIR 1 (non diagnostic), TIR 1C (cystic), TIR 2 (Non malignant/benign), TIR 3A (LRIL), TIR 3B (HRIL), TIR 4 (suspicous for malignant neoplasm) and TIR 5 (malignant neoplasm). In this form the IRS is perfectly comparable with the most important national reporting systems, especially the American Bethesda system (2008) and the British RCPath classification (2015). A prospective multicenter trial is planned to validate the clinical effectiveness of the present classification system.

**References**


**POLMONE**

G. Troncone - E. Vigliar

**GINECOLOGIA**

A. M. Buccoliero - M. R. Giovagnoli

**THE PARIS SYSTEM FOR REPORTING URINARY CYTOLOGY**

M. Bonzanini\(^1\), F. Pierconti\(^2\)

\(^1\) Università Cattolica A. Gemelli - Roma; \(^2\) OUM Anatomia Patologica Ospedale S. Chiara – Trento

The lack of uniformity in reporting urine cytology has led to a new system of reporting urine cytology which is comparable to the Bethesda system for reporting cervical cytology and thyroid cytology. The Paris System (TPS) Working Group, composed of cytopathologists, surgical pathologists and urologists, was formed at the 18th International Congress of Cytology held at Paris in May, 2013 and produced “The Paris System for Reporting Urine Cytology”.

The main purpose of urine cytology is to detect high-grade urothelial carcinoma (HGUC). With this principle in mind, TPS Working Group has proposed and published a standardized reporting system that includes specific diagnostic categories and cytomorphologic criteria for the reliable diagnosis of HGUC. The categories of The Paris System are:

1) Unsatisfactory/Non diagnostic.

The specimen is considered adequate if atypical, suspicious or malignant, if there is appropriate benign urothelial cellularity and if is adequate volume in absence of appropriate benign urothelial cellularity.

The specimen is considered inadequate if non-urothelial factors are obscuring urothelial cells and there is no appropriate benign urothelial cellularity in instrumented specimen.

2) Negative for High grade Urothelial Carcinoma (NHGUC).

This does not exclude the possibility of low grade urothelial...
neoplasms. These patients should be again screened in the next scheduled check-up.

3) Atypical urothelial cells (AUC).
The criteria for AUC are: non-superficial and non-degenerated urothelial cells with a N/C ratio of >0.5 along with one of the three below mentioned features: hyperchromasia or irregular coarse, clumped chromatin, or irregular nuclear membrane contours. This category of patients should be followed up closely. In the context of previously documented urothelial neoplasm, this category should be subjected to ancillary studies like FISH, U-cyt etc.

4) Suspicious for High Grade Urothelial Carcinoma (SH-GUC).
The criteria for this category include: N/C ratio of >0.7 and hyperchromasia. Along with one of the two below mentioned features: irregular clumped chromatin or irregular nuclear contours.

5) High Grade Urothelial Carcinoma (HGUC).
The criteria for this category are the same as SHGUC but according to the Paris System consensus, a cellular cytologic urine specimen with a minimum of 5 to 10 viable malignant cells will qualify as HGUC.

6) Low Grade Urothelial Neoplasm (LGUN).
The features for this category are subtle and easily missed. The important feature that can be relied upon is the presence of well defined fibrovascular cores with capillaries within.

7) Other malignancies primary and secondary malignancies and miscellaneous lesions.

Prospective studies to establish successful prediction of HGUC by all categories, and clinical outcomes relative to each morphologic category, will be essential to the successful acceptance and implementation of TPS. For urologists, understanding the diagnostic criteria, their clinical implications, and appreciating the limitations of TPS is necessary if we are to utilize urine cytology and ancillary tests in a thoughtful and practical manner.

Reference

BREAST CITOLOGY


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Fine needle aspiration (FNA) has traditionally been regarded as the simplest, less invasive and less expensive diagnostic procedure for the definition of breast lesions. In expert hands, it allows obtaining an accurate diagnosis in most breast lesions. The introduction of widespread mammographic screening programmes and the consequent detection of a large number of small, non-palpable lesions have increasingly brought to the routine use of other minimally invasive biopsy methods using heavier gauge cutting needles – the so-called core biopsies (CB) and other automatic, imaging-guided devices, such as vacuum assisted biopsies (VAB) – partially obscuring the central role of FNA. These new opportunities lead to a greater autonomy of the radiologists, who don’t feel anymore the need of having the on-site cytopathologist attending the sampling session. Indeed, this professional is not always available in the “spoke” healthcare centres, although it is an indispensable element for the rapid on-site evaluation (ROSE) of sample’s adequacy and the “fast-track” diagnostic process. For this reason, CB, previously considered as a second-level exam, available in cases with inadequate (C1) or indeterminate (C3-4) cytology, has increasingly been used as a primary diagnostic method without resorting to cytology. Another reason for the spread of CB in breast diagnostics is the possibility of performing the biomolecular analyses for the preoperative characterization of T2+ tumours, which can benefit in some cases from neoadjuvant treatments. Recently, new recommendations propose to definitively abandon FNA, restricting all morphological investigations to CB.

But is this true? Has FNA completely lost its role as to let us to remove it from the routine diagnostic practice? From a careful reading of contemporary literature, it is evident that FNA maintains to this day a role as first level exam in all those centres where the cytopathologists grant their constant presence in the radiology units, actively taking part in the sampling sessions, make a constant use of a clear and standardized report, including diagnostic categories, and integrate their diagnoses in the context of a multidisciplinary team. This allows eventually completing the morphological diagnosis with ancillary techniques, when truly necessary, by resorting to further sampling. Moreover, this close collaboration between radiologist and pathologist leads to better evaluate the single situation, deciding together whether to extend the examination on multiple lesions and axillary lymph nodes, and eventually resort directly to CB, if needed. In current recommendations, “the use of FNA should be limited principally to mass-forming breast lesions, whether clinically or radiologically evident, or for the evaluation of axillary lymph adenopathy”. It is clear that FNA should be proposed in centres where compliance to the quality standards is assured and can be demonstrated, as it is stated in the European guidelines. Compliance to these standards allow accreditation of this method as first choice diagnostic technique in certified breast unit centres, as demonstrated also by the experience of Trieste’s breast unit. This is possible if an expert cytopathologist is available in the centre, and if an adequate workload is assured, which should preferably be higher than 100-150 cases per year. Most important is the possibility of integrating the sample with supplementary material, especially when there is the possibility of a neoadjuvant treatment. Routine cyto-histological comparison is critical to the maintenance and improvement of the diagnostic performances, for its key role for the synthesis between preoperative diagnostic methods. A rational management of the diagnostic process of each patient and each lesion is possible only in the breast units where all diagnostic techniques, including FNA, are available, and if these fulfill the required quality criteria. In our personal experience from the breast unit of Trieste, where there systematic use of FNA is available, it is proved that this method may bring to a definitive diagnosis 2/3 of the lesions investigated. The unjustified abandonment of the technique by breast healthcare centres must be avoided, since it could only have negative repercussions on the whole preoperative diagnostics. A reduced confidence of pathologists with breast cytological samples could only negatively impact on the ability to recognize also axillary lesions, for which FNA is still widely used. The advent of new molecular methods and the introduction of genomic sequencing (not only NGS) should conversely increase the use of FNA, for the undoubtedly quality
advantage of cytological material compared with paraffin-embedded material. Thus, it is necessary for the pathologists to guarantee the maintenance of their professional expertise in this field, and to transmit it to the young.

**SALIVARI**
J. Klijanienko

**SEROUS EFFUSIONS**
V. Ascoli
Dipartimento di Scienze Radiologiche, Oncologiche e Anatomia-Patologiche, Università Sapienza, Roma

Serous effusions are a common medical problem with several causes including benign conditions (collagen diseases, organ dysfunction and drugs) or malignancy. Because serous fluids are easy to collect, any pathology laboratory may deal with effusion specimens. If malignancy is suspected, cytological examination of the effusion is a rapid way to obtain a diagnosis. The diagnostic yield for malignancy is related to specimen collection and processing and depends on the experience of the cytopathologist and on tumor type. The diagnostic rate is higher for adenocarcinoma than for mesothelioma, squamous cell carcinoma, lymphoma and sarcoma [1].

A systematic approach to the investigation is required. There are cytology textbooks and several publications on effusions but there are no established practical recommendations to optimize the processing methods of fluid samples, or dataset for the cytological reporting. An exception is the inclusion of cytology in the investigation of pleural effusions provided by professionals with an interest in pleural diseases [2]. Recently, a group of cytopathologists with an interest in the mesothelioma field contributed to compile the guidelines for malignant mesothelioma cytodiagnosis [3].

Cytopathological diagnosis of effusions is mainly focused on malignant effusions that can be diagnosed up to 90% of cases. The yield from sending more than two specimens is not high. Immunocytochemistry should be used to differentiate between malignant cell types.

Aspirated fluid should immediately be drawn into a plain container with anticoagulant (EDTA 1mg/mL or heparin 3 units/ml of fluid) and transported fresh to the laboratory as quickly as possible at room temperature. If a delay in processing is likely, the specimen can be refrigerated at 4 °C. Addition of fixative is not recommended. After separating aliquots for microbiology (5 ml) and biochemistry (2-5 ml), the sample should be sent to for cytological analysis (maximum volume from remaining available sample).

A dedicated technical approach is crucial and highly recommended for serous effusions. The protocol for processing fluids should include:
- Gross examination: volume, color, viscosity to be noted.
- Bloodstained effusions require hemolysis. Hemolysis solution is composed of 1 gr potassium carbonate + 8.3 gr di ammonium chloride + 0.037 gr EDTA in 1 liter of distilled water. After centrifugation, add 5-10 ml of working solution to the sediment. Let incubate for 10 minutes at 4 °C, and rinse cells with isotonic saline.
- Any clot should be fixed in formalin.
- Centrifuge the whole sample to concentrate cells and separate the supernatant from the cell deposit.

If the sediment is scarce prepare cytopsins.
If the sediment is abundant prepare both wet-fixed, and air-dried direct smears. Stain with Papanicolaou and May-Grünwald-Giemsa. Prepare cytopsins and/or direct smears for ancillary staining techniques. It is strongly recommended to process the residual cell deposit. Routinely add formalin to the prepare cell block. Sediment can be stored at -80 °C as dry cell pellet, or as vital cells in DMSO. Supernatant aliquots can be stored at -80 °C.

Cytomorphology is the basis for diagnosis coupled with the evaluation of macroscopic appearance of the fluid and the sediment, and ancillary tests. The cell block is a routine procedure that has gained importance because of its key role in cytodagnosis and ancillary studies.[4]

Effusion samples can be divided into four categories: 1) Non-diagnostic effusions. 2) Benign effusions. 3) Effusions that require some form of ancillary testing to establish or exclude malignancy (not always possible!). 4) Malignant effusions on cyto-architectural grounds requiring the origin of these malignant cells to be defined.

When it is difficult to distinguish reactive mesothelial cells from malignant ones, the absence of Desmin immunoreactivity in Calretinin positive cells is a strong indicator of malignancy. EMA with clear-cut reactivity at the cell membrane is often used to support the diagnosis of mesothelioma. Loss of expression of BAP1 protein is a useful adjunct supporting a diagnosis of mesothelioma in effusion cytology. To demonstrate/exclude the mesothelial lineage of a tumor cell population, it is widely recommended to use a panel of antibodies, both in favor (Calretinin, Podoplanin (D2-40), WT-1 and Cytokeratin 5/6 (positive markers)) and against mesothelioma (CEA, BerEp4, MOC-31, and CD-15 (negative markers)). The use of antibodies indicating primary site depend on the gender of patient, the site of effusion and clinical history.

Reporting of cytology results should reflect the four abovementioned categories: 1) Inadequate (for no mesothelial cells are seen or only degenerate cells are present). 2) No malignant cells seen (an adequate sample without evidence of malignant cells. One should be aware that it does not exclude malignancy!). 3) Atypical cells or suspicious cells for malignancy (the sending of an additional sample may be helpful; occasional cells with not definitively malignant features). 4) Malignant (unequivocal malignant cells present which require typing by immunocytochemistry). This can be done on cell blocks (reliable and reproducible) as well as on smear/cytospin preparations.

**References**
Venerdì, 25 novembre 2016
Aula Ponente – 08:30 - 13:30

PATOLOGIA PLEUROPOLMONARE
Hot topics in patologia pleuropolmonare
SESSIONE I

Moderatori: Mattia Barbareschi (Trento) – Marco Chilosi (Verona) – Giulio Rossi (Aosta)

UTILITÀ DELLA CRIOBIOPSIA NELLA DIAGNOSI DELLE INTERSTIZIOPATIE POLMONARI
A. Dubini
For many years cryoprobes have been used in the management of lung disease mainly for resection of endobronchial tumours; in the past decade the technique has been used primarily for the diagnosis of diffuse interstitial lung diseases (ILD). The aim, in this context, is identification and characterization of the histologic findings, putting them in the right context taking into account clinical and radiological features, and, in some cases, other findings such as rheumatologic and serologic data. The correct handling of samples is very important and it starts in endoscopic room where cryobiopsies are taken from the cryoprobe and transferred from saline into formalin; is also important to embed specimen in paraffin block oriented to maximize tissue surface area in cut slides. Minimum of two levels in E&E slides are suggested, with multiple cuts of the specimen(s) on each slide. It is also important to take in account that there are some artifacts that may be encountered in cryobiopsies and tissues other than lung (pleura and chest wall skeletal muscle).
In consideration of the high diagnostic yield in diffuse parenchymal lung disease (up to 80%) and of the low morbidity and mortality rate of cryobiopsies, the technique has been suggested as an alternative to have a diagnosis in some patients with diffuse parenchymal lung disease.

IPF VERSUS PATTERN UIP
A. Cavazza

PATOLOGIA NON-NEOPLASTICA CHE SIMULA I TUMORI
A. Cancellieri

MARCATORI IMMUNOMOLECOLARI NELLE INTERSTIZIOPATIE POLMONARI
F. Calabrese
Interstitial lung diseases - ILDs - (or named diffuse parenchymal lung diseases - DPLDs) are a large group of lung disorders having a generalized involvement of lung interstitium. Currently more than 200 different diseases are recognized with different aetiologies but they share the same acronym because of common clinical, radiological and histological features. The ILD diagnosis is mainly based on routine histology and requires high expertise/experience and overall a multidisciplinary approach. Idiopathic pulmonary fibrosis (IPF) is considered the most common and severe form of the fibrosing interstitial lung diseases, with a median survival of approximately three years because no effective therapy is available and important complications develops (acute exacerbation, pulmonary hypertension and lung cancer). Although our understanding of the pathogenesis of IPF has greatly improved, at present time we have limited information on reproducible and sensitive immunohistochemical/molecular biomarkers to facilitate early detection, best prognostic accuracy and targeted therapies. Considering the microscopic spatial and temporal heterogeneity of pathological changes and complexity of the pathogenesis of IPF, it is clear that a single biomarker assay (“cherry-picking approach”) is unlikely to have transformative effects on clinical practice. Implementation of multi-marker panels, assessing the crucial pathobiological processes involved in IPF, may provide clinicians with the information needed to improve patient care.
Moreover, a careful characterization of IPF patients is mandatory also for those candidate to lung transplantation, leading to a better early and late graft outcome.
Tissue evaluation remains, however, the bedrock to guide subsequent molecular testing and extrapolate the most clinically fitting biomarkers. Centers with tissue biobank are strongly encouraged to collaborate in order to increase the diagnostic and prognostic value of new markers.

NUOVE ENITÀ PATOLOGICHE NELLA WHO 2015
G. Pelosi

SESSIONE II

Moderatori: Camilla Comin (Firenze) – Oscar Nappi (Napoli) – Bruno Murer (Mestre)

FATTORI PREDITTIVI NEL NSCLC: IL RUOLO DEL PATOLOGO TRA TESSUTO E BIOLOGIA MOLECOLARE
P. Graziano

MOLECULAR BIOMARKERS IN MALIGNANT PLEURAL MESOTHELIOMA
L. Righi
Pathology Unit, Department of Oncology, University of Turin at San Luigi Hospital, Orbassano (Torino)
Malignant pleural mesothelioma (MPM) is a highly lethal, chemo- and radio-resistance asbestos-related neoplasm, which usually presents as an unresectable disease, frequently in elderly patients with severe co-morbidities and poor performance status. Diagnosis is usually performed at advanced disease stage on video-thoracoscopic biopsies. The first-line treatment is chemotherapy with cisplatin and antifolates such as pemetrexed (PEM), whereas standardized agent for second-line chemotherapy are still lacking. Many studies are ongoing to identify novel effective prognostic and predictive tissue or serum biomarkers and molecular targets to better select
THORACIC MALIGNANCIES MIMICKING BENIGN PROCESSES

G. Rossi, 1 R. Patetta, 1 P. Carassai, 1 L. Caramanico, 1 P. Bernardi, 1 G. Jocollè, 1 M. Cecilia Mengoli, 2 P. Baderna, 1 G. Donati, 1 A. Cavazza 3

1 Azienda USL Valle d’Aosta, Regional Hospital “Parini”, Aosta (Italy); 2 Pathology Unit, University Hospital Policlinico, Modena (Italy); 1 Azienda S.Maria Nuova, IRCCS, Reggio Emilia (Italy)

Malignant processes mimicking and then misinterpreted as benign/reactive lesions in the lung are an underrated phenomenon and may be observed in several pathologic entities with different cell differentiation.

The epidemic increase of lung adenocarcinoma in the last decades has lead to encounter more frequently well-differentiated adenocarcinomas with lepidic growth that may create several diagnostic problems, especially on cytology and small biopsy. A meticulous morphologic examination of some robust criteria (e.g., the uniformity and monotony of the glandular proliferation lacking primary cilia) together with a striking correlation with the imaging studies represent the best approach in order to do reach the correct diagnosis.

Another major issue concerns the spindle cell proliferation in the lung. In some cases, sarcomatoid carcinomas, particularly spindle cell carcinoma, may show foci of necrosis and cavitation, a prominent interstitial growth coupled to an important inflammatory infiltrate with reactive fibroblastic reaction. Such cases may be likely mistaken as organizing inflammatory processes. A careful attention to the atypical cytological features and subtle invasion of the vascular wall also using immunohistochemical support by pan-cytokeratins stain do represent helpful findings in recognizing the malignant process and rule out benign processes as lung infarction, interstitial lung diseases, inflammatory myofibroblastic tumor, IgG4 syndrome and other benign or low-grade conditions.

Lymphoid infiltrate in the lung may be encountered in various lesions, particularly extra-nodal marginal zone lymphoma, chronic lymphocytic leukaemia, lymphomatoid granulomatosis and Hodgkin’s disease. Differential diagnosis needs the knowledge of the medical clinical history of the patient and adequate use of immuno-molecular investigations demonstrating neoplastic lymphoid cells and clonal rearrangement. Intravascular lymphoma and pleural lymphomas may be mis-interpreted as an interstitial pneumonia and reactive chronic pleuritis, respectively. A careful search for atypical lymphoid cells within the alveolar capillaries and immunomolecular demonstration of viruses (e.g., EBV, HHV8) and clonal rearrangement are key features in the differential diagnosis.

Among mesothelial proliferations, the desmoplastic variant of sarcomatous mesothelioma is extremely difficult to diagnose, because large portions of the tumors are composed of bland-appearing fibrous tissue and a bland disorganized growth of mesothelial cells often lacking immunohistochemical expression of mesothelial markers. Demonstration of invasion of pleural soft tissue or lung parenchyma is the best criteria to evidence malignancy in desmoplastic mesothelioma, but close correlation with imaging studies (e.g., CT scan and PET) is always necessary.

Vascular neoplasms, as Kaposi’s sarcoma, epithelioid haemangioendothelioma and well-differentiated angiosarcomas, although rarely occurring in the lungs, may be misinterpreted as pulmonary hemorrhage or organizing infarction. The clinical and radiographic pattern, usually mimicking metastatic disease, and the fact that atypical spindle cells occlude small pulmonary arteries with surrounding alveolar hemorrhage are clues to the recognition of these lesions.

Essential references


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LE ALTERAZIONI MOLECOLARI NEI TUMORI POLMONARI DEI GIOVANI

A. Marchetti

TUMORI NEUROENDOCRINI POLMONARI: RIPRODUCIBILITÀ DIAGNOSTICA E STANDARDIZZAZIONE DEI REFERTI

R. Monaco
The term “intraductal carcinoma of the prostate (IDC-P)” describes a proliferation of malignant secretory cells that grows within and expands prostatic ducts and/or acini. IDC-P is included as a distinct entity in the 2016 WHO classification. In the majority of cases, IDC-P represents a retrograde spread of prostatic adenocarcinoma into prostatic glands; in rare cases, where an invasive component is not present, it represents a precursor lesion to prostatic adenocarcinoma. IDC-P is defined as malignant epithelial cells filling large acini and prostatic ducts with at least partial preservation of basal cells and forming either 1- solid or dense cribriform (in which punched-out luminal spaces account for <50% of the central cellular mass) pattern or 2- loose cribriform or micro-papillary pattern with either marked nuclear atypia (nuclear size 6X normal size or larger) or non-focal comedonecrosis. The basal cell layer is present or at least partially preserved. Stains for 34βE12 and p63 can be used to confirm the presence of basal cells. Minor criteria (non diagnostic) include atypical irregular glands or right angle branching, involvement of > 6 glands and/or ≥1 mm size, two cell populations with an outer perimeter cell group composed of tall, pleomorphic, and mitotically active cells and as a central group that is cuboidal, monomorphic, and quiescent. Lesions that fell short of the criteria for IDC-P, but are more ominous than high-grade prostatic intraepithelial neoplasia (HGPIN) are labeled as atypical intraductal proliferations.

Distinguishing IDC-P from cribriform HGPIN in prostate biopsy is the most important differential diagnosis, because management for these two conditions is considerably different. Loose cribriform and micropapillary patterns can be seen in both HGPIN and IDC-P, but dense cribriform, solid patterns and comedonecrosis are not seen in HGPIN. To establish the diagnosis of IDC-P in the loose cribriform and micropapillary patterns, other cytologic features are required, such as markedly enlarged and pleomorphic nuclei and non-focal comedonecrosis. These criteria can reliably distinguish IDC-P and HGPIN in most cases. HGPIN and IDC-P share immunohistochemical profiles, including positive staining for PSA, AMACR, and basal cell markers; therefore, none of these stains is helpful in the differential diagnosis of these two entities. Our group has reported ERG rearrangement by FISH in 75% of 48 cases of IDC-P, but in none of the 16 cases of isolated cribriform HGPIN. Recently Lotan et al. have reported PTEN loss and ERG expression (by IHC) in >80% and 58%, respectively of IDC-P, compared to 0% and 13%, respectively of HGPIN lesions. PTEN and ERG may be helpful in distinguishing IDC-P and HGPIN. Infiltrating cribriform acinar adenocarcinoma (Gleason pattern 4 or 5 with comedonecrosis) closely mimics cribriform IDC-P. Invasive cribriform prostate cancer, unlike IDC-P, lacks basal cell lining. Ultimately, the presence of a basal cell layer rules out infiltrating acinarPCA. There is significant morphological overlap between ductal adenocarcinoma of the prostate and IDC-P. Distinguishing features in ductal adenocarcinoma include tall pseudostratified columnar epithelium, arranged in cribriform patterns with slitlike spaces and/or true papillary fronds. In contrast, IDC-P has cuboidal cells, cribriform pattern with rounded lumina, and micropapillary tufting without fibrovascular cores. Intraductal spread of urothelial carcinoma may mimic IDC-P. Urothelial carcinoma is typically more pleomorphic than IDC-P cytologically. A panel of immunostains can often resolve the diagnostic ambiguity. IDC-P stains positive for prostate specific markers, including PSA, PAP, PSMA, NKX3.1 and P501S, while stains for basal cells, such as CK5/6, 34βE12 and p63, are positive only in the basal cells at the periphery of the cancer glands. In contrast, urothelial carcinoma is negative for prostate specific markers and often is positive for GATA3 and basal cells markers. IDC-P is typically associated with high-grade and high volume cancer (with adverse prognostic features, such as extraprostatic extension, seminal vesicle invasion, and positive margins) and has been found to be of prognostic significance independent of Gleason grade, pathological stage and tumor volume. Regardless of the treatment, tumors with an IDC-P component are associated with worse outcome. The presence of IDC-P should be sought and reported in both prostate biopsy and radical prostatectomy (RP) specimens. IDC-P in prostate biopsy should be reported even when associated with extensive and high-grade PCA, as it may provide additional prognostic value (improves accuracy of postoperative monograms to predict PSA recurrence after RP). Grading of IDC-P is not recommended. When IDC-P is associated with a Gleason pattern 3 component on prostate biopsy, its presence should be documented and its poor prognostic significance should be mentioned. When IDC-P is identified on prostate biopsy without concomitant invasive component, pathologists should report its presence with a comment stating that IDC-P is usually associated with a high-grade and high-volume prostate cancer. An immediate repeat biopsy within 3 months or definitive therapy should be recommended, depending on the extension of the lesion.
I CRITERI DI REPORTING ICCR NEI TUMORI UROGENITALI

M. Colecchia
Uropathology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano

The International Collaboration on Cancer Reporting (ICCR) represents an international effort to produce “common, internationally validated, and evidence-based pathology datasets for cancer reporting for use throughout the world, through broad collaboration between Pathology Colleges, Societies, and major cancer organizations internationally.” The recommendations have been reviewed by members of an international expert panel but not yet gone through open consultation and should be considered provisional. After evaluation of these documents, discussions via telephone conference calls on these documents among the panel members were made. Several corrections and a consensus document was edited in the end. The experts had to treat first an element name, then to decide whether this item was required or recommended, a literature research was done, the final document was then sent out for corrections. In this presentation the datasets developed for carcinomas of the genitourinary tract will be presented. The committees developed many data sets embracihing the fields of renal core /wedge biopsy; the invasive carcinoma of renal tubular origin, prostate core needle biopsy, prostate cancer histopathology reporting guide radical prostatectomy specimen, (2nd edition), prostate cancer-transurethral resection and enucleation, testis tumors, penile cancer datasets and urothelial carcinomas of the urinary tract (from renal pelvis to urethra) in biopsy or transurethral resection specimens and individual data sets for resections of the renal pelvis and ureter, the urinary bladder and the urethra. In developing the datasets, the committees considered reporting items to be required if there was evidence at level III-2 that the element was prognostically significant or if it was the consensus of the committee members that the element was necessary for clinical management, staging or prognosis. Other elements that were considered of interest or reasonable to include in a complete pathology report were included as recommended items.

For all datasets there were a number of required elements that were common to all. These included documentation of the operative procedure and the tissues submitted. Although recording clinical information is obviously critically important, given the difficulty and variability in its availability and that it is largely out of the control of the reporting pathologist, the ICCR has placed this as a recommended rather than required element in all its datasets. Histologic tumour type (by WHO 2016), presence of variant histology, histological grade, extent of invasion, the presence or absence of lymphovascular invasion are also required in the majority of the data sets. Grading using the 2016 WHO is required. Reporting of grade using alternate systems is optional. For the resection datasets the common required elements also include the tumour location, maximum tumour size and extent of invasion (as estimated grossly). Surgical margin status and lymph node status are also required elements. The datasets also include a number of recommended elements that vary somewhat depending on the specific dataset. Lastly, to date there are no ancillary items that have sufficient data to support inclusion as required elements in the majority of these datasets. For those cases where ancillary studies are performed, it is recommended to include the results in the pathology synoptic dataset.

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http://www.iccr-cancer.org/

GRADE GROUPING IN PROSTATE CANCER: UPDATE ON REPORTING

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Numerous studies have confirmed the prognostic value of Gleason grading. In the last 40 years, prostate cancer management and reporting have changed. As a result of increasing number of cases coupled with the importance assigned to Gleason grading in modern predictive models, pathologists have accumulated a large experience in applying the grading system that has led to a gradual evolution in contemporary surgical pathology practice. During the 2005 ISUP Consensus Conference on Gleason Grading of Prostatic Carcinoma, the Gleason grading system underwent a major revision reflecting the modern practice. The changes proposed at the meeting and implemented in the modified Gleason grading system pertain both to pattern interpretation and reporting. There was a consensus that a diagnosis of Gleason score 2-4 on needle biopsy should be made rarely if ever. It is likely that with the use of immunoperoxidase staining for basal cell markers many of the original Gleason score 1+1=2 carcinomas would currently be regarded as adenosis. The modified Gleason grading system defines pattern 3 prostate cancer as composed of single, well-formed glands infiltrating among nonneoplastic prostatic acini. A departure from the original Gleason classification system is that “individual cells” would not be allowed within Gleason pattern 3. At the conference, it was also agreed that most cribriform glands be diagnosed as Gleason pattern 4; subsequently it has been recommended that all tumor glands showing cribriform architecture should be classified as Gleason pattern 4. These revised guidelines for Gleason grading have made Gleason score 6 cancer a more homogeneously indolent disease, which may be relevant in the era of active surveillance. The marked contraction of Gleason pattern 3 has resulted in a significant expansion of Gleason pattern 4, including fused microacinar glands, ill-defined glands with poorly formed lumina, and all glomeruloid and cribriform glands. Fused glands may be under-graded when present in small foci. On the other hand, careful evaluation of multiple tissue levels may be necessary to determine whether few glands are truly fused or simply tangentially cut. Ill-defined glands with poorly formed glandular lumina should be graded as pattern 4; however caution should be applied in distinguishing them from very small “well-formed” glands.

The grading of morphologic variants of prostate cancer was also addressed at the 2005 consensus conference: prostatic tumors containing clear vacuoles should be graded by only evaluating the underlying architectural pattern; atrophic and pseudohyperplastic cancers should be graded as pattern 3; ductal adenocarcinomas and colloid (mucinous) carcinomas with irregular cribriform glands floating within a mucinous matrix should be graded as pattern 4; sarcomatoid and signet ring cells should be graded as pattern 5. Small cell/neuroendocrine, squamous and basaloid carcinoma should not be graded. Important recommendations regarding reporting can be summarized as follows: in the setting of high-grade cancer...
one should ignore lower grade patterns if they occupy <5% of the tumor; high-grade tumor (identified at low to medium magnification) of any quantity on needle biopsy should be included within the Gleason score; in cases in which there are three different patterns on needle biopsy, but the third pattern is lower grade, the lower grade pattern should be ignored; for tumors with patterns 3, 4, and 5 both the primary pattern and the highest grade should be recorded. Using the modified grading system (narrowing the definition of Gleason pattern 3 and expanding the definition of pattern 4) has resulted in disease migration or upgrading with more cancers assigned a Gleason score ≥ 7 than in the past. It also resulted in a more homogeneous Gleason score 6, which has an excellent prognosis when the disease is organ confined. Biopsy Gleason score 6 tumors have decreased from 48% to 22% and from 68% to 55%, whereas Gleason score 7 has increased from 25% to 68% and from 30% to 43%. After the modified Gleason grading, the agreement of Gleason score between needle core biopsy and radical prostatectomy specimens for Gleason score 7 has improved from 45% to more than 85%. The inter-observer reproducibility among pathologists using the modified Gleason system has improved compared to the old conventional Gleason grading (from 60% to 80%). The vast majority of studies using both systems have shown that Gleason grading of prostate adenocarcinoma on needle biopsy and radical prostatectomy specimens is strongly associated with pathologic stage, status of surgical margins, metastatic disease, biochemical recurrence, and cancer-specific survival, with the modified system outperforming the original one in some large series. Patients with original Gleason score 6 upgraded to modified Gleason score ≥ 7 had intermediate values of pathologic stage, biochemical progression and metastases compared with original and modified Gleason score 6 and original Gleason score 7-8.

At the 2014 International Society of Urological Pathology (ISUP) consensus conference, a previously proposed concept of five prognostic grade groups, from 1 to 5 was adopted with 90% consensus. The Grade Groups, used in parallel to the modified Gleason grading system, translate Gleason scores in five distinct risk categories where Grade Group 1 is defined as Gleason score ≤ 6, Grade Group 2 as Gleason score 3+4=7, Grade Group 3 as Gleason score 4+3=7, Grade Group 4 as Gleason score 4+4=8, and Grade Group 5 as Gleason score 9/10. This five-tiered grade system better reflects biologic behavior and guides clinical care. The Grade Groups have been endorsed by the ISUP and the World Health Organization (WHO). The new Group Grade system was validated in a multi-institutional study of over 20,000 radical prostatectomy specimens. Recently, the distinct risk of biochemical recurrence based on each Grade Group has been confirmed in a nationwide population-based cohort. The importance of future additional studies evaluating external validation in different patients’ cohorts should be emphasized. At the 2014 consensus conference it was recommended to report the percentage pattern 4 in GS 7 tumors (particularly in 3+4=7), since patients with minor pattern 4 component (<5%) may be deemed eligible for active surveillance. GS 3+4=7 prostate cancer with minimal pattern 4 on biopsy is associated with low-risk tumor on radical prostatectomy. Authors advocated including the presence of cribriform pattern 4 and IDC-P in routine pathology reporting, since any amount of large cribriform GP4 and IDC-P is a significant prognostic factor after adjusting for GS and pathologic stage in determining patient outcome.

### SESSIONE III

**Moderatori:** Enrico Bollito (Orbassano) – Maria Rosaria Raspolini (Firenze)

**DIAGNOSI PRESENTATE DA GIOVANI PATOLOGI**

**TERATOMA WITH SOMATIC-TYPE MALIGNANCY OF THE TESTIS WITH METASTATIC LOCALIZATIONS AT THE ONSET: A MORPHOLOGICAL AND MOLECULAR STUDY**

S. Massa, B. Paolini, M. Colecchia

Fondazione IRCCS Istituto Nazionale dei Tumori di Milano.

A painless testicular swelling occurred in a 21-year-old male: CT scan confirmed a neoplasm in the left testicle and bulky metastatic retroperitoneal lymph nodes involving perirenal soft tissue. A CT scan showed metastases in the lungs, liver and in the supraclavicular lymph node. Plasma protein levels were: AFP 6564 U/ml, betaHCG 5661 mU/ml and LDH 1349 U/L.

Four cycles of neoadjuvant cisplatinum based chemotherapy (PEB) were performed, six month after orchiectomy and excisions of retroperitoneal and left renal masses were carried out.

Two months after surgery, the patient is alive with normalization of serum LDH, beta HCG and AFP levels. No additional chemotherapy has been scheduled while anticoagulant therapy needed for vascular stenosis.

Macroscopically a cm 18x11x10 testicular greyish tumour occurred, showing cystic, necrotic and firm areas. The multiple metastases in the retroperitoneum and in the perirenal soft tissue showed greyish-white, hemorrhagic and necrotic areas with an infiltrative pattern of growth.

Microscopically, a testicular post pubertal teratoma occurred together with a somatic malignant component showing a proliferation of atypical spindle cells (high-grade pleomorphic sarcoma). Areas with lipomatous and cartilagineous differentiation were mixed with poorly differentiated pleomorphic spindle cells. Atrophic tubules with scant spermatogenesis and Sertoli hyperplasia were observed in surrounding parenchyma. Both in the testis and in the metastesis fibrosis, necrotic areas and scattered siderophages or foamy cells were evidence of post therapeutic regression of the germ cell tumoral components.

Immunohistochemical stains were performed: somatic malignancy was positive focally for actin, desmin and mdm-2, while SALL4 was positive in the epithelia of glands in the postpubertal teratoma. The diagnosis was metastatic teratoma with somatic type malignancy of the testis with a predominant high grade sarcoma in the somatic type malignant component.

A NGS analysis revealed PTEN mutation. MDM2 has been investigated by CISH, but no amplification has been found. Germ cell tumor with somatic type malignancy is associated with poorer cancer specific survival than traditional germ cell tumor. The chemoresistance associated with SM necessitates a distinct therapeutic approach. Traditional GCT prognosticators such as stage and IGCCCG risk category failed to maintain prognostic significance in this patient population. Incompletely resected tumor and distant metastases is as-
associated with poor prognosis. Tumor grade demonstrated prognostic importance, which appeared to be confined to sarcomas and sarcomatoid yolk sac tumors. Tumor grade is an important prognostic factor in sarcomas. Aggressive and serial resections are often necessary to optimize cancer specific survival. Successful management of GCT with SM requires aggressive and often serial resections at high volume centers. Multidisciplinary surgical approaches are often necessary to ensure complete resection. Furthermore, specimen review by an expert gynecological pathologist is essential to establish the appropriate diagnosis and determine prognosis.

References

Venerdì, 25 novembre 2016
Aula Scirocco – 08:30 - 13:30

GINECOPATOLOGIA
La patologia ginecologica tra il vecchio e il nuovo

SESSIONE I
Moderatori: Gian Luigi Taddei (Firenze) – Gian Franco Zannoni (Roma)

PRACTICAL ISSUES IN THE DIAGNOSIS OF SEROUS CARCINOMA

E. D’Angelo

Introduction
Serous carcinoma (SC) is the prototype of type II endometrial cancer and accounts for less than 10% of all endometrial carcinomas (EC) (1). It is a very aggressive tumor, unrelated to estrogen stimulation, arising occasionally in endometrial polyps or from precancerous lesions developing in atrophic endometrium that mainly occur in older women (2). It is regarded as high grade tumor, by definition. SC has a high tendency to develop lymph node metastasis and peritoneal spread. SC and endometrioid carcinoma (EEC) grade 3 have been compared using the surveillance, epidemiology and End Results (SEER) program data from 1988 to 2001. They represented 10% and 15% of EC respectively, but accounted for 39% and 27% of cancer death respectively. SCs have also been reported in association with a history of breast cancer, and a possible role for tamoxifen has been proposed. SC is usually diagnosed at advanced stage; 46% of patients are at stage II to IV at presentation (3-5). The estimated 5-year overall survival is between 18 and 27%. Approximately 60 to 70 % of women with SC present with disease outside the uterus.

Serous carcinoma is described in WHO 2014 as characterized by complex papillary and/or glandular arrangement, with diffuse marked nuclear pleomorphism (6). The typical microscopic features and its aggressive behavior were well described by Drs Hendrickson and Kempson’s group, from Stanford (7). However, Factor previously described the existence of papillary adenocarcinomas of the endometrium with psammoma bodies, noting their similarity to ovarian cancer (8). In 1981, Lauchlan designated the tumors as “tubal” or “serous” (9).

Pathologic features
The tumor shows thick, fibrotic or edematous papillae with prominent stratification of tumor cells, and cellular budding (10-14). The papillae are lined by anaplastic cells with large, eosinophilic cytoplasm, with striking cellular pseudo-stratification, micropapillary formation and cell budding. Occasionally, groups of detached tumor cells may be present lying between papillae. The nuclei are hyperchromatic or vesicular, and contain prominent nucleoli. Over one third of serous carcinomas contain clear cells and elements with hobnail configuration. In some areas, the tumor may show a solid pattern, and in other a glandular arrangement with slit-like and irregular spaces. Mitoses are frequent, and many of them are abnormal. Psammoma bodies may be present. The tumor is usually associated with deep myometrial invasion, and extensive lymphatic invasion. When there is myometrial invasion, a “gaping gland” appearance often unaccompanied by any stromal reaction is frequently seen.

SC does not arise from preexisting endometrial hyperplasia. Different stages of precursor lesion have been described, from p53 signature to Endometrial glandular dysplasia (15-16). These lesions usually develop in the setting of atrophic endometrium or endometrial polyps. P53 is one of the molecular abnormalities involved in development and progression of this type of tumors; and tumor cells usually are strongly positive for p53 immunostaining. Endometrial Intraepithelial Carcinoma (EIC) is characterized by replacement of the surface endometrial epithelium by highly atypical cells with extension to endometrial glands, with overlapping cytological features to invasive SC, but without stromal invasion. However, the diagnosis of EIC is discouraged, since it may be associated with high-stage disease and a fatal outcome. EIC cells may spread to the peritoneal surface via transtubal spread of tumor cells from the uterine cavity, even in absence of myometrial invasion. EIC may be found in isolation or, more commonly, adjacent to a focus of invasive serous carcinoma. (17-21).

Uterine serous carcinomas are aggressive tumors (3-5). Patients often have advanced stage tumors at initial presentation. Surgical staging should include total hysterectomy with bilateral salpingo-oophorectomy, pelvic and para-aortic lymph node dissection, omentectomy and peritoneal biopsies. The overall survival rate for all stages is approximately 30-40% (22-28). Patients with FIGO stage I disease have the highest 5-year survival rates (29). Size and the extent of lymphovascular invasion are also prognostically important (30-32). The tumor may spread to cervix, fallopian tubes, ovary, lymph nodes, peritoneum and pleura (33). The microscopic appearance of endometrial SC metastasizing to the peritoneum is very similar to metastatic ovarian serous carcinoma. WT-1 immunostaining is helpful in this differential diagnosis. How-
ever, it is worth mentioning that WT-1 is also expressed in a reduced number of uterine SC, but staining is usually weak to moderate.

**Differential Diagnosis:**

**High Grade EEC**

SC may exhibit a prominent solid pattern of growth. These cases show problems in differential diagnosis with high grade EEC. Identification of typical features of EEC or typical characteristics of SC is helpful. It is important to pay attention to the presence of glandular differentiation, and also presence of squamous, mucinous or secretory change, which favors EEC. Coexistence with complex endometrial hyperplasia (endometrioid intraepithelial neoplasia favors an EEC, while the presence of EIC would support the diagnosis of SC (34-37). There are reports suggesting that high-grade EEC with mutations in POLE may be particularly prone to exhibit features mimicking SC (38-40).

**Low-grade EEC**

This is a very important problem (41). There is a subset of SC that shows a prominent glandular pattern, simulating at low-power magnification a low-grade EEC (35-37). There is usually great discordance between the architectural and cytological features of the tumor. Tumor cells show high-grade atypical features with high nuclear to cytoplasmic ratio, prominent nuclear pleomorphism, enlarged hyperchromatic nuclei, and prominent nucleoli. Presence of obvious endometrioid differentiation, such as squamous, mucinous or secretory change goes against the diagnosis of SC. In contrast, SC shows marked pseudosтратification, and lack of polarity. It is important to pay attention to the myoinvasive front. Peculiar patterns such as the MELF (microcystic elongated fragmented) favor EEC, while the typical “gaping gland” appearance is characteristic of SC.

An important differential diagnosis of SC is EEC with papillary (villoglandular) pattern (42-43). It shows elongated villous projections, but the nuclei lack the striking pleomorphism, macronucleoli of SC. Moreover, villoglandular carcinoma lacks the complex papillary pattern of SC, and does not show prominent cell stratification and papillary tufts. Finally, SC may be confused with EEC with small nonvillous papillae (44). This variant accounts for 8% of EEC. The tumors contain small papillae admixed with endometrioid glands. The papillae are composed of buds of cells with large eosinophilic cytoplasm, which typically show lower grade cytological features.

**Mixed Endometrioid-Serous Carcinoma**

SC usually occurs in pure form. However, occasionally, SC may coexist with conventional EEC (45-47). It has been suggested that the serous component may arise as a result of progression of the endometrioid elements. When one of these components is present in at least 5% of the tumor, the tumor is diagnosed as a mixed endometrial carcinoma, being mixed endometrioid and serous carcinoma (mixed EEC-SC) the most frequent tumor type. The correct diagnosis of the second component is crucial to determine treatment options and outcome for these patients, since it has been suggested that the presence of as little as 10% of a type II component could adversely affect patient’s outcome. There is some interobserver variation in histological typing in endometrial carcinoma. This is partly due to the fact that some pure EEC may exhibit papillary arrangements and may be erroneously mistaken as SC. On the other side, some pure SC may show glandular pattern of growth, and may be misinterpreted as EEC. Inappropriate interpretation of these unusual pathological patterns may lead to incorrect diagnosis of mixed EEC-SC. To avoid incorrect diagnosis of pure EEC and pure SC as mixed EEC-SC, rigorous criteria should be used, and diagnosis should be confirmed with the help of immunohistochemistry or molecular pathology.

**Clear Cell Carcinoma**

SC may exhibit cells with clear cell change. These cases should not be interpreted as clear cell carcinoma or mixed se-rous-clear cell carcinoma. Rigorous interpretation of the clear cell component is recommended when analyzing a SC (48). Endometrial clear cell carcinoma is similar to clear cell carcinomas of the ovary. Microscopically, clear cell carcinomas are characterized by a variety of patterns such as solid, papillary, glandular, and tubulocystic. Most clear cell adenocarcinomas show mixed patterns. Tumor cells may exhibit a prominent clear appearance, with abundant glycogen. Tumor cells may also show a hobnail configuration, which results from secretion of the cytoplasmic contents into the lumina, so the nuclei appear bulbous, and protrude into the central aspects of the glands. Cystic spaces are common. Hyaline bodies are very frequent, and very characteristic. Clear cell adenocarcinoma usually contains complex papillae with hyalinized cores, and does not show the typical pseudo-stratification of SC. In contrast to SC, mitotic index is low. The lumen of the glandular spaces usually contains mucus. The nuclei are pleomorphic, with prominent nucleoli. Clear cell adenocarcinoma of the endometrium may contain sheets of large cells with abundant eosinophilic (oncocytic or oxyphilic) cytoplasm, which may predominate in occasional cases. Clear cell carcinoma is usually (but not always) negative for estrogen receptor, and shows a wild-type pattern for p53. AMACR and Napsin immunostaining are helpful techniques in confirming the diagnosis of clear cell carcinoma (49-50). Positive staining for Napsin was seen in 87% of clear cell carcinomas, while only in 8% SC and 0% EEC. Moreover, AMACR immunoreactivity was seen in 75% of clear cell carcinomas, but only in 22% of EEC and 15% of SC. It has been shown that hepatocyte nuclear factor 1-beta immunoreactivity lacks of specificity for endometrial clear cell carcinoma (51).

**Carcinosarcoma**

The differential diagnosis between malignant mixed mullerian tumor and SC may be very difficult in small biopsies and curettages. The recognition of a biphasic pattern, with an obvious typical sarcomatous component, is the most important criteria for the diagnosis.

**Diagnosing SC in Small Biopsies**

The diagnosis of SC may be difficult in small endometrial biopsies. It is important to look for typical features of SC, such as papillae, cell stratification and budding, slit-like spaces and highly atypical cells. It is also important to look for lack of EEC features, such as squamous and mucinous areas. The presence of marked discordance between the architectural gland arrangement and the high grade cytological features should alert one to the possibility of SC. Rigorous interpretation of clear cell elements, and appropriate assessment of tumor stroma may be helpful in the differential diagnosis with clear cell carcinoma or carcinosarcoma. Immunohistochemistry (p53, p16, ER, IMP2, IMP3, PTEN, Arid1A) is very helpful, but individual cases may show discordant features. A diagnosis of **High grade endometrial carcinoma, serous carcinoma component cannot be excluded**, is acceptable as a managerial diagnosis in endometrial biopsies.

**Immunohistochemistry and Molecular Pathology in Distinguishing SC from endometrioid Carcinoma**
The Cancer Genome Atlas Research Network (TCGA) has recently performed an integrating genomic characterization of EC (52). Exome sequence analysis revealed four groups of tumors. Group 1, with EEC with mutations in PLOD, associated with good prognosis. Group 2, including EEC with microsatellite instability and group 3 tumors including EEC with low copy number alterations, both showing similar progression-free survival rates. Group 4 (Serous-like) showed p53 mutations, and worse prognosis, and was composed of most (but not all) SC, but also some EEC (many EEC3, but also some EEC1-2). In other words, there are tumors that are morphologically EEC but molecularly similar to SC, and also tumors that are microscopically SC, but molecularly similar to EEC. These ambiguous tumors show discordant microscopic/molecular features.

Several IHC markers have been shown to be differentially expressed in EEC, including EEC 3, as opposed to SC. Some of these markers were previously found to be differentially expressed between EEC and SC by Cdnna analysis. For example, several studies have shown decreased expression of ER in SC, and also a lower frequency of PTEN alterations in SC in comparison with EEC. Additional proteins that are differentially expressed in EEC and SC, and that were used in this study are HER2 (53), claudin 3 and 4 (54), Nrf2 (55), p53 (56-57), p16 (58), FOLR-1 (59), HMGA-2 (60-61), cyclin E (62), IMP2 (63), IMP3 (64). Several authors have attempted the use of panels of antibodies to help in diagnosis and prognosis. Alkushi et al (65) examined the IHC expression profile of twelve markers (bcl-2, ER, p53, p21, p27, HER-2, E2F1, p63, PTEN, Gfi-1, Bcl72.3, CK5/6) in 200 EC (including 156 EEC and 13 SC) using a TMA. Seven of the 12 markers (p53, ER, bcl-2, HER-2, p27, E2F1 and PTEN) showed prognostic significance in univariate analysis. The cluster group designation was performed based on eight markers (p53, ER, bcl-2, HER-2, p27, E2F1, PTEN, and p21), and correlated with tumour grade, stage and cell type. Inter-laboratory reproducibility was verified. Reid-Nicholson examined 126 EC (including 42 EEC 1-2, 40 EEC 3, and 24 SC) by using a IHC profile of 5 markers. Substantial immunophenotypic diversity was observed (66). Only 70% of EEC 1 and 2 and 26% of EEC 3 exhibited the typical phenotype of EEC (p16+, ER+, PR+, Mca-, vimentin+). Alkushi (67) also assessed the usefulness of a panel of 6 antibodies (ER, IMP3, p16, p53, PR and PTEN), in a series of 180 EC. A subgroup of 58 high grade carcinomas was also studied, which included 34 EEC 3, and 15 SC. They found that p16, PTEN and IMP3 were statistically significantly more frequently expressed in SC in comparison with EEC 3, while ER and p53, approached but did not reach significance. A combination of p16 and PTEN predicted EEC 3 versus SC with a sensitivity of 90.0%, and specificity of 96.8%. Han and coworkers (68) assessed the inter-observer agreement on histological type in high grade EC, by morphology, but also by using IHC marker combinations with 6 routine (ER, PR, Ki-67, vimentin) and 6 experimental markers ( PTEN, ARID1A, CTNNB1, IMP3, HNF1B, and TFF3). Consensus about histological type based on morphology reached 72%, but increased to 100% when IHC markers were used. A three panel marker composed of p53, ER and p16, was recommended. Wei (69) reviewed 358 biopsies of EC, and assessed the usefulness of IHC (ER, PR, p53, p16, vimentin, and in some selected cases also beta-catenin, WT-1, HER-2, HMGA-2 and Ki-67) in 41 cases with differing interpretation regarding EEC and NEEC between the referring pathologist and second reviewer. Of the 41 cases, 10 were classified as EEC, 18 as SC, and diagnosis remained indeterminate in 5 cases. Recently, McConehy et al (70) performed target enrichment sequencing on 393 EC, by sequencing nine genes (ARID1A, PPP2R1A, PTEN, PIK3CA, KRAS, CTNNB1, P53, Braf and PPP2R5C), and suggest that the nine-gene panel may be useful as an adjunct to morphological classification of EC. In a recent study, we have used IHC for several proteins, previously shown to be differentially expressed in EEC and SC by Cdnna and protein analysis. As a result, we identified nine conditions that allowed prediction of EEC (IMp3>=2, IMp2>=15, p53>=20, HMGA2>=30, FolR1>=50, p16>=170, CycEl>=720, nuclear PTEN>=2, and ER<=50). The performance of this signature was remarkably solid, with good good interobserver agreement (71).

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In the past, four different pathogenetic theories have been proposed for this disease: the collision theory suggests that the spindle cell component only rarely resembles leiomyosarcoma or low-grade endometrial stromal sarcoma. The homologous stromal component is the preferred term in the ISGP/WHO classification for these tumors despite recent evidence indicating that they have features more in common with carcinomas than sarcomas, so the term MMMT is widely used in literature. By definition, these are neoplasms composed of an admixture of malignant epithelial and mesenchymal components. Both the components may show a wide pattern of differentiation: the carcinomatous component may have endometrioid, serous, undifferentiated or clear cell morphology while the sarcomatous component may show diverse histotypes resembling both typical uterine sarcomas such as leiomyosarcoma and endometrial stromal sarcoma(homologous) and other soft tissues tumors (heterologous) such as chondrosarcoma, liposarcoma, osteosarcoma.

Pathogenesis

In the past, four different pathogenetic theories have been proposed for this disease: the collision theory suggests that the carcinoma and sarcoma are two independent neoplasms; the combination theory suggests that both components are derived from a single stem cell which undergoes divergent differentiation early in the evolution of the tumour; the composition theory suggests that the sarcomatous element derives from the carcinoma during the evolution of the tumour; the three theories share that the spindle cell component is a pseudosarcomatous stromal reaction to the presence of the carcinoma. Recent studies have shown that uterine carcinosarcoma should be regarded as a metaplastic carcinoma: indeed, the carcinomatous component is considered the ‘driving force’ of the disease, being the most frequently found element in tumor-involved lymph-vascular spaces, metastatic lesions, and representing, above all, the major determinant of clinical outcome. The emergence of sarcomatous elements would therefore represent the evolution of subclones arising within an aggressive, poorly differentiated endometrial carcinoma with endometrioid, serous or clear cell histology. Even in metastases, endometrial carcinoma can progress to carcinosarcoma. All these findings seem to support the conversion theory which considers MMMT a particular subtype of endometrial carcinoma rather than a sarcoma. MMMT should therefore be included in the Type II subgroup having a p53-mediated pathogenesis, an aggressive behavior and adverse prognosis. Recently Biscuola et al. found 23 mutations in 9 different oncogenes in 44% of MMMTs; PP13K/AKT was the most common altered pathway, present in 32% of tumors.

Risk Factors

The risk factors for MMMT have been difficult to determine as robust epidemiologic studies have not been done due to the low prevalence of the disease. That MMMTs may share risk factors (body weight, exogenous estrogen use, and nulliparity) with endometrial carcinoma. Tamoxifen therapy has also been noted as a possible contributor to the development of MMMTs. MMMTs, along with high-grade endometrial carcinomas, have been reported to arise in patients previously treated with pelvic irradiation for rectal or cervical carcinomas.

Gross Appearance

MMMTs are frequently polypoid with fleshy cut surface and usually fill the entire endometrial cavity. Generally they are big tumors and they often protrude through the cervical os, simulating a cervical neoplasm. The protruding tip of the mass can be necrotic, making diagnosis based on biopsy of this portion of the tumor difficult. The tumors are variably soft to firm and tan with vast areas of necrosis and hemorrhage.

Microscopic Appearance

MMMTs are composed of an admixture of histologically malignant epithelial and mesenchymal components but the epithelial component of MMMT is frequently difficult to subclassify. It is still debated what histological type of carcinoma is the most common, but in almost cases it is an high grade carcinoma including endometrioid, serous, clear cell, mucinous, squamous, and mesonephric carcinoma. Approximately half the cases demonstrate a homologous stromal component, usually a nonspecific highgrade sarcoma resembling an undifferentiated endometrial sarcoma. The homologous stromal component only rarely resembles leiomyosarcoma or low-grade endometrial stromal sarcoma. When heterologous elements are present, rhabdomyosarcoma and chondrosarcoma are the most common types encountered. Some MMMTs are composed of cells that contain cytoplasmic eosinophilic globules that should not be misinterpreted as evidence of rhabdomyoblastic differentiation. Uncommon to rare components include adenosarcomalike foci, neuroectodermal (including glial) tissue, yolk sac tumor, malignant rhabdoid tumor, and melanocyt.
**Immunostains**

Immunostains are rarely needed for diagnosis, but can highlight both components. Carcinomatous elements typically stain for epithelial markers (CK, EMA), vimentin (like most endometrial adenocarcinomas), and in some cases, CD10. The sarcomatous elements typically stain for vimentin, CD10, CD34, and often actin and/or desmin. Focal positivity for epithelial markers can occur in the sarcomatous areas, in some cases likely representing isolated carcinomatous elements. Myogenin and MyoD1 facilitate recognition of rhabdomyosarcoma and S-100 can highlight foci of chondrosarcoma and liposarcoma. Usually p16 and p53 staining in both the carcinomatous and sarcomatous component in most MMMTs, the p16 Strong expression of VEGF, her2, WT1, and EGFR are common in MMMTs, potentially allowing for targeted treatment. c-kit expression in MMMTs has varied widely among studies (from 0–83%); improved progression-free survival in one study. ER and PR expression is uncommon and if present is usually within the epithelial component.

**Differential Diagnosis**

Monophasic tumors should not be diagnosed as a MMMT, although it is acknowledged that either the mesenchymal or epithelial components of MMMT might predominate in small samples such as biopsies and scant curettage specimens. For these reason a wide sampling is recommended. The recently described dedifferentiated endometrial carcinoma is an example of a biphasic tumor containing differentiated and undifferentiated components. However, unlike MMMTs the differentiated carcinoma is usually well-differentiated endometrioid and the undifferentiated component is composed of small, tightly packed, round cells of uniform size instead of spindle shaped or obviously pleomorphic cells often separated by intercellular substance.

Another relevant differential diagnosis is represented by Müllerian adenocarcinoma. Unlike MMMTs, these tumors have a pure or predominantly benign glandular component and a usually low-grade sarcomatous component that forms periglandular cuffs and intraglandular projections. Adenosarcomas with sarcomatous overgrowth often have a high-grade sarcomatous component but foci of typical adenocarcinoma are also present.

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**VECCHI E NUOVI FATTORI PROGNOSTICI NEL CARCINOMA ENDOMETRIALE: LVI SI E LINFONODO SENTINELLA**

I. Montagnani

**SESSONE II**

Moderatori: Luigi Coppola (Roma) – Giovanni Negri (Bolzano)

**I TUMORI OVARIICI A CELLULE TRANSIZIONALI: UN’ENTITÀ IN ESTINZIONE?**

N. S. Losito

Transitional Cell Ovarian Tumours are characterized, by definition (WHO 2003), by histologic resemblance to urethelium and its neoplasms. Traditionally, they included two categories: Brenner Tumours and Transitional Cell Carcinomas (non-Brenner-type). This distinction appeared in 1999 WHO classification and was retained in 2003 WHO classification. Every pathologist knows the morphologic criteria to distinguish benign from borderline from malignant Brenner Tumours: entity of epithelial proliferation, presence of stromal invasion, grade of nuclear atypia, number of mitoses. Anyway, any entity reminds to the correspondent urethelial neoplasm.

But, do Brenner Tumours show true urethelial differentiation? In the years, many studies have demonstrated immunostchemical similarity between Brenner Tumours and bladder urethelial neoplasms (expression in Brenner Tumours of ureplaksins, thrombomodulin, p63, S100P and GATA 3). On the other hand, Transitional Cell Carcinomas (non-Brenner-type), notwithstanding morphologically resemblance to urothelial carcinomas, don’t express urethelial markers like ureplaksins and thrombomodulin and intensively stain with WT1, typically expressed in Sierous-papillary Ovarian Adenocarcinoma. Moreover, TCCs are high-grade carcinomas and show mutation and overexpression of p16 and p53. In many papers, transitional growth pattern has been associated to BRCA mutations, endometrioid and sierous-papillary adenocarcinoma, so it should be considered a morphologic variant of sierous-papillary adenocarcinoma and not a true, distinct entity.

For this reason, the new 2014 WHO classification includes only Brenner Tumours among Epithelial Ovarian Tumours. But, a recent study showed a molecularly distinct pathogenesis for Brenner Tumours, for the absence of TERT promote mutations commonly expressed in bladder transitional tumours. So, the question is still opened; anyway, the differential diagnosis between malignant Brenner Tumours and transitional variant of sierous-papillary adenocarcinoma is important for the better prognosis of the first histotype.

**CARCINOMA OVARIICO: CLASSIFICAZIONE CON BRCA1 E IMMUNOSTOIChIMICA**

L. Resta
The histological assessment of activity and inactivity in IBD is still a problem (2,3,4,5). However, notwithstanding this effort, in our opinion some concepts remain still unexplained in particular:

- no observation are made on the exact number of the biopsies necessary for a precise evaluation. In our opinion must be 2 for any segment of the colon and terminal ileum as highlighted in the ECCO statements 4A and 4B: “Pathologist should receive at least two biopsies for each segment of the colon and terminal ileum with a precise identification of the site of biopsies, avoiding all the circumstances that can damage the tissue” (6) and is extremely limited the biopsies only in the rectum as in Mosli paper.

- Fundamental is a correct orientation of the biopsies for a precise evaluation of the different morphological aspects in particular basal plasmocytosis.

- Histological remission in ulcerative colitis (UC) and also in Crohn’s disease (CD) are considered or not a clinical target useful for the gastroenterologists.

- There is a need for a standardized histological scoring system for IBD which must be validated, reliable and reproducible in which subjectivity is reduced to zero.

As indicated in the ECCO Statement 4C, (6) the presence of basal plasma cells in the histological evaluation of colonic mucosa with morphological features suggestive for IBD has a high predictive value for the first diagnosis of IBD [EL 3, RGC] and is considered an important marker especially in the differential diagnosis with other forms of colitis. Moreover, we recently (9) demonstrated that this feature, in addition to the presence of eosinophils intermingled with basal plasma cells in the same anatomical position has an high predictive value for the first diagnosis of IBD and is present in all phases of the disease, either in the active and in the quiescent phases as a marker of an “inflammatory bowel disease”. Thus, we feel that the hypothesized requirement of absence of basal plasma cells in the so called “mucosal healing” of IBD is contradictory, because the presence of basal plasma cells also in this phase of the disease is a sign of the preexistent IBD also Eosinophils, as basal plasma cells, are present in all phases of the disease.

As in the experience of Mosli et al at the end the most important limitation is the subjectivity among the different pathologists.

For this reason, we want to highlight at a glance some concepts on this topic that can be elements for future studies:

- Histologically, the presence or absence of neutrophils in the crypts (with the consequent development of crypt abscesses) and in the lamina propria markers of disease activity. The concept is similar in others inflammatory diseases of the gastrointestinal tract; e.g., in the stomach the presence of neutrophils aggressive on the crypts is the morphological sign of an active gastritis.

- As indicated in many experiences (4, 7,8,11) one of the most important problems is represented by the absence of a validated and correct histopathological evaluation of the colonic mucosa and the subsequent inappropriate use of terms such as “resolving IBD” or “quiescent IBD” as indicative of the so called “mucosal healing in IBD”. In particular, the histological treatment target for UC or CD can induce absence of neutrophils (both in the crypts and the lamina propria); while are problematic to induce the absence of basal plasma cells and/or reduce lamina propria plasma cells to normal (what is normal?) and in similar way for eosinophils.(11)

- In our opinion are necessary some clarifications:

  - The first point is the presence or absence of neutrophils in the crypts (with the consequent development of crypt abscesses) and in the lamina propria markers of disease activity. The concept is similar in others inflammatory diseases of the gastrointestinal tract; e.g., in the stomach the presence of neutrophils aggressive on the crypts is the morphological sign of an active gastritis.

  - As indicated in the ECCO Statement 4C, (6) the presence of basal plasma cells in the histological evaluation of colonic mucosa with morphological features suggestive for IBD has a high predictive value for the first diagnosis of IBD [EL 3, RGC] and is considered an important marker especially in the differential diagnosis with other forms of colitis. Moreover, we recently (9) demonstrated that this feature, in addition to the presence of eosinophils intermingled with basal plasma cells in the same anatomical position has a high predictive value for the first diagnosis of IBD and is present in all phases of the disease, either in the active and in the quiescent phases as a marker of an “inflammatory bowel disease”. Thus, we feel that the hypothesized requirement of absence of basal plasma cells in the so called “mucosal healing” of IBD is contradictory, because the presence of basal plasma cells also in this phase of the disease is a sign of the preexistent IBD also Eosinophils, as basal plasma cells, are present in all phases of the disease.

- As in the experience of Mosli et al at the end the most important limitation is the subjectivity among the different pathologists.

- For this reason, we want to highlight at a glance some concepts on this topic that can be elements for future studies:

  - Histologically, the presence or absence of neutrophils should be considered the hallmark for the differentiation between the active and the quiescent (resolving) phase of the disease, as expression of the efficacy of the therapy (mucosal healing).
pathologist it is necessary to avoid any form of morphological score in the evaluation of colonic mucosa, because, as widely demonstrated in this review also, these are extremely complicated and subjective.

We hope that in the next future the histological “mucosal healing” will be considered as a target for therapy in IBD and as an important endpoint of remission that must be achieved together with clinical, laboratory and endoscopic data.

References


EVOLVING APPROACH AND CLINICAL SIGNIFICANCE OF DETECTING DNA MISMATCH REPAIR DEFICIENCY IN COLORECTAL CARCINOMA

M. Fassan

THE PATHOLOGY OF SCREENING COLONOSCOPY: EPITHELIAL AND MESENCHYMALE LESIONS

C. Langner

IMMUNOHISTOCHEMISTRY IN THE BIOPITIC DIAGNOSIS OF WELL DIFFERENTIATED HEPATOCELLULAR NEOPLASMS

C. Sempoux

FROZEN SECTIONS IN PANCREATIC LESIONS: TRICKS AND TRAPS

G. Zamboni

LIVER METASTASIS OF COLORECTAL CARCINOMA AFTER CHEMOTHERAPY: HISTOLOGICAL REPORT

M. Guido

GENOTYPING AND IMMUNOHISTOCHEMISTRY OF GIST: AN UPDATE

R. Ricci

Pathology Department, Università Cattolica del Sacro Cuore, Roma.

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. The discovery of the occurrence of activating KIT mutations and KIT expression in GISTs opened the way to the unequivocal diagnosis of these tumors and to their successful treatment with imatinib, a tyrosin kinase inhibitor. Since then, research progress revealed molecular GIST triggers alternative to KIT, implying heterogeneous analytic approaches and prognostic expectations. Several targeted therapies, variably specific for each GIST trigger, have been developed or are being investigated. Thus, GISTs eventually revealed a family of diseases rather than a single tumor type. All these events had an unprecedented impact on pathology practice, constituting at the same time a heavy burden and an exciting challenge, ultimately putting pathologists in the spotlight as never before. Hot topics concerning GIST are discussed, including: novel findings in GIST pathogenesis; GIST precursors and related lesions; some recent advances in GIST molecularly targeted therapy; and potential pitfalls in the differential diagnosis of GIST.
ANALISI DELLE MUTAZIONI DI EGFR NEL DNA LIBERO CIRCOLANTE: È PRATICA CLINICA NELLA DIAGNOSTICA DEL CARCINOMA POLMONARE

A. Marchetti

LIQUID BIOPSIES TO MONITOR MOLECULAR HETEROGENEITY AND DRUG RESISTANCE IN COLORECTAL CANCERS

A. Bardelli

When metastatic colorectal cancers are challenged with targeted agents almost invariably a subset of cells insensitive to the drug emerges. As a result, in most instances, targeted therapies are only transiently effective in patients. Strategies to prevent or overcome resistance are therefore essential to design the next generation of clinical trials. How can we overcome the near-certainty of disease recurrence following treatment with targeted agents? To address this question a deeper understanding of the evolutive nature of cancer cells is necessary. We used colorectal cancer (CRC) as a model system to test the hypothesis that by understanding tumor’s evolution the emergence of drug resistance can be controlled. We find that clonal dynamics can be monitored in real time in the blood of patients, and liquid biopsies can be used to intercept the emergence of resistant clones before relapses are clinically manifest. We discovered that a multistep clonal evolution process driven by progressive increases in drug fitness underlies the development of resistance in cells and patient avatars. To have long-term efficacy, the use of targeted therapies must take into account the continuous evolution of cancer cells, that is to say, therapies must adapt to tumor evolution. One possibility is to anticipate the changes the tumors will make. For example, we propose to use liquid biopsies to know early the mechanisms of resistance to EGFR blockade in individual patients, and devised further rounds of therapy accordingly.

ANALISI PREDITTIVE SU CELLULE TUMORALI CIRCOLANTI: A CHE PUNTO SIAMO?

P. Gazzaniga

Although a predictive biomarker, in the molecular age of personalized medicine, should be defined as one the presence of which is required for the treatment to work, still confusion is engendered by the non-appropriate use of the term “prognostic” and “predictive when referred to a biomarker. A clear example is provided by circulating tumor cells (CTCs) enumeration, for which the attribute “predictive biomarker” has been widely confused with their prognostic information. More recently, the implementation of molecular and genomic characterization of CTCs has largely contribute to improve the treatment selection for a more personalized treatment approach. The characterization of AR-V7 as a biomarker to guide treatment selection in prostate cancer, as well as the presence of PD-L1 as a biomarker of resistance to immunotherapy in lung cancer are two examples of how CTCs are slowly moving from being a mere prognostic to a predictive biomarker.

TUMOR EDUCATED PLATELETS

Nik Sol1,2,3, Myron G. Best1,2,4, Sjors G.J.G. In ’t Veld2,3, Adrienne Vancura2,3, Aniko V. Fejes2,3,5, François Rustenburg2,3, Pepijn Schellen2,3, Heleen Verschueren2,3,6, Edward Post2,3,6, Laurine E. Wedekind2,3, Mirte Muller7, Anna-Larissa N. Niemeijer8, Cyra Leurs1,9, Tessa Y. Le Large10, Jihane Tannous11, Hanne Meijers-Heijboer12, Geert Kazemier10, Elisa Giovannetti13, Jaap C. Reijneveld1,3, Sander Idema1, Joep Killestein1,9, Christine Mannhalter1, Harm-Jan Bogaard1, David P. Noske2,3, W. Peter Vandertop2,3, Daan van den Broek14, Bauke Ylstra1, R. Jonas A. Nilsson16, Rafael Rosell15, Adrianus J. de Langen4, Egbert F. Smit7,8, Michel M. van den Heuvel7, Pieter Wesseling1,9,16, Bakhos A. Tannous13, Thomas Wurdingen2,3,6,13
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Blood platelets are well known for their role in hemostasis. Recently, it has been shown that they play a major role in the immune system and several diseases including cancer. Blood platelets act as local and systemic responders during tumorigenesis and cancer metastasis, thereby being exposed to tumor-mediated platelet education, and resulting in altered platelet behavior. Blood-based liquid biopsies, including these tumor-educated blood platelets (TEPs), have emerged as promising biomarker sources for non-invasive detection of cancer and therapy selection. In our work we have analyzed over 1000 platelet mRNA samples from different kinds of cancer and diseases. Selecting different gene panels, makes it possible to separate healthy individuals from patients, pinpoint the type of cancer, find the mutational background of a tumor, predict treatment response and monitor patients. All from a single blood tube.
This development of molecular genetics from a technique specific to study of prenatal conditions or diseases into an instrument to investigate pathologies of the patient throughout the whole life brings the TSLB to face new challenges and acquire new skills in order to apply new techniques to these ever diagnostic technologies.
TUMORS OF THE HEART AND GREAT ARTERIES: DIAGNOSTIC APPROACH

S. Rizzo, C. Basso
Department of Cardiac, Thoracic and Vascular Sciences, University of Padua Medical School, Padua, Italy

Cardiac masses are rare and usually non-neoplastic. The most likely aetiology of a cardiac mass is a thrombus or vegetation in patients with predisposing factors (atrial fibrillation, native valve disease, prosthetic valves, intracardiac devices, hypercoagulable states). Cardiac tumors are mostly secondary (20 times more frequent). Primary cardiac tumors are benign in up to 90% of cases. Myxomas and papillary fibroelastomas are the most common benign cardiac tumors in adults, fibromas and rhabdomyomas in the paediatric age. Primary malignant neoplasms are represented mainly by sarcomas followed by lymphomas. Diagnostic approach to cardiac masses requires careful evaluation of the clinical setting, including past medical history, age at presentation, location in the heart, histology based likelihood and imaging features. Cardiac noninvasive imaging through echocardiography, magnetic resonance and computed tomography easily detects heart masses. However, histology of percutaneous or surgical biopsy is still the diagnostic gold standard to differentiate and characterize cardiac masses and to plan the best treatment.

Sabato, 26 novembre 2016
Aula Tramontana – 08:00 - 12:00

NEUROPATOLOGIA
Hot topics in neuropatologica: novità introdotte dalla classificazione WHO 2016
Moderatori: Felice Giangaspero (Roma) – Gianluca Marucci (Bologna)

INTRODUZIONE CON PUNTI SALIENTI DELLA NUOVA CLASSIFICAZIONE WHO 2016 E DISCUSSIONE DI CASI CLINICI PERTINENTI
F. Giangaspero

TUMORI EMBRIONALI
M. Antonelli

EPILEPSY-ASSOCIATED TUMORS: CONFIRMATIONS AND NOVELTIES
G. Marucci
Department of Neuropathology, IRCCS Foundation, Neurological Institute “C. Besta”, Milan, Italy

Epilepsy-associated tumors (EATs) represent a common pathologic substrate in the setting of medically intractable chronic epilepsy. These tumors most commonly arise in the temporal lobe, mainly in the tempo-ro-anterior-basal-mesial site. The spectrum of EATs encompasses an increasingly wide variety of lesions, e.g. glioneuronal tumors (ganglioglioma [GG], dysembryoplastic neuroepithelial tumor [Dnet], papillary glioneuronal tumor) and glial tumors (pilocytic astrocytoma, pleomorphic xanthoastrocytoma [PXA], diffuse astrocytoma, oligodendroglioma, angiocentric glioma). Moreover, compound EATs (i.e., GG + PXA, GG + Dnet) have been observed. EATs are frequently a cause of focal epilepsy, especially in children and young adults, with epilepsy as the primary and almost always dominant symptom. For this group of tumors the acronym “LEATs” (long term epilepsy associated tumors) has been proposed. By definition, the adjective “long term” means that drug resistant seizures have lasted for 2 years or more. The biological behavior of these tumors is not completely understood, but it is well known that tumors like PXA or diffuse gliomas tend to recur and may become high-grade gliomas. Recently, the knowledge about molecular features of EATs has rapidly improved and, at the same time, the molecular alterations that characterize diffuse gliomas have been recognized as important diagnostic, prognostic and predictive factors. These neoplasms, particularly GG and Dnet, may be associated with malformations of cortical development, in particular focal cortical dysplasia [FCD] (30–80% of cases). The most recent internationally adopted FCD classification introduced the group of FCD type IIIb, identifying cases of cortical lamination abnormalities adjacent to a glial or glioneuronal tumor. An essential task would be the identification of those EATs with a higher propensity for recurrence or even malignant progression, and also the characterization of molecular features associated with risk of recurrence and anaplastic progression may be very helpful. It has been recently evidenced that somatic activating mutations in mTOR are associated with different epileptogenic conditions under the term of ‘mTORopathies’, such as GG, tuberous sclerosis, FCD and hemimegalencephaly. Advances in morphological and biomolecular knowledge allow more precise histological definition and a better comprehension of oncological behavior, also considering that today these lesions are early recognized and operated on, and a long-term epilepsy history (that in the past actively contribute to characterize these lesions) is more and more frequently absent.

EPENDIMOMI
P. Nozza

NEOPLASIE MENINGEE: NUOVI ASPETTI CLASSIFICATIVI E MOLECOLARI
V. Barresi

GENETIC AND PREDISPOSITION TO DEVELOP BRAIN TUMORS
P.L. Poliani
Primary malignant brain tumors are rare but extremely aggressive lesions and despite modern advances in the understanding of their biology prognosis remains poor with surgery, ra-
diation and chemotherapy options being often only palliative leading to incomplete cure of the tumor and carrying long lasting morbidity for patients. Early detection of the tumor is extremely important to reduce brain tissue damage and to increase success of treatment and patient survival. Albeit their etiology is still unknown, improvements in genetics and DNA screening allowed to identify a wide range of brain tumor associated gene alterations that also helped to better understand the genetic risk factors predisposing to the develop of brain tumors. Patients harboring a germline mutation in a cancer-related gene will be also predisposed to develop brain tumors. Moreover, genome-wide association studies have successfully identified a large number of single-nucleotide polymorphisms influencing glioma risk whose frequency could be either independent of tumor genetic profile or associated with genetic profiles strictly related to specific molecular pathways involved in brain tumor development. Inherited variants in chromosomal regions have been also shown to be associated with increased risk for brain tumors. In this presentation, it will be discussed and reviewed the available literature on the most common predisposition disorders carrying a high lifetime risk to the development of brain tumors, such as neurofibromatosis type 1 and 2, schwannomatosis, tuberous sclerosis complex, von Hippel-Lindau, Li-Fraumeni, Gorlin and Turcot syndromes. Recent findings of genetic risk for brain tumor development and newly identified glioma related gene alterations will be also discussed. Tumor predisposition syndromes are rare but raise important diagnostic, clinical and psychological challenges to both clinicians and families. Effective and comprehensive surveillance schedules for mutation carriers may be extremely useful to the early detection of the tumor with survival benefit.

UPDATE SUI TUMORI DEL SISTEMA NERVOSO PERIFERICO

S. Rossi
La diagnosi dei tumori maligni delle guaine nervose periferiche (MPNST) al di fuori del contesto della Neurofibromatosi di tipo 1 ha da sempre rappresentato una sfida per il patologo. In particolare, la diagnosi differenziale include da un lato altri tumori neuroectodermici come lo schwannoma cellulare, il neurofibroma atipico ed il melanoma a cellule fusate metastatico e dall'altro le neoplasie mesenchimali maligne a cellule fusate, prime tra tutti il sarcoma sinoviale ed il tumore fibroso solitario maligno. La recente comprensione dei meccanismi molecolari che sottendono alla biologia degli MPNST, fornisce dei preziosi marcatori immunoistochemici che possono essere utilizzati dal patologo per la diagnosi differenziale, come la perdita di espressione dell'istone H3.3 trimetilato in corrispondenza della K27 (H3.3K27me3), la perdita di espressione di p16, la ridotta espressione di SOX10 e della porzione C-terminale della neurofibromina (clone NFC). Per quanto riguarda le neoplasie benigne del sistema nervoso periferico, la pubblicazione di serie recenti ha messo a fuoco le caratteristiche morfologiche delle varianti più rari dello schwannoma, come lo schwannoma cellulare, lo schwannoma melanotico, lo schwannoma epitelioide e reticolare/microcisti, ognuno con il suo spettro di diagnosi differenziali. Inclusi come entità a sé stante nella nuova classificazione WHO 2016, sono i tumori ibridi delle guaine nervose periferiche.

Sabato, 26 novembre 2016
Aula Scirocco – 08:00 - 12:00

PATOLOGIA PEDIATRICA
L'adolescente: la nuova terra di mezzo

Moderatori: Emanuele Stefano Giovanni d’Amore (Vicenza) – Angela Rita Sementa (Genova)

HEPATOCELLULAR ADENOMAS IN CHILDREN AND ADOLESCENTS: ASPECTS CLASSIFYING AND CLINICAL REFLECTIONS

P. Francalanci, F. Callea, I. Giovannoni, R. Boldrini
Anatomia Patologica - Osp. Pediatrico Bambino Gesù - Roma

Hepatocellular adenomas (HCA) are rare benign tumor for long time considered a homogeneous entity. The majority of HCA occurs in fertile women taking oral contraceptive (OC), however HCA can also be found in pediatric age and aged persons, in women not taking OC and in men. Molecular biology and immunohistochemistry have demonstrated that HCA is a heterogeneous tumor. Genotype classification has allowed the identification of 3 subtypes: 1) HNF1A-mutated HCA, 2) CTNNB1 mutated HCA and 3) IL6ST-STAT-JAC-mutated inflammatory HCA. HCAs without any mutation and any specific marker are defined unclassified HCA. Phenotypic classification applies immunohistochemical markers derived from molecular characterization: lost of expression of liver fatty acid binding protein (LFABP), product of HNF1A; nuclear β-catenin expression in case of CTNNB1 mutation and overexpression of serum amyloid A and C-reactive protein in hepatocytes of inflammatory HCA. The aim of the genotype-phenotype classification of HCA is to better characterize the natural history of the different subtypes of HCA in terms of growth, bleeding, malignant transformation and familial counseling.

TRANSLOCATION CARCINOMA RENALE

P. Collini

SPITZOID NEOPLASMS: DIAGNOSTIC PROBLEMS WITH MORFOLOGY, IMMUNOISTOCHEMISTRY AND GENETIC ALTERATIONS

C. Urso
SOS Anatomia Patologica di Firenze - Ospedale S. Maria Annunziata - Ospedale S. Giovanni di Dio – Firenze - Azienda USL Toscana centro

Spitzoid neoplasms are quite common in pediatric patients. These lesions may pose significant diagnostic problems, because in a fraction of them it is quite difficult or impossible to establish if they are benign or malignant lesions. An extraordinarily large number of studies have been made in attempts to solve the problem of the differential diagnosis of spitzoid neoplasms [1]. Regrettably, the proposed histological criteria have often proven to be ineffective in making this distinction
with confidence, and the concordance of dermatopathologists is excessively low [1-2]. Moreover, immunohistochemistry and other special techniques, including polymerase chain reaction (PCR) and in situ hybridization (ISH), have proven to be either totally ineffective for the diagnosis or useful just as complementary tools [3]. Finally, genetic studies, including analysis of gene mutations, fluorescence in situ hybridization (FISH) analysis, array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) [4-7], are useful, but do not still play more than an ancillary role, often failing to achieve a consistent distinction between malignant from benign forms [8-9]. The reasons for the diagnostic failure are not clear but they may be related to the current classification. Spitzoid neoplasms are classified as Spitz naevi and spitzoid melanomas; additionally, it is (not universally) recognised a poorly defined category of lesions, labeled as atypical Spitz tumours (ASTs), variously regarded, as intermediate, borderline, ambiguous, problematic, uncertain, undetermined or low-grade tumours [7-9]. It has been hypothesized that spitzoid neoplasms may have been regarded by a wrong or inadequate perspective [10]; the existence of a poorly understood diagnostic category, such as ASTs, would seem to reinforce this hypothesis. It is probable that, without better understanding ASTs, the entire category of spitzoid neoplasms cannot be fully understood. The analysis of available data and a rational review of old and new concepts may suggest a different representation that may help in overcoming the difficulty in diagnosis of these tumors [11].

References


TRAPS OF ADOLESCENT TESTIS

R. Boldrini

Germ cell tumors are the most common testicular tumors accounting approximately for 95% of all testicular tumors. They represent a vast spectrum of tumors of different malignant potential.

As treatment varies by histologic type, accurate diagnosis of these neoplasias is critical.

The 2004 WHO classification classified germ cell tumors into seminomas and non-seminomatous tumors. The upcoming WHO 2016 classification is based on germ cell tumors biological behavior, distinguishing post-puberual tumors that are aggressive and typically associated with in situ germ cell neoplasia and cytogenetic anomalies, and pre-puberual tumors that are benign and lack association with in situ neoplasia and cytogenetic aberrations.

Because of possible histologic al overlaps of germ cell tumors differential diagnoses are discussed and the importance of the use of immunohistochemistry profiles is emphasized. Although classic seminoma may be an easy diagnosis, sometimes, especially when improperly fixed, the neoplastic cells lack the well defined cell borders and nuclear morphology and display microcystic or glandular patterns mimicking those of yolk sac tumor.

Yolk sac tumor has a wide variety of histologic patterns but may be difficult to recognize when the tumor has myxoid or microcystic features.

The proportion of embryonal carcinoma in mixed germ cell tumors can influence treatment decisions thus recognizing morphology is critical: immunohistochemistry can be extremely helpful in this regard.

Germ cell tumors, particularly seminoma, show architectural growth patterns that may overlap with sex-cord stromal tumors: the most common difficulty is with malignant Sertoli cell tumor which may show predominantly solid or nested growth patterns. Immunohistochemical markers for seminoma (OCT3/4, SALL 4) are negative in sex cord stromal tumors, whereas inhibin an calretinin are positive in these tumors and negative in seminoma.

Post-puberual germ cell tumors of the testis differ from the pre-puberual one for biological behavior and cytogenetic aspects. A correct diagnosis is crucial to assure the most appropriate treatment.

PATOLOGIA INFIAMMATORIA DEL GROSSO INTESTINO

C. Mescoli

IMMUNOMARKERS FETALI: APPLICABILITÀ DIAGNOSTICA IN ONCOEMATOLOGIA PEDIATRICA

G. Magro

NEOPLASIE SURRENALICHE

R. R. de Krijger
The term “small round blue cell tumor” (SRBCT) is a morphological encompassing an heterogeneous group of neoplasms, often highly aggressive, frequent in childhood and adolescence. Their morphologic hallmark is a mononuclear proliferation of small cells with nuclei two to three times larger than a red blood cell and scant cytoplasm. This denomination is classically associated with some peculiar entities such as neuroblastoma, Wilms tumor, lymphomas, small cell carcinoma, small cell melanoma, and some sarcomas. Since this term represents a working diagnosis rather than a real one, several clinical, radiological, morphological, immunophenotypical and molecular features are deployed to reach the diagnosis. A precise histological diagnosis is of paramount importance since different and distinct effective therapies can be applied.

Age is very helpful in approaching these patients since some neoplasms are typical of the younger ages (neuroblastoma, rhabdomyosarcoma, Ewing and Ewing-like sarcomas, desmoplastic small round cell tumor, poorly differentiated synovial sarcoma, small cell osteosarcoma) whereas other are more common in adults (melanoma, small cell carcinoma, Merkel cell carcinoma). Also the clinical presentation and imaging are helpful in the differential diagnosis. Though, sometimes they can be misleading. A rapid and precise diagnosis is very often required in this group of neoplasms.

Here we report a case of a 4-year-old female presenting with rapidly worsening ascites. A computed tomography scan showed a large mass extending from the right lung hylus through the diaphragm, and superficially growing around the liver and involving the peritoneum. Clinical presentation, age and imaging were suggestive of an high grade lymphoma, likely Burkitt’s lymphoma, hence ascites fluid and cerebrospinal fluid (CSF) were immediately gathered and a trucut biopsy of the peritoneal mass (16G), bone marrow (BM) aspirates and biopsies were performed and sent to the pathologist with the request of a rapid diagnosis, since the worsening conditions of the baby. On day two the small patient progressed with bilateral pleural fluid. A cytological diagnosis of SRBCT was made on the smears of ascites fluid; CSF and BM aspirate were negative. Cytofluorimetry and a fluorescent in situ hybridization (looking for cMYC break-apart) were urgently performed in order to confirm the clinical diagnosis of Burkitt’s lymphoma, but they were both not diagnostic of lymphoma. On day three, in the early morning the slide of the trucut biopsy was available and immediately examined. A small round blue cell proliferation was present, showing the focal presence of more spindled cells and a myxoid stroma. Mitotic index was 2/10 high power fields and necrosis was focally present. Based mainly on the presence of spindle cells and the low mitotic index the diagnosis of Burkitt’s lymphoma was discarded. An urgent panel of immunohistochemistry was performed. On the bases of morphology and a diffuse desmin and myogenin positivity together with negativity for CD45, Hu, NB84a, WT1(180), and CD99 and only focal weak positivity for WT1(C19), a diagnosis of embryonal rhabdomyosarcoma was performed on day three and chemotherapy started accordingly (EpSSG RMS 2005).

The disease is in partial remission and the patient is alive with disease at 7 months of follow-up.

Small round blue cell tumor is a descriptive diagnosis encompassing different entities, often highly aggressive, that deserve different therapies. A fast integration of clinical, imaging, morphological, immunohistochemical and molecular data, with the coordinated efforts of all the actors of the play, is mandatory to reach a rapid, meaningful and effective diagnosis.

Acknowledgements
We thank the ‘Associazione Bianca Garavaglia’ for their support in diagnostic equipment and personnel.

RENAL CELL CARCINOMA IN PEDIATRIC AGES: WHO 2016 CLASSIFICATION APPLIED TO 42 CASES IN CHILDREN UP 18 YEARS OF AGE ENROLLED IN THE ITALIAN PROTOCOLS DURING A 23-YEAR PERIOD.


From the Department of Diagnostic Pathology and Laboratory Medicine, the Pediatric Oncology Unit of Fondazione IRCCS Istituto Nazionale dei Tumori of Milan, the AIEOP TW2003 and SIOP Protocols, TREP Project, and all the Pathologists of the AIEOP and SIOP Renal Tumour Study Group

Background
Renal cell carcinomas (RCCs) are rare tumors in children (patients <18 years). Their annual incidence is very low, being more common in adolescents (11-14 years of age). The clinical presentation of pediatric cases is represented by signes and symptoms such as pain, abdominal mass and haematuria. Diagnostic imaging uses techniques such as ultrasonography, complemented by CT or less commonly MRI techniques. It’s possible to evaluate tumor size, renal vein thrombosis, and extent of disease (locoregional lymph nodes or distant metastases). For a better definition of metastatic disease, the CT / PET or bone scans are applied. There are no standardized treatment protocols for RCC in children. Treatment recommendations are based on small retrospective case series or case reports, or taken by the guidelines for RCC of adults. Surgery, when possible, is the treatment of choice and is curative if the disease is localized and totally excised. Radiotherapy and immunotherapy treatments do not have a clear role and even multidrug chemotherapy treatments show only a small activity in clinical trials.

The 5-year survival is better in children than in adults (50-70% compared with 40% of adults), and the most important prognostic factor is the stage.

From the pathological standpoint, pediatric RCCs have distinctive characteristics: many varieties have papillary features and about half of the cases are RCCs with translocation of the gene family of transcription factors associated with microphthalmia (MiTF) (rRCC).
**Aims.** Study aims are a better definition of morphology, immunohistochemical profile and the molecular alterations of the pediatric RCCs, reclassifying the various entities under the criteria of the new WHO classification of 2016, for a large part based on the Vancouver 2012 classification.

We also correlate the data of the immunohistochemical expression of TFE3 with the FISH analysis results to assess the reliability of TFE3 immunohistochemistry.

Another aim is to evaluate the role and representativeness of tissue microarrays (TMAs) in the study of this type of tumors. Furthermore, we performed a correlation between the clinical and pathological data to better understand the trend of the disease and to open new scenarios on the development of possible biological therapeutic targets.

**Materials and methods**

We collected 42 cases sent by various Italian centers participating in the 1992 Italian CNR Protocol, in the Protocol AIEOP / TW2003, in the SIOP 2001 Protocol, and in the TREP project of the Italian Association of Pediatric Hematology and Oncology (AIEOP).

On all cases, FISH analysis for translocation of TFE3 gene (Xp11.2) and immunohistochemistry using a panel of predetermined antibodies were carried out to better characterize tumors.

**Results**

In our series there were 42 cases with a M / F ratio of 20/22 and with a median age of 11 years (range 9 months-17 years). After FISH analysis, 20 cases were classified as tRCCs and 22 cases as non-tRCC. All translocated cases (tRCC) showed immunohistochemical expression of TFE3; 8 non-tRCC cases were positive for TFE3 immunohistochemistry.

tRCC showed a variable expression of vimentin and carbonic anhydrase IX, while they had a decreased expression of cytokeratins compared to non-tRCCs. In addition, 2 translocated cases showed positivity for HMB45.

3 cases with translocation and 3 cases without translocation showed focal positivity against succinic dehydrogenase beta subunit (SDHB), while a case without translocation was negative.

2 not translocated cases, one classified as type 1 papillary RCC and one classified as unclassified RCC, showed positive immunohistochemistry for ALK, and ALK gene rearrangement was confirmed by FISH analysis.

1 non-translocated case showed focal positivity for melanoma antigens.

The cases were reclassified as follows: 20 RCC with translocation of TFE3, 22 cases of RCC without translocation including 8 papillary carcinomas, 2 clear cell carcinomas, 1 collecting duct carcinoma, 11 unclassified carcinomas. These 11 cases were further analyzed by molecular biology techniques for a better definition of the histotype.

The creation of TMA was a good surrogate for translocated cases, while it was of not completely satisfactory for non-translocated cases where positivity for some antibodies was focal and TMA was therefore not representative.

In 17 cases, clinical data were collected and correlated with pathological and molecular data.

**Acknowledgments**

This study was done with the support of the ‘Associazione Bianca Garavaglia’ and ‘5x1000 MIUR 2012’

**References**


the total number of renal and liver transplantations reached 376 cases in the two project centers. The digital pathology and its proper and timely application to build a telepathology network, are expected to improve significantly the transplantation workflow. Firstable it will allow the real time second opinion between pathologists in order to assess the suitability of the donor organ, avoiding the slide transfer, potential damage or loss. Our technical partners delivered two slide scanners, the software solutions to enable the virtual microscopy and the web-based digital slide sharing and storage resources. In addition, the project comprises an online survey which focuses on the accountability of the system, the user perception and the concordance study for the entire project outcomes evaluation.

REQUISITI MINIMI ISTOLOGICI DELLA VALUTAZIONE DELLA BIOPSIA EPATICA DURANTE IL PROCESSO DONATIVO

C. Mescoli

TAVOLA ROTONDA

“Requisiti minimi che deve avere una diagnosi frozen nell’attività trapiantologica”

Moderatori: Antonietta D’Errico (Bologna) - Andrea Giannelli Castiglione (Genova)

IPMN: LIMITI DIAGNOSTICI

D. Malvi

RENAL NEOPLASIA DURING ORGAN TRANSPLANTATION

Brunelli M, Martignoni G and Eccher A.
Department of Diagnostics and Public Health, University of Verona.

Intraoperative consultation rarely is requested for lesions in the kidney, however during the processes of organ transplantation the appropriate diagnostics of neoplastic nodules may be required from clinicians and surgeons in order to proceed to graft allocation.

The shortage of donors in the face of the increasing number of patients wait-listed for renal transplantation has prompted several strategies including the use of kidneys with a tumor, whether found by chance on harvesting from a deceased donor or intentionally removed from a living donor and transplanted after excision of the lesion. Current evidence suggests that a solitary well-differentiated renal cell carcinoma, Fuhrman nuclear grade I-II, or with low nucleolar grade (ISUP/WHO 2016) less than 1 cm in diameter and resected before grafting may be considered at minimal risk of recurrence in the recipient who, however, should be informed of the possible risk and consent to receive such a graft (Frascà et al. 2016, J Nephrol). Few studies report findings at frozen sections for diagnostics. The study evaluating at frozen most cases was performed by Krishnan et al. who reported a frozen section intraoperative consultation on 324 renal lesions. 199 specimens were submitted for gross consultation only. The clinical implications and diagnostic pitfalls in 125 specimens submitted for frozen section were the focus of their study. Most frequent requests were “to evaluate surgical margins in partial nephrectomy specimens, solid renal mass in an unusual clinical or radiologic setting, synchronous renal and extrarenal masses, cystic renal lesion, ureteral surgical margins for transitional cell carcinoma, multiple renal masses, solid mass in a diffusely cystic kidney, and renal injury”. Among the 125 cases, the diagnoses were deferred in 17; the frozen section diagnoses were incorrect owing to limited sampling in 5 and misinterpretation in 4 (melanoma vs angiomylipoma, lymphoma vs angiomylipoma, benign cyst vs cystic renal cell carcinoma, metastatic renal cell carcinoma vs pheochromocytoma) (Krishnan et al. Am J Clin Pathol 2003).

Awareness of distinctive indications for frozen section intraoperative consultation and diagnostic pitfalls should improve diagnostic accuracy and facilitate proper management of these lesions.

References

LA DIAGNOSTICA DEI GIST IN FROZEN SECTION DURANTE L’ESPIANTO DI ORGANI

L. Novelli

Sabato, 26 novembre 2016
Aula Maestrale – 08:00 - 12:00

APOF

I giovani patologi oltrefrontiera

SESSIONE I

Moderatori: Stefano Guzzetti (Torino) - Laura Viberti (Torino)

INFORMARE, COINVOLGERE, PARTECIPARE: WEB E SOCIAL NETWORK PER DIFFONDERE LA MISSION DI APOF

L. Viviano

Il primo assioma della comunicazione afferma che non si può non comunicare. Per capire meglio il contenuto della comunicazione di APOF, andiamo ad analizzare uno strumento che abbiamo a disposizione, il web, che sicuramente ci permette di raggiungere un gran numero di persone. Abbiamo sviluppato due grandi canali che sfruttano la rete, ovvero il sito web e la pagina Facebook di APOF. Il sito www.apof.eu è accessibile a
tutti, non solo in italiano, ma anche in inglese e francese. Vengono riportate in breve le notizie più recenti ed i progetti attivi con il link alle relazioni complete. C’è poi la sezione dedicata alle newsletter, che raccoglie tutte le newsletter prodotte da APOF nel corso degli anni. La newsletter è uno strumento agile che utilizza la posta elettronica per far avere a quanti lo desiderano, un aggiornamento sulle iniziative di APOF e sullo stato di avanzamento dei progetti attivi. Abbiamo poi pensato che sarebbe stato interessante rendere il contenuto della vita di APOF, fruibile ad un vasto pubblico non direttamente coinvolto e che, pertanto, non si sarebbe mai mosso per conoscere i patologi oltre frontiera. A tal fine abbiamo rivolto parte della nostra attenzione ai social network. Ciò che emerge dai resoconti dei progetti, visibili sul sito e da post della pagina Facebook, è il vivace dei protagonisti della missione di APOF. Per quanto è stato e continua ad essere il loro contributo allo sviluppo del Sud del Mondo, altrettanto hanno ricevuto dai nuovi amici incontrati per strada. Per permettere a chiunque di poterne beneficiare, è necessario comunicarlo.

SESSIONE II
Il coraggio di partire

Moderatori: Stefano Guzzetti (Torino) - Laura Viberti (Torino)

DIECI ANNI FA: I PRIMI PASSI DELLA TELEPATOLOGIA IN AFRICA

F. Pagni

DIECI ANNI DOPO: PROVE DI TELEPATOLOGIA SOSTENIBILE

L. Molinaro

INSEGNARE IL PAP TEST ALL’EQUATORE

A. Fornari

THE ROLE OF THE LABORATORY TECHNICIAN: APOF’S EXPERIENCE IN PALESTINE

T. Zanin
E-O- Ospedali Galliera, Genova – Italy

The Associazione Patologi Oltre Frontiera (APOF: Association of Pathologists Beyond Borders) is an NGO made up of pathologists, biologists and laboratory technicians. Through the various skills of its members, it promotes projects aimed at fighting cancer by setting up pathology facilities in developing countries. Founded in 1999 by a group of SIAPEC pathologists, the Association has implemented, and is currently undertaking, several projects. One of these is ongoing in Palestine, a controversial territory that needs firm support for its development. In 2005, following a series of contacts with the Province of Venice, the Association was asked to set up a team to carry out maintenance of the technological equipment installed in the Palestinian territories. A small group made up of two doctors, a technician and an assessor from the Province of Venice set off to conduct a fact-finding and feasibility study. The project was to be implemented under the patronage of the Italian government’s International Cooperation programme. During this first mission, the team visited the Beit Jala quarter of Bethlehem, where the government hospital was seen to house a laboratory annexed to a paediatric intensive care unit. The laboratory was endowed with modern equipment, but none of it was working! Following this inspection, the project was modified in order to meet the new needs that had been ascertained; indeed, we were asked to activate the laboratory and to make it run efficiently.

In this phase of the project, a very important role was played by the laboratory technician, whose task was to assess the type of technology available on site, to implement a training programme for local technical staff and to teach the latest immunohistochemical techniques. Over the years, several pathologists and technicians have spent various periods in the hospital in order to render the staff of the Pathology Department perfectly autonomous. With regard to this activity, our thanks go to Elisabetta Petretto, who undertook several missions and made a precious contribution to the success of the project. During one such mission, in 2009, a few organisational problems were encountered; these were finally overcome and the laboratory was rendered clean, efficient and operational.

Two technicians are working in the histopathology service, Adel and Ines, who are efficient and work well together. Soon after our arrival in the hospital, we realised that they were both very willing to listen to us and keen to improve their operational skills. In addition, the pathologist, Dr Riad Shraim, always welcomes our visits enthusiastically and evinces great hopes for the results that our mission can achieve. Dr Shraim is accompanied in the laboratory by another doctor, Dr Ahmed Itmezeh, a pathologist who specialised in Russia and who follows the activity of Dr Shraim in order to develop his own expertise. For three days a week, they are flanked by Dr Yousef Abu Ghosh, whose 60 years of age and vast experience enable him to act as a practical and spiritual guide to his colleagues. The professionalism of Dr Abu Ghosh and his passion for his work struck us immediately. During our stay, we were assisted from Italy by another technician, Stefano Simonazzi, who provided us with an Italian version of the software used to run the automatic equipment for immunohistochemistry, as the English version that had previously been installed was causing serious problems. Indeed, we should underline the importance of “distant collaboration”, in that precious support is provided not only by those who undertake on-site missions, but also by those who make their skills available to the Association while remaining at their own workplace.

During their missions in Bethlehem, the various laboratory technicians drew up methods, procedures and other specifications needed for the efficient running of the laboratory. This is a “snapshot” of what happens in all the APOF projects, in which a variety of skills and competencies are harnessed in order to provide the possibility of a diagnosis for those who would otherwise be left without.
USE OF TELEPATHOLOGY IN CYTOLOGICAL DIAGNOSIS: EXAMPLES OF SOME AFRICAN CASES DIAGNOSED IN REMOTE FROM ITALY

E. Caselli
MD, Resident pathologist, Department of Experimental Medicine, Section of Pathologic Anatomy and Histology - “S. Maria della Misericordia” Hospital, Perugia (Italy)

The Hospital of Mungbere, a small town in the forest of the eastern province of Democratic Republic of Congo, is run by Combonian missionaries and provides health care to a population of over 60,000 people, with 3,000 patients annually hospitalized.

The director, Father Dr. Gian Maria Corbetta, in collaboration with “Pathologists Beyond Borders Association, NGO (APOF)”, planned a training course for cytotechnicians to prepare cytological specimens, identify more significant areas for pathological diagnosis and get static digital images of these areas of slide. These trained cytotechnicians can storage digital images in a web platform, called Sinapto, and pathologists all over the Europe can enter in this database and share observations to realize finally a diagnosis. In Sinapto system it is possible upload a file for every patient and it is possible add clinical data and selected digital images with region of interest (ROI) to allow remote cytopathological diagnosis. A final shared diagnosis between pathologists can be formulated and recorded in Sinapto for every file, improving patient care in Developing Countries.

This system represents an interesting example of telepathology with 190 cases of extravaginal cytology in a period of 27 months (June 2014 – September 2016). This system was used successfully also in specimens with different sampling techniques (fine needle aspiration, imprint cytology and exfoliative cytology).

The aim of our slide seminar is to show static images of some interesting African cases and demonstrate potentiality of telepathology in cytological diagnosis.

USE OF TELEPATHOLOGY HISTOLOGICAL DIAGNOSIS: A SLIDE SEMINAR ON SOME AFRICAN CASES DIAGNOSED IN REMOTE FROM ITALY

E. Prosperi
MD, Resident pathologist, Department of Experimental Medicine – Section of Pathologic Anatomy and Histology, Hospital “S. Maria della Misericordia”, Perugia, Italy

Pathologist Beyond Borders Association (APOF) is an NGO founded in 1999 with the aim to implement projects aimed at the development of pathologic anatomy and diagnostic oncology in developing Countries.

In the Hospital of Mungbere, a town in the Democratic Republic of Congo, trained staff (which includes a doctor and technicians) prepare the histological specimens, providing sampling and preparation of histological slide. Afterward, the personal scans and store digital images of these histological specimens in a cloud platform, called Dropbox. These images are not static, but can be viewed as a “virtual slide” by pathologists from all over the Europe, only with a simple web access. Pathologists allowed entering in the cloud platform could, after share observations and concerns with each other, make a final diagnosis. Cases of difficult interpretation and also random cases are physically sent to Italy as a quality control.

This represents an efficient example of telepathology and was used to make 319 diagnoses in a period of 27 months (June 2014- September 2016).

The aim of this slide seminar is to show some cases of histological diagnosis made using telepathology, with particular regard to Kaposi’s sarcoma, an extremely frequent pathology in Africa.
The study of these mummies allow to advance in the reconstruction of the embalming art by crossing the literary sources with the results carried out by direct observations of the mummies. As a matter of facts, in the Renaissance period the religious embalming becomes a surgical practice. The most part of authors prescribe a round craniotomy and a longitudinal jugular-pubic incision to obtain the total evisceration. Generally, the authors indicate these procedures: total evisceration; stripping of fleshes; baths and washes with brine or wine-spirit, or turpentine; filling and sprinkle the cavities with embalming material; wrapping the corpses with bandages and dressing; deposition in a sarcophagus. The evisceration carried out with a vertical and other transverse incisions seems a typical tradition of Neapolitan Kingdom. This kind of embalming practice is described by Neapolitans Renaissance authors, such as the surgeons Cintio D’amato (XVII sec.) and Marco Aurelio Severino (1580-1656), who suggests that "essendosi tagliato il ventre prima di lungo, et poi di largo cioè pertraverso, si esprimono l’intestini staccate con lo stomaco, i reni, il fegato, et la milsa. Di poi aperto il petto dall’un et dall’altra parte, dove le costi si terminano in cartilagine, si cacciano fuora i membri spiritali, qual sono il cuore, il pulmone, l’esofago, tagliati infino all’epiglottide". Cintio D’amato prescribes to perform a cross incision on the chest and another one on the abdomen, cutting ‘primieramente il ventre prima per lungo, et poi per largo, cioè pertraverso’, for the evisceration.

The great number of artificial mummies, examined by G. Fornaciari and his equipe, allowed to reconstruct the real embalming techniques in the Renaissance, by crossing the data with the medical literature.

**LEONARDO DA VINCI AND THE ANATOMICAL KNOWLEDGE IN HIS TIME**

L. Cataldi

Alumni Cattolica “Ass. L. Necchi”, GSSP della Società Italiana di Pediatrica

Associazione Clemente Sussini perla Storia della Medicina

Leonardo da Vinci has long been recognised as one of the great artists of the Renaissance, but he was also a pioneer in the understanding of human anatomy. He intended to publish his ground-breaking work in a treatise on anatomy, and had he done so his discoveries would have transformed European knowledge of the subject. Author presents the results of his own research performed on the Leonardo’s drawings in the Royal Collection at Windsor Castle, and on a lot of ancient books reporting anatomical studies around the 1500s years, comparing the anatomical knowleges in that time with those obtained on the basis of the Leonardo’s studies.
MOLECULAR IDENTIFICATION AND FACIAL VIRTUAL RECONSTRUCTION OF G.B. MORGAGNI

F. Zampieri, A. Zanatta

Department of Cardiac, Thoracic and Vascular Science, University of Padua, Padua, Italy.

Morgagni died on December 5th 1771, 89 years old, and was buried in Saint Maxim church in Padua, where his wife and five of his 15 children, four daughters and one son, were already buried. In 1868 and 1900 the tomb was opened to identify Morgagni. Between the remains of several adult individuals, two skulls considered of very old persons were identified and replaced in an earthenware jar inside the sepulcher. In 2011 we opened the tomb and found the remains described during the first two identifications, but additionally we found the skulls fragments of three very young individuals which could have been Morgagni’s children. An anthropological analysis confirmed that one of the skulls inside the earthenware jar belonged to the oldest individuals (“senilis”) between those found in the tomb. A genetic analysis proved a kinship between this skull and the fragments of young individuals (one male and two females), supporting the hypothesis that they were Morgagni and his children. In conclusion, thanks to the interaction between historical studies, anthropological research, and molecular analysis that reinforce each other, we can assume that the skull is Giovanni Battista Morgagni’s and that the series of skull fragments are from his children who were buried together with their parents. Having obtained the identification of Morgagni, we performed a forensic facial reconstruction with new 3D technology. The intent was to compare the facial reconstruction with Morgagni’s portraits done when he was living and near to his death, as to be closest to his real resemblances. We performed a superimposition test with busts and portraits, as to achieve a further confirmation of the molecular identification.

References


SESSIONE II

Moderatori: Gino Fornaciari (Pisa) – Ezio Fulcheri (Genova)

NEONATAL AND POSTNATAL MORTALITY IN ROCCEPALEOGO THROUGH THE STUDY OF PARISH RECORDS AND HISTOLOGICAL EVIDENCE

M. Traversari1, C. Figus1, A. Vazzana1, G. Grupponi1, F.M. Galassi2, V.G. Vellone2, E. Fulcheri1

1Laboratori di Antropologia e DNA Antico, Dipartimento di Beni Culturali, Università di Bologna-Campus di Ravenna; 2 Institute of Evolutionary Medicine (IEM), University of Zurich; 3Anatomia Patologica, Dipartimento di Scienze Chirur-}

"richte e Diagnostiche Integrate, Università di Genova

Due to the restoration of the church of the Conversion of St. Paul, located in Roccapelago a small village of the Emilian Apennines, it was found a lost chamber containing the remains of over 300 individuals who lived between the sixteenth and seventeenth century AD. Some of these were natural mumified. Mumification was made by the particular location of the crypt, built on the ruins of the medieval fortress of Roccapelago, equipped with ventilation slots. Simultaneously to archaeological research, were recovered parish records of the church. During the excavation of these skeletal and mumified remains, a considerable amount of disarticulated nonadult skeletal elements was recovered. The nonadults were retrieved also from the same areas of the adults and not only from separate burials, during the excavation. This suggests that no preferential treatment was given to children’s burials, despite the citation of a “grave of the angels”, found in the parish records. Parish registers of death and birth were digitized and organized in into digital matrices. The death records starting from 1578 until 1891. Deaths were recorded through the year of death, name and surname, parents, relatives and spouse, age at death, cause of death, notes, for a total of 2,036 records. The birth records starting from 1593 until 1916. births were recorded through the year of birth, period of conception, name and surname, parents, notes, for a total of 4,585 records. It was also analyzed an osteological sample composed of 76 nonadults (Minimum Number of Individuals or MNI was calculated from the most numerous category of skeletal elements), to verify the correspondence of the documents with anthropological data. Estimation of age at death was based on several methods: level of epiphyseal closure and/or fusion of ossification centres (Scheuer and Black 2000); dental eruption and development (Ubelaker 1989; AlQahtani 2010). Long-bone length was used the age of lower classes. (Maresh, 1955; Fazekas and Kósa, 1978; Black and Scheuer, 1996). The sample was divided into 5 classes of age, according to the table proposed by Scheuer and Black (2000). Pathologies was evaluated through macroscopic examination and compared to reference atlas (Mann and Hunt, 2005; Aufderheide and Rodriguez-Martín, 1998; Ortner 2003). The analysis of parish records showed a high percentage of deaths, referable to the age class 0-1 year, the anthropological analysis of the postcranial skeleton showed a concentration of deaths that goes from 36 weeks up to 1 year, with increased frequency around 38th weeks, corresponding to the perinatal age. Mortality distribution evidence obtained on the base of teeth eruption, gives a different result from the one obtained upon the base of the postcranial skeleton, data based upon dental eruption assure mortality rates between birth and first year of life, with increased frequency around 5th months. An interesting indicator to highlight complications during delivery, were the recordings of “emergency baptisms”. The frequency of this particular type of rite on births with only one born in Roccapelago, is 3.02%. It’s instead of the 13.73% of the administration of emergency baptisms on twin births. The frequent perinatal deaths, show that it was risky the moment of birth, any complications that could arise during the phase of expulsion, could be lethal. Emergency baptism phenomenon is a clear example: none of the twins who received this particular sacrament is survived. The difference shown by two methods of Estimation of age at death, considered the best to clarify the liability of the dental eruption. It may explain a growth delay due to poor living conditions. Further histological analyses will be carried out on the remains in an attempt to better clarify this growth delay.

"richte e Diagnostiche Integrate, Università di Genova

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References

GEORGIOS PAPANICOLAOU AND THE HISTORY OF UTERINE CERVIX SCREENING

V. G. Vellone

DISC-Anatomic Pathology, University of Genova

Since the end of the 19th century, exfoliated cancer cells had been described in all of the types of specimen in which we find them today. However, it was not until Drs. Papanicolaou and Traut published their account of the diagnosis of uterine cancer from exfoliated cells (1941 and 1943) that cytopathology acquired the strength to develop into the powerful presence that it has in human medicine today.

Born to a physician father on May 13, 1883, at Kyme Greece, he received MD from University of Athens in 1904 and served as an assistant surgeon in the military until 1906. After his military service, Dr. Papanicolaou returned to Kymi and reluctantly practiced medicine with his father. He was not interested in clinical medicine, but yearned for a career of scientific research.

In the spring of 1907, Dr. Papanicolaou left for Jena, Germany, to begin his postgraduate studies under Professor Ernst Haeckel, and earned a PhD in zoology from University of Munich in 1910. Returning to Greece, he met and married Andromache Evaliyen, later known as Mrs Mary Pap, who became a pillar of support for him. Prospects of better opportunities brought the couple to US on Oct 19,1913 where he became assistant in Pathology Department of New York Hospital and later in 1914 as assistant in Anatomy at Cornell Medical School.

It was at Cornell where Dr. Papanicolaou worked examining vaginal smears of guinea pigs to determine the existence of a menstrual cycle in them. In 1920, he started studies on human vaginal cells. He observed cancer cells in vaginal smears and realized great potential of this simple test in early diagnosis of cancer.

He introduced this low cost screening test for early detection of cancer at a medical conference in Michigan in 1928, which met skepticism and resistance from medical community.

Diagnostic potential of vaginal smear in early detection of cervical cancer was validated by scientific studies culminating in in publication of famous monograph titled “Diagnosis of Uterine Cancer by Vaginal Smears” in 1943 by Dr Papanicolaou and Dr Herbert Traut. This revolutionary diagnostic test was named Pap smear which has saved lives of millions of women by early diagnosis of cancer of cervix.

Although Georgios Papanicolaou is generally credited for the invention of the cervical cancer screening test by cervical cytology the Romanian physician Aurel Babes was the true pioneer in the cytopathologic diagnosis of cervical cancer. Babes presented his findings to the Romanian Society of Gynaecology in Bucharest on 23 January 1927. His method of cancer diagnosis was published in a French medical journal, Presse Médicale, on 11 April 1928, but it is unlikely that Papanicolaou was aware of it. Furthermore Babes’ method is radically different from Papanicolaou’s method. Differences included the sampling method, the fixation and staining technique, and the interpretation of the results regarding cases of cervical cancer.

The cytopathological examination of the uterine cervix smears according to Papanicolaou was introduced in Italy in the early fifties by the efforts of Professor Mario Tortora in Naples and then in Ferrara

Dr Papanicolau died on February 18, 1962 of heart failure and was buried in New Jersey. This great cytopathologist was honoured by USA in 1978 and by Greece in 1973 and 1978 by releasing commemorative stamps.

PALEOGENOMIC AND ANCIENT DNA

R. Gaeta1, S.R. Tasha2, R.Cano2, G. Fornaciari2

1Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; 2Center for Applications in Biotechnology, California Polytechnic State University, San Luis Obispo, CA, United States of America

Introduction

The DNA is a nucleic acid that contains the genetic information necessary for RNA and protein biosynthesis. DNA extracted from past samples (teeth, bones, faeces, etc.) is defined ancient DNA (aDNA) and needs, to preserve, low level of oxygen, fast decrease in water content and, above all, according to the Arrhenius equation, low temperature. From 1985 (first aDNA extracted from a mummy) with the introduction of the Polymerase Chain Reaction (PCR) several studies of paleogenomic were born, but it is essential to identify some possible errors such as fragmentations, contaminations and post-mortem mutations [1]. The applications of the paleogenomic are: evolutionary biology, population studies, studies of the pathogens and microorganism.

Materials and methods

We report 3 cases studied by the Division of Paleopathology of Pisa:

a) Ferrante I, king of Naples (1431-1494). The natural mummy showed round white formations of the pelvis infiltrating the abdominal wall (1a-b).

b) Maria of Aragon (1503-1568). The well-preserved artificial mummy had a small pedunculate arborescence neof ormation in the right inguinal region (fig. 2a-b).

c) Andean female mummy (so-called ‘Fi9’) dated 10th–11th century AD by radiocarbon analyses. The natural young mummy presented a marked megavisceral syndrome characterized by megacolon, megaesophagus and cardiomegaly (fig. 3a-b).

It was possible to perform complete autopsies and collect tissue samples utilized for histological analyses and DNA extraction.
Results
a) Histology performed on the round formations confirmed the diagnosis of colorectal adenocarcinoma. Amplification of aDNA highlighted a point mutation of the codon 12 in K-Ras oncogene responsible for the cancer [2] (fig. 1c).
b) Macroscopic and histological aspects seemed peculiar of condyloma acuminatum, a papillomavirus-induced squamous lesion also called “venereal wart”. Molecular study revealed the presence of HPV 18, a virus with high oncogenic potential. Automated sequencing of several clones revealed 100% similarity sequences of both HPV 18 and JC9813 DNA, a putative novel HPV with low oncogenic potential [3] (fig. 2c)
c) Analysis of the gut microbiome (paleofeces, descending, transverse and ascending colon) underlined the massive presence of Clostridiceae. Sequences homologous to HPVs in the mummified gut (descending colon) was particularly surprising. It was detected also the Tripanosoma cruzi; by comparing a partial sequence homologous to the large ribosomal subunit alpha of the presumptive ancient T. cruzi with modern strains, we suggest that this pathogen may have a more remote origin than previously expected. We also found sequences associated with putative beta-lactamases, penicillin-binding proteins, resistance to fosfomycin, chloramphenicol, aminoglycosides, macrolides, sulfa, quinolones, tetracyclines and vancomycin, and multi-drug transporters [4] (fig. 3c).

Conclusion
a) The alimentary “environment” of the Neapolitan court of the XV century, with its abundance of natural alimentary alkylating agents (red smoked meat), well explains the acquired mutation of K-Ras.
b) This represented the first molecular diagnosis of HPV in mummies. HPV is a very old virus that evolved together with man.
c) Streptococcus, Staphylococcus, Bacillus and Pseudomonas sequences were identified in the mummified gut, opening the opportunity to investigate possible mechanisms by which these bacteria are preserved. The detection of sequences homologous to those of pathogens such as T. cruzi and HPV indicate their presence in the Americas prior to European colonization. The presence of antibiotic-resistance genes in an 11th century pre-Columbian Andean mummy is intriguing as antibiotics were introduced recently. The presence of beta-lactam antibiotic resistance is certainly not unexpected in any culture, as would be in the case of resistance to any natural rather than a semi- or completely synthetic antibiotic as a result of exposure to natural antibiotic-producing microbiota originating from the environment (e.g. soil); however, vancomycin, particularly, was discovered more than 50 years ago, and vancomycin-resistance genes have been mainly implicated with the increased use of this antibiotic. The presence of antibiotic-resistance genes in the ancient human gut microbiome clearly indicates that these genes pre-date therapeutical use of these compounds and that they are not necessarily associated to a selective pressure of antibiotics use. Identification of pathogens and antibiotic-resistance genes in ancient human specimens will aid in the understanding of the evolution of pathogens as a way to treat and prevent diseases caused by bacteria, microbial eukaryotes and viruses.

References
Introduction
Human remains can give us a huge amount of information about disease in past times, helping us to better understand contemporary pathological conditions. As a multidisciplinary science, paleopathology is widely based on modern investigation techniques and pathologists involved in the study of ancient diseases should be aware of the countless options available today. Beside the well-known morphology-based methods (such as anatomical dissection, histology, radiology and endoscopy) there is a growing number of analytical techniques that may be used to extract information from human remains (1). Among the different approaches employed, advanced morphologic and compositional methods play an important role in paleopathological investigation (2). The purpose of this speech is to illustrate the personal experience in the application of such methods through a series of practical examples (3-6), in order to outline a basic guideline for pathologists.

Methods
Different ancient materials were studied using the following techniques: binocular stereomicroscopy (BSM), phase-contrast microscopy (PCM), scanning electron microscopy (SEM), also with energy dispersive X-ray analysis (EDX), X-ray diffraction (XRD) analysis, and Fourier transform infrared (FT-IR) spectroscopy. The features of the samples and the information needed addressed the choice of the most suitable method from case to case.

Results
The renal stones found in the mummies of Pandolfo III Malatesta, Lord of Fano (1370-1427) and of an anonymous nobleman from Popoli (XVIII century) were investigated using BSM, SEM/EDX, and XRD (Fig. 1). Such methods enabled us to disclose the morphological details of the surface and the inner portions of the stones, along with their elemental and chemical compositions (ammonium acid urate and weddellite for Pandolfo; whewellite and hydroxylapatite for the nobleman from Popoli).

The content of four canopic jars from the Egyptian Museum of Florence, belonged to an anonymous individual of the New Kingdom (1550-1069 BC, XVIII-XX Dynasties), underwent investigation by BSM, PCM, and SEM/EDX (Fig. 2). Paraffin and methacrylate histology allowed to identify lungs with silico-anthracosis, and intestinal content with starch particles. One sample melted away after processing for methacrylate embedding leaving only entwined fibers related to the linen fabrics used to wrap the organs. Furthermore, chemical constituents of natron salts (sodium chloride, sulphate and carbonates) used during embalming were identified.

A XX century female mummy of 32-40 years from the church of San Michele Arcangelo in Sermoneta displayed all but one (the right fifth) clear, white fingernails. The fourth left nail was carefully extracted from its bed and submitted to BSM, and SEM/EDX (Fig. 3), in order to establish chemical composition of the white substance. BSM allowed to appreciate differences between dorsal (polished) and ventral (unstained) surfaces, SEM evidenced nail root and free edge contours details. EDX measurements displayed O, S and Ca in the pigmented areas, suggesting the presence of calcium sulphate (CaSO₄) used as a nail polish. Al, Fe and Si in the free edge of the nail, were referred to remnants from manicure devices.

The partially skeletonized body of the Blessed Sante Brancorsini (1343-1394) contained multiple calcified nodules near the lumbar vertebrae. One of these nodules underwent BSM,
SEM-EDX, XRD, and FT-IR to distinguish between calcified lymph nodes, phleboliths, renal stones or restoring material. The composition in apatite revealed by EDX and XRD, along with the histologic evidence of a fibrous capsule, allowed to rule out the latter two issues. The presence of DNA and serum protein between the organic components displayed by FT-IR confirmed their biological nature, but did not allow to establish the actual origin of the nodules.

Conclusions
Advanced morphologic and compositional investigation methods may be of great help in understanding ancient diseases (1-2). They are classified in invasive and non invasive, as well as in quantitative and qualitative techniques. Although one may be discouraged by the great number of acronyms (SEM-EDX, XRF, XRD, FT-IR, AAS, ICPAES, HPLC and so on) the paleopathologist should be prepared to choose the best method in every instance. We must be aware of the following basic rules: there are many different techniques, one should know how to choose the most suitable to the specific problem and be able to interpret results.

References

DENTO-ALVEOLAR DISEASES IN ANCIENT POPULATIONS
S. Minozzi

Introduction
The study of dental diseases in ancient populations has been given special attention by paleopathologists, considering that oral health is a critical component of overall health and well-being. Moreover, the study of ancient dentition can help archaeologists and historians to reconstruct the lives and lifestyle patterns of past populations.

The relationships between dental pathology, conditions of life, health and nutritional habits of a population have been largely investigated in the anthropological and dentistry literature, which has shown the importance of these investigations to reconstruct the biological history of past generations.

Teeth are one of the most important parts of any anthropological study and, fortunately, they are the most resistant than any other part of the skeleton to chemical and physical destruction and to taphonomic conditions.

Methods
The prevalence of dentoalveolar diseases in past populations has been investigated on the basis of literary sources. Many works are available in paleopathological literature because dental diseases, along with other archaeological and ecological data, represent a valuable element for the study of dietary habits and related social and cultural factors (Hillson, 2008; Larsen, 1997).

Results and Conclusions
Bioanthropological researches conducted in the last few decades have emphasized to the relationship between dental disease and social and environmental phenomena, enhancing the already strong connection between lifestyle and health conditions during history.

Caries is one of the infectious diseases that has plagued humans since the beginning of recorded history and can be easily observed in the human remains retrieved from archaeological excavations.

Many factors influence the spread of caries, such as dietary habits, oral hygiene, changes in the salivary flow (PH alteration), as well the composition and alteration of oral microbioma (bacterial plaque), but the major cause of caries is a diet rich in fermentable carbohydrates or simple sugars.

Epidemiological studies carried out on various populations have documented an increase in caries frequency when the amount of carbohydrates, sugar and sticky nutrients increases within the diet. For this reason, a sensitive increase in the frequency of caries was evident in the transition from the hunting-gathering economy of the Paleolithic to the agricultural economy of the Neolithic. In more recent times, between the seventeenth and nineteenth centuries, there was a high increasing of caries, closely associated with the consumption of refined carbohydrates, especially sugar (Moore, Corbett, 1975).
Other pathologies affecting the teeth and alveoli are periodontal diseases related to inflammatory processes involving the tissues that surround and support the tooth, generally linked to dental plaque deposits. These cause a progressive bone retraction of the alveolar edges, exposing the tooth root to the oral environment and facilitating caries as well as the infiltration of infections resulting in alveolar pockets, abscesses and, finally, tooth loss. These infections, especially periapical, are also caused by exposure of the dental pulp to bacterial attack, resulting from dental caries, heavy wear and traumas (Hillson, 1996).

Dento-alveolar diseases have been present over the millennia and ecological, social, and cultural environments have modulated their incidence, so that a comparative study of the historic prevalence of these diseases in past populations worldwide can provide important data about their related factors and etiology.

References
PATOLOGIA ULTRASTRUTTURALE

ULTRASTRUCTURAL ANALYSIS OF RENAL TISSUE RECOVERED FROM BIOPSIES PREPARED FOR HISTOLOGY AND/OR IMMUNOFLOUORESCENCE IN THE DIAGNOSIS OF NON-NEOPLASTIC RENAL DISEASES

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Introduction. Routine preparation of the renal tissue includes a fragment for light microscopy (LM), fixed in formalin or a Bouin and embedded in paraffin, a second fragment quick frozen for immunofluorescence (IF) and a fragment fixed in glutaraldehyde and embedded in epoxy resin for electron microscopy (EM). The last is not necessary in all cases, but can be important when the diagnosis is not obtained (remain undetermined) with the two other methods. When the fragment for EM is not available at a first time, and a diagnostic definition needs ultrastructural examination, is possible to retrieve tissues sample by recovering a fragment of renal tissue from samples prepared for LM and/or IF analyses. There may be several reasons for the lack of the fragment for EM examination at the time of biopsy:

<table>
<thead>
<tr>
<th>Identification of immune complex deposits</th>
<th>Formaline fixative</th>
<th>Bouin fixative</th>
<th>Recovery from paraffin slide</th>
<th>Recovery from frozen samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural analysis of immune complex deposits</td>
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<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Amyloid identification and characterization</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Immune complex deposits distribution</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Cellular alterations</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Basal membrane alterations</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Fabry disease deposits identification</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

During the biopsy procedure, a very small fragment is obtained, because of technical problems. This fragment is intended for LM and IF analyses, according to clinical data Cost/benefit analysis which do not include the EM examination. A deliberate decision, at the beginning of biopsy procedure was taken, excluding EM (i.e. less invasive procedure). The reason for asking the EM at a second time are different: Absence of glomeruli in the samples for IF and/or histological analyses IF result is not conclusive, for example IF staining is very weak or questionable IF is in disagreement with the clinical suspicion. (This can be true for cases with a high degree of sclerosis, diabetic nephropathy, renal amyloidosis, Immunecomplex- related GN with a scanty deposition, because of very early stage of disease). A false-negative IF suspected on the base of the observed histology A retrospective analysis is asked in case of a new diagnostic hypothesis.

Methods. We describe 70 cases in which ultrastructural examination was performed retrieving fragment from the tissues remaining after preparations for histological examination or / and IF. Method and result of these analyses will be reviewed and the limits and potential diagnostic use in the different preparation will be discussed for immunocomplex and non-immune renal disease.

Results. Table summarizes the results, comparing different situations. Our results indicate that recovery renal tissue from fixed of frozed biopsies for EM is possible although some limitations have to be considered.

Main results:
If the tissue is recovered from paraffin blocks, peripheral
and mesangial electrondense deposits are easily recognized. Deposits along the glomerular and tubular basal membrane, in cases of light chain deposition disease (LCDD), are also recognized. The same for structured deposits in fibrillary/ immunotactoid GN. Other structural deposits such as tubuloreticular inclusions (TRI) are also possibly identified. By contrast, finger prints deposits and structured deposits in cryoglobulinemic GN are not seen and recognized in such samples.

In tissue recovered from frozen fragment used for IF, immune deposits are possibly identified, and also cryoglobulin aggregates are seen. Interestingly, in cases of Fabry disease, intracellular deposits are easily identified in podocytes, endothelial and mesangial cells. Inclusions can be seen also in interstitial cells, useful in cases of absence of glomeruli. Frozen tissue recovered for EM shows that cells are damaged, the integrity of cell membranes being lost in most cases.

In cases with EM examination performed with recovery from glass, a lower sensitivity in identifying and localizing the immune deposits is present, compared with paraffin recovery.

**PATOLOGIA TESTA E COLLO**

**MIRNA205P DETECTION USING IN SITU HYBRIDIZATION IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA**

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**Introduction.** The presence of cervical lymph node metastases in oral squamous cell carcinoma patients is a very important in therapy choice and prognosis, with great impact on overall survival. Therefore, an accurate molecular marker to identify cervical lymph nodes status is necessary.

MicroRNAs are post-transcriptional regulators of gene expression frequently dysregulated in a range of human malignancies. They can act as tumor suppressors or oncogenes. Interestingly, in oral squamous cell carcinoma, miR-205 acts as both a tumor suppressor and oncogene.

The aim of this study was to evaluate the expression of miR-205p by In situ hybridization and the expression of some transcription factors involved in epithelial-mesenchymal transition in oral squamous cell carcinoma (OSCC).

**Method.** We used locked nucleic acid probe in situ hybridization (LNA-ISH) to visualize, in prognostic tissue micro-arrays (TMAs) of 119 formalin-fixed paraffin-embedded (FFPE) archival OSCC tumors, the expression of miR-205p. Moreover, we evaluate the expression of EMT-associated proteins Twist, Snail and Slug in different grades of OSCC by immunohistochemistry. We also investigated the clinical significance of miR-205p expression on OSCC.

**Results.** We confirmed a high cytoplasmic expression of miR-205 in tumor tissue of oral squamous cell carcinoma. Conversely, the miR205 level does not show any significant association with overall survival. Moreover, miR-205 expression in non-metastatic primitive OSCC group was significant-ly lower than the metastatic group (P < 0.05). Interestingly, the level of miR-205 was directly associated with the expression of Twist and Snail (P < 0.05).

**Conclusion.** Our results suggest that miR-205 could be involved in the Epithelial-mesenchymal transition process. The miR-205 expression can effectively predict and estimate the cervical lymph node metastasis in OSCC. Therefore the evaluation of miR205 in primitive OSCC could be an important tool for the surgery management of patients, contributing to an improved quality of life.

![Figure 1. In situ Hybridization (ISH) analysis of miR-205p. a) high expression of miR-205 in OSCC, b) negative expression of miR-205 in OSCC, c) ISH with a negative control probe (scrambled miRNA), d) ISH with a positive control probe (U6 small nuclear RNA).](image)

**References**


**DIAGNOSTIC CYTOLoGY ASSOCIATED WITH DNA HPV TESTING FOR ORAL CANCER SCREENING**

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Introduction. The survival rate for squamous cell carcinoma of the oral cavity (one of the leading causes of cancer related deaths worldwide) remains low, as it is often diagnosed late due to no reliable diagnostic method of selecting subjects with lesions at high risk of malignant transformation. To date, the diagnosis of oral cavity squamous cell carcinoma (OSCC) and precancerous potentially malignant lesions (PMLs) has been based exclusively on the scalpel (surgical) biopsy. Liquid based oral cytology alone can provide useful information on oncologically relevant morphologic alterations (sensitivity, specificity and positive predictive values were reported to be 94.7%, 98.9% and 95.9% respectively in 411 patients) and gives good first level screening results (1, 2, 3, 4). However, oral diagnostic cytology alone, whilst providing useful information (sensitivity is higher than the Pap test and specificity is similar) does not suffice for the diagnosis of OSCC and PMLs in patients with highly keratinized lesions. The most important risk factors identified so far are tobacco and alcohol. Moreover, a potential role of oral HPV (human papilloma virus) infection in the onset of OSCC, hypothesized but still to be proven, could represent an adjunctive predictive factor. The presence of HPV DNA in oral cytological samples were investigated so as to determine the virus incidence, the genotype distribution and its association with PMLs and OSCC.

Methods. Screening of apparently normal subjects is ongoing with a first level method, i.e. liquid-based cytology combined with investigation for the DNA-HPV with a high sensitivity RDB test able to identify and genotype both of low and high grade HPV. Samples are obtained by a cytobrush on the most common involved sites for oral carcinoma, i.e. the floor of the mouth, tongue, gums and cheek lining.

Results. Sixty-seven subjects were enrolled: 65/67 had normal cytology results and 2/67 had a low-grade oral lesion (OIN 1). Two patients had a positive HPV-DNA test on oral mucosa: HPV was found only in female samples. A high grade HPV was identified in the first case and a co-infection of high and low grade HPV in the second. Cytology detected normal mucosa in the first case and a low grade lesion (dyskeratosis) in the second.

Conclusions. By combining “liquid based” diagnostic oral cytology and HPV infection testing it seems that a subgroup of patients with potential predictive factors for PMLs and OSCC development may be selected. Therefore, these subjects are to have regular follow-ups even in absence of visible oral lesions.

The prospective evaluation of healthy subjects with HPV infection may well provide useful information as to its role in the development of OSCC. HPV becomes the main risk factor in those subjects where alcohol and tobacco are less representative. It is also important to choose the appropriate HPV test in as much as many HPV commercial kits on the market nowadays were originally developed for cervical carcinoma screening. To date, the classification of high and low grade HPV genotypes has been based on cervical cancer evidence, but this nomenclature could be misleading in the presence of an OSCC. Further studies are needed to better understand the HPV distribution in oral mucosa and the real grade of risk in the cancer etiology. However, if the causal role of HPV is proven, a combination of oral cytology and tests for HPV infection could represent a significant diagnostic step towards an early diagnosis of OSCC, as has already been demonstrated in uterine cervical carcinoma screening with the PAP test and DNA-HPV.

As the mortality rate for oral carcinoma in industrialized countries is now higher than that of uterine cervical carcinoma, which has been drastically reduced thanks to screening, the introduction of screening programmes also for oral carcinoma, as advocated for years by numerous epidemiologists, including Silvia Franceschi et al (5), is a must.

References
5 Franceschi S. et al., “Screening for cancer of head and neck: if not now, when?” Oral Oncol 1997; 33: 313-316.

PATOLOGIA ENDOCRINA

CYTO-HISTOLOGICAL COMPARISON OF THYROID FINE-NEEDLE ASPIRATION BIOPSIES PROCESSED BY LIQUID-BASED CYTOLOGY CLASSIFIED WITH THE 2014 ITALIAN REPORTING SYSTEM: PRELIMINARY RESULTS OF AN INSTITUTIONAL SERIES

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Background. Nodular lesions represent a very common problem for the clinicians as well as a diagnostic challenge for the pathologists. Up to 5% of the general population has a palpable thyroid nodule though only approximately 5% of these clinically apparent thyroid nodules actually harbour malignancy. The real challenge facing general practitioners, endocrinologists, surgeons, and pathologists is to reach an accurate preoperative diagnosis of malignancy and to ensure the patient receives a timely and appropriate treatment. FNA is the only test that can provide a definitive preoperative diagnosis of malignancy. The sensitivity and specificity of FNA are reported to be 68–98% and 56–100%, respectively. FNAB is also regarded as the most accurate method for the selection of patients with thyroid nodules for surgery or for the ‘wait and see’ management and it can be considered a very cost-effective diagnostic test. The aim of the present study is to evaluate the institutional series of the Catholic University of Rome classified according to the 2014 Italian six-tiered reporting system for thyroid cytology. This recent classification, in addition to the classic 5 categories of the previous system of 2007 (TIR I- non diagnostic; TIR 2 - negative for neoplasia; TIR 3 - indeterminate/follicular neoplasia; TIR 4 - suspicious for malignant neoplasm; TIR 5 - positive for malignancy) includes the subgroup of TIR 1C (cystic) for the non-diagnostic group and the subdivision of the indeterminate category in TIR 3A (low-risk indeterminate lesion) and TIR 3B (high-risk indeterminate lesion).

Materials and methods. From April 2014 to July 2016 in
the Division of Anatomic Pathology and Histology of the Catholic University, Foundation “Agostino Gemelli” Hospital of Rome 3140 patients underwent an ultrasound-guided fine-needle aspiration cytology (FNAC). The aspirated specimens, performed with 25-gauge to 27-gauge needles, were processed using the ThinPrep 5000 liquid-based cytology (LBC) method (Hologic Co., Marlborough, MA, USA). The resulting slide was fixed in 95% ethanol and stained with Papanicolaou. All cases were diagnosed and classified according to the morphologic criteria of the new Italian reporting system for thyroid cytology.

Results. The 3140 cases were classified as follows: TIR 1 = 218 (7%); TIR 1C = 70 (2.2%); TIR 2 = 2223 (70.8%); TIR 3A = 217 (6.9%); TIR 3B = 148 (4.7%); TIR 4 = 79 (2.5%); TIR 5S = 185 (5.9%). We analysed the ROM (risk of malignancy) in 194 cases submitted to surgery in the newly included indeterminate categories TIR 3A and TIR 3B which resulted respectively as 6 out of 40 (15%) and as 27 out of 89 (30.3%) malignant neoplasms at histology. For the category TIR 1C only 6 cases underwent surgery with no cases of malignant neoplasm. The TIR 4 and TIR 5 categories showed a ROM of respectively 81.6% (40/49) and 99.3% (154/155).

Conclusions. This investigation emphasizes the reliability of the 2014 Italian reporting system regarding the mutual frequency of the diagnostic categories. The ROM for the different classes, although the surgical series is still limited, is perfectly within the range of the estimated values.

OVEREXPRESSION OF AXL IS ASSOCIATED TO RADIOACTIVE IODINE REFRACTORINESS AND DISEASE RECURRENCE VIA P65 NFkB IN THYROID CANCER

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Background. Papillary thyroid carcinoma (PTC) represents 80% of thyroid malignancies. Considering the generally favorable behavior, identification of patients who need aggressive treatment and a close follow-up to reduce PTC recurrence are needed. In fact, 20–30% of PTC patients experience recurrence and/or metastasis with subsequent increased mortality. In addition, approximately 5% of metastatic PTC lose their differentiated features and evolve to radioactive iodine-refractory (RAIR) diseases. The Axl proto-oncogene is a receptor tyrosine kinase member of TAM receptors family that regulates the cancer cell viability, invasiveness and chemoresistance in various human carcinomas, including PTC. The aim of this study was to evaluate the Axl expression in a series of PTC and define its relation with prognostic parameters and its association to apoptosis and proliferation key proteins.

Methods. 110 samples of PTC were included in a prognostic Tissue MicroArray and the proteins expression analysis were performed by immunohistochemistry. Apoptosis was evaluated by TUNEL method. All the statistical analysis were carried out using the SPSS version 20.0 software (SPSS Inc., Chicago, USA). Thyroid cell lines were transfected with custom-synthesized siRNA, treated with recombinant GAS-6 and, in selected experiments, with Bosutinib or PI3K/AKT or p65 inhibitors and then subjected to the TUNEL reaction. Lysates containing comparable amounts of proteins were subjected to Western blot.

Results and Conclusion. We found a high expression of the receptor in 60.8% of cases, while normal thyroid tissues were negative. AXL high expression segregated with PTC RAIR and disease recurrence. The concomitant occurrence of AXL expression and BRAF V600E mutation reinforced the correlation with RAIR refractoriness and disease recurrence. AXL overexpression positively correlated with p65 NFkB phosphorylation Ser536-p65 NFkB levels. Consistently, AXL stimulation of TC cells with its ligand GAS6 induced p65 NFkB phosphorylation in Ser536 in an Akt-dependent manner. AXL or Akt blockade inhibited p65 NFkB phosphorylation and impaired GAS6-induced cell survival. Moreover, the blockade of p65 NFkB nuclear translocation inhibited GAS6-mediated resistance to apoptosis of TC cells. Thus, AXL could represent a potential marker of aggressiveness and a novel therapeutic target in PTC.

References

MOLECULAR CHARACTERIZATION OF A COHORT OF PREOPERATIVE THYROID NODULES IN A DIAGNOSTIC SETTING

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Introduction. Fine-needle aspiration cytology (FNAC) represents the most reliable procedure to diagnose thyroid nodules. Most thyroid nodules are benign(85%) and in most cases FNAC is determinant in the discrimination of benign thyroid nodules from malignant ones. However, in the cytological examination there remains a “gray zone” comprising thyroid nodules classified as Thy 3-Thy 4 (according to the classification of the British Thyroid Association), the diagnosis of which is indefinite between benignity and malignancy (25% of Thy 3 and 70% of Thy 4 cases result malignant on final
histology). Management of such a kind of patients is challenging since they may undergo to potentially superfluous thyroid surgery. Thus the risk to perform diagnostic surgery on benign thyroid nodules is high and in parallel lobectomy surgery may be inadequate for diagnosed cancer > 1 cm. Recently incorporation of molecular assay together with cytology has the role of helping accuracy of pre-operative diagnosis of cancer in thyroid nodules. In papillary thyroid cancer (PTC), which represents the majority of thyroid cancer (about 90%) and follicular thyroid cancer the most common mutations are BRAF and RAS genes alterations together with RET/PT and PAX8/PPARY rearrangement. Their detection is now being used for the identification of malignancy in thyroid nodules with indeterminate FNAC and to guide the therapeutic approach more appropriately.

In this study, we set up a strategy to perform molecular analysis of BRAF and KRAS-NRAS-HRAS genes directly on the routine air-dried stained FNAC smears in a cohort of patients affected by Thy 3 or Thy4 FNAC diagnosis. Specificity and sensitivity of the molecular assays were evaluated and compared with the final histological diagnosis.

**Materials and Methods.** 272 thyroid fine-needle aspiration cytological samples (FNAC), fixed with cytfix on slides, were collected prospectively for molecular diagnostics. All the samples have been classified as Thy3 or Thy4 category (198 Thy3 cases and 74 Thy4 cases) according to Bethesda guidelines by experienced Pathologists. EE and/or Giemsa stained smears were further reviewed by Pathologist for cellular adequacy of the sample and areas with the highest number of thyocytes cells carefully selected. The optimal number of cells suitable for molecular studies should be 100 or more. Samples with less of 100 cells were excluded from the molecular analysis. DNA was extracted from the selected areas by a “home-made” buffer (pH 8, 1%Tween) and digestion with Proteinase K or by a commercial kit (Pinpoint Slide DNA Isolation System™, Zyomed). BRAF V600 mutations were detected by using the highly sensitive and specific BRAF therascreen PCR Real Time (Qiagen). In contrast, conventional PCR followed by direct Sanger sequencing was the test of choice for sequence analysis of the entire exon 15 of BRAF, KRAS (exons 2 and 3) and N-HRAS genes (exon 3).

**Results.** Cytological smears consisting of 198 Thy3 category and 74 Thy4 category were collected between 2013-2016 and subjected to molecular testing. The DNA purified directly from the stained smears as described in MM was sufficient and of good quality for execution of the molecular assays in 262/272 slides, thus showing that the methodological procedure employed for DNA extraction was enough robust to make 96% of the cytological slides valuable. FFPE samples with a certainty of histological diagnosis were available for 160/262 patients. All of the cytological samples were primarily tested for mutations in BRAF gene, KRAS-NRAS-HRAS genes. Seventy-one different mutations were found: 44 alterations in BRAF V600E, 2 substitutions in BRAF K601, one 9bp duplication (V600_K601insAThV) in BRAF gene and 24 RAS mutations. Since number of RAS mutated cases is low, a correlation between mutations found on cytological smears and final histological diagnosis will be detailed only for BRAF mutated cases. Histological diagnosis of PTC was assigned to 60 cases, 44/60 of these were V600E mutated and 16 were BRAF wt, thus indicating that sensitivity of the BRAF PCR Real Time test was 73%, a percentage very close to the one reported in literature for PTC. Interestingly 8/44 V600E BRAF mutated PTC were classified on FNA cytology as thy3 indeterminate category, thus indicating that the genetic characterization of BRAF status increase the preoperative diagnostic accuracy of about 18%. Thirteen out of 60 PTC were diagnosed as follicular variant and 10/13 FVPTC were BRAF mutated. Among the BRAF wt PTC, 5/16 cases were classified on FNA analysis as thy3 (31%). The remaining 70% (11/16 samples) of the cytological slides even with a thy4 category resulted BRAF wt. These data together indicated that detection of BRAF V600E mutation, irrespective of the cytological diagnosis is a strong predictor of malignancy, with a 100% of specificity. In our cohort of FNAC smears, additional 14 cases after surgery showed a mixture phenotype, being composed of follicular adenoma and PTC (micro or macro papillary carcinoma). All these cases were BRAF V600E negative, with the exception of one showing the BRAF duplication. It is of note that 11/14 of these mixed histological cases were categorized on FNAC as Thy3, a finding that may suggest that aspiration procedure might undertake more benign lesion -mutation negative than the malignant nodule. Studies are in progress to perform BRAF mutation test on isolated distinct benign and malignant areas from tissue. Preliminary data show that in some samples a BRAF mutation could be detected in the area of papillary cancer but not in the follicular adenomatous area. In 31 cases (24/31 of thy3 category), histology attributed the diagnosis of Follicular Adenoma, 2/31 express a BRAF mutation in codon K601, but not in codon 600. These results are in line with the data in literature about the molecular characterization of Follicular Adenomas.

In addition, in our cohort 7 cases BRAF wt were Follicular Carcinoma, and interestingly, 46 patients were diagnosed with solely hyperplasia, the majority of them being BRAF wt. Finally 102 cases did not undergo to surgery, thus the molecular screening on cytology cannot be compared with a final diagnosis. However, none of this patients showed BRAF mutation. The combination of FNA cytological evaluation of thy2/3 classification together with the negativity of BRAF mutation determined a follow up of these patients without any other invasive intervention. The 24 cases which resulted RAS mutated in our cohort were distributed among mixed type Adenoma/PTC (6 cases), Follicular adenoma (3 cases), hyperplasia (7 cases) and 7 among the 102 cases that have not undergone under surgery. Although the number of cases in this study is too low for any statistical analysis, these results suggest that the detection of RAS mutation has low sensitivity and specificity as molecular marker of malignancy and that so far, its presence has a not yet clear significant.

**Conclusions.** Detection of V600E BRAF mutation appear feasible using routine FNAC, even if expertise in treating with poor cellular samples, management of the low quantity of DNA extracted and molecular test with high sensitivity may be required. Correlation between results of molecular test performed on FNAC and final histological diagnosis may give an indirect prove of accuracy of the approaches used for detection of mutations on this kind of samples. Discrepancy between molecular testing on cytological Thy3/Thy 4 samples and corresponding FFPE histology may often be due to mixed benign and malignant lesions in the tissue. While BRAF mutation can be considered an high specific molecular marker for malignancy, in contrast RAS mutations are still of uncertainty value since they are present both in benign lesions (Follicular Adenomas) and in reactive normal tissue (hyperplasia). These data indicate that a follow up of these kind of patients may reveal in long run if RAS mutational status has a role in evolution of these thyroid nodules.
In conclusion, the accuracy of cancer diagnosis in thyroid nodules by FNAC could be improved significantly testing for a panel of mutations, including BRAF, RAS. Moreover, correct molecular classification of the FNAC, regardless of diagnosis, will help the subsequent choice of surgery approach.

References

RANK AND RANK LIGAND EXPRESSION IN PAROTID GLAND CARCINOMAS

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Introduction. Salivary gland carcinomas are rare tumours, representing less than 5% of all head and neck malignancies1. The majority of salivary gland carcinomas occur in the parotid gland, and currently over 20 different histological subtypes are recognized in the WHO Classification. The conventional approach to these tumours is the surgery, associated to radiotherapy on the basis of clinical and pathological risk factors2. The prognosis still remains poor and new treatment strategies, possibly based on the identification of specific targets, are needed.

Recently, it has been reported that deregulation of the Receptor Activator of NFkB Ligand (RANK)/RANK signalling axis results in salivary gland carcinoma development in a mouse transgenic model, while early blockade of RANK/ RANK signalling markedly reduces the development of these neoplasms3. Based on these results, the RANK/RANKL pathway may represent a novel therapeutic target in salivary gland carcinomas.

The aim of this study is to investigate the expression of RANK and RANKL in primary parotid gland carcinoma.

Material and methods. Forty-six patients treated for parotid gland carcinoma between 2001 and 2011 were selected for this study. In each case, all available histological slides were reviewed and tumours were classified according to the 2005 WHO Classification scheme4, and assigned to low-risk and high-risk categories5. For comparison, we examined a group of 40 randomly chosen parotid gland adenomas (pleomorphic adenomas, myoepitheliomas and Warthin tumours). A tissue microarray was prepared using the Beecher Tissue Microarrayer, with three cores of different tumour areas. Serial sections were stained with haematoxylin and eosin or employed for the immunohistochemical analysis. The results of immunohistochemical study were evaluated with a semi-quantitative method, considering both the staining intensity and the percentage of positive cells6. The staining intensity was evaluated on a four point scale (0-3), while the proportion of positive cells was evaluated according to the scale 0, 1 (1%), 2 (2-10%), 3 (11-30%), 4 (31-60%), 5 (100%)7. The two values were summed to obtain a total score ranging between 0 and 8. For statistical analysis, cases with score > 4 were considered positive. All statistical tests were performed using SPSS software (release 12.0). Associations between categorical variables were assessed by chi-squared test. For analysis of survival, the endpoints considered in this study were rates of developing first local recurrence and rate of any disease-related mortality. Local recurrence-free survival and disease-specific survival were modelled using the Kaplan-Meier method and analyzed by the log rank test. Chi square P values < 0.05 were considered significant.

Results. Overall, 33 carcinomas (71.7%) were scored as positive for RANK and 25 (54.3%) for RANKL. The expression of both RANK and RANKL was significantly higher in carcinomas than in adenomas since only 6 (15%) adenomas were positive for RANK, and RANKL was negative in all benign tumours (P < 0.001 for both, Fisher’s exact test). Among carcinomas, no significant differences between the histological types was observed, even if mucoepidermoid and carcinoma ex-pleomorphic adenoma presented a high frequency of the two markers. High grade tumours tended to expressed more frequently RANKL than low grade ones (66.6% vs 36.8%; P = 0.07).

In 5 of the 9 cases of carcinoma ex-pleomorphic adenoma differences in RANK-RANKL expression between the benign and malignant component were evaluated. In 3 cases the malignant component was positive for the markers, while the benign one was negative. In 2 cases both the components of the tumour were negative and in the remaining two cases an increase in RANKL expression from adenoma to carcinoma was observed.

Conclusions. The results of our study indicate that the expression of RANK and RANKL is associated with the acquisition of a malignant phenotype in parotid gland tumours, since carcinomas showed a significantly higher expression than adenomas. Interestingly, some histological types presented a high prevalence of positive cases, including salivary duct carcinoma, mucoepidermoid carcinoma and carcinoma ex-pleomorphic adenoma. Another important aspect is that the inhibition of the RANK/RANKLsignalling axis could be useful in the treatment of salivary glands tumours, either alone or in combination with other therapies. Denosumab, a monoclonal antibody directed against RANKL, is currently used in the management of osteoporosis, bone metastases and giant cell tumor of bone, mainly for its role of inhibitor of bone resorption through its effects on osteoclastogenesis8. However, there is increasing evidence that inhibition of the RANK/RANKL axis may offer new therapeutic chances in cancer patients8. Further studies on larger series of patients are needed to explore this attractive hypothesis.

References
PDCD4 AND BRAF IN PAPILLARY THYROID CARCINOMA: CLINICO-PATHOLOGICAL AND MOLECULAR CORRELATION

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Introduction. Papillary thyroid carcinoma (PTC) constitutes almost the 80% of all thyroid cancers and females are much more affected rather than males (M: F = 1:2.5). PTC is an indolent neoplasm and its long-term survival rate appears to be very good. Nevertheless, the stratification of the risk is available only after surgery, which can be a total thyroidectomy or a hemi-thyroidectomy. Therefore, researchers are studying novel pre-surgical markers in order to choose the most appropriate surgical intervention and the best case management. To reach this purpose the mutation of BRAF and the expression of PDCD4 represent two important targets that we evaluated here for their potential role in the presurgical phase.

Methodology. The present study aims to investigate: a) the nuclear expression of PDCD4 amongst a larger number of PTC cases; b) the existence of a possible interaction between the PDCD4 expression and BRAF mutations; c) the relationship between these two potential markers and other prognostic factors of PTC d) the role of PDCD4 nuclear expression and mutations of BRAF as pre-surgical markers in PTC patients. We evaluated 127 patients with PTC, studying the mutation of BRAF in pre-surgical fine-needle aspiration and the nuclear expression of PDCD4 through immunohistochemistry in surgical specimens. Therefore, we statistically analysed the relationship between these two potential markers and other clinical and pathological variables.

Results. The mutation of BRAF and the absence of PDCD4 resulted to be highly related (p < 0.0001). Furthermore BRAF appears to be also related with other variables, such as: histological variants (p = 0.002) and staging of PTC (p = 0.010). On the other hand, the loss of the expression of PDCD4 at nuclear level seems to be related to pT (p = 0.0338) and pN values (p = 0.0039) and also to histological variants (p = 0.0017). In our study we concurrently evaluated BRAF status and PDCD4 expression using a simple algorithm (0 = BRAF wild type [wt] and PDCD4 +; 1 = BRAF mutated or PDCD4-; 2 = BRAF mutated and PDCD4-). The value 2 of this algorithm resulted correlated to age (cut-off 55 y.o.) (p = 0.0271), size of neoplasia (p = 0.046), extra-thyroid aspiration (p = 0.016), histological variants, pT (p = 0.04), pN (p = 0.027), lymph nodal ratio (p = 0.039) and staging (p = 0.027).

Conclusions. Individually, BRAF mutation and the absence of PDCD4 resulted to be related to different malignant prognostic factors. This study highlighted for the first time that the analysis of both these markers, using a single algorithm, is strongly related to many different prognostic factors. This evidence, if confirmed by future studies, suggests the combined evaluation of BRAF and PDCD4 as pre-surgical markers, indicative for the aggressiveness of PTC.

Mercoledì, 23 novembre 2016
Aula Levante – 12:30 – 13:30
Moderatori: Angela Rita Sementa (Genova) – Oscar Nappi (Napoli)

PATOLOGIA PEDIATRICA

LONG TERM CHILDHOOD CANCER SURVIVORS: A PILOT STUDY IN ITALY

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Background. Childhood cancer survival has increased significantly during the last decades. As a result, an increasing number of adults require appropriate follow-up care due to recurrences and late effects of early treatments. This study contributes to the ongoing discussion about the design and delivery of care to long term childhood survivors: by estimating their number, features and age distribution in the Italian cancer registry areas.

Methods. We apply the CHILDPREV method to limited duration prevalence of 15 Italian registries, and obtain complete prevalence of people of all ages who were diagnosed during their childhood of one of the following cancers: Acute Lymphocytic Leukemia (ALL), Brain and Central Nervous System cancer (CNS), Hodgkin lymphoma (HL), and all cancer types combined but non-melanoma.

We also reconstruct the patterns of care in adult age of individuals diagnosed with cancer in childhood age in Veneto and Piemonte, by linking at individual level cancer registry data with hospital admissions archives. We compute hospitalization and incidence rates for diseases possibly related with childhood cancer treatment and compare results with those obtained for the general population with same age, gender, residence area.

Results. In Italy we estimate about 44,000 survivors of childhood cancer at January 1, 2010 out of 2.6 million people living with a cancer diagnosis; ALL accounts for 23% of prevalent cases, CNS for 24% and HL for 7%.

Severe diseases possibly related with childhood cancer treatments are present in 3.4% and 2.6% of childhood cancer survivors in Piemonte and Veneto, respectively; these proportions are significantly higher than those measured in regional populations.

Conclusions. Adults with a childhood cancer diagnosis represent a relevant target from the public health perspective. Providing for them specific health care monitoring is appropriate for early detection and timely treatment of late effects and severe diseases.

PEDIATRIC ONCYCOTY ADRENOCORTICAL TUMORS: WHAT SCORING SYSTEM SHOULD BE APPLIED?

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Introduction. Rarely adrenocortical tumors (ACTs) occur in pediatric patients. They can be classified in adrenocortical adenomas, ACTs with indeterminate malignancy (borderline tumors), or adrenocortical carcinomas. Their diagnosis is often challenging and pathologic criteria (Weiss scoring system) applied to adult tumors are not always reliable in children. Therefore, other parameters, such as age, signs of endocrine dysfunction, volume, and weight tumor, as well as disease stage, have been proposed over time to predict prognosis. In addition, only rarely “oncocytic ACTs” occur in children. The extreme rarity of pediatric oncocytic ACTs makes difficult to predict their biological behavior. To the best of our knowledge, based on the Lin-Weiss-Bisceglie score system (1-2), only six cases of oncocytic ACTs have been reported in children, five cases were classified as benign (adrenocortical oncocytomas) and one case as “borderline tumor”, while none oncocytic carcinoma has been reported in literature.

Materials and methods. We herein report a rare case of oncocytic ACT in a 7-year-old boy with pseudo-precocious puberty. Radiological examination revealed a suspicious solid mass in his right adrenal gland and adrenalecctomy was performed, accordingly. The Lin-Weiss-Bisceglie scoring system used for adult counterpart was applied to classified the present case (1-2). The scoring system is based on the identification of major criteria (high mitotic rate: >5 mitoses per 50 high-power fields; any atypical mitosis; venous invasion) or minor criteria (large size and huge weight; necrosis; capsular invasion; sinusoidal invasion). If an oncocytic tumor shows at least one (or more) major criterion is classified as “malignant”; while if a tumor exhibits one to four minor criteria, it should be considered as “uncertain malignant potential” (borderline tumor). When none of the above-mentioned major or minor criteria are identified the tumors are classified as “benign”.

Results. Grossly, the mass, weighed g 80 and measured cm 6x5x3.5, was almost entirely surrounded by a capsule. The cut surface showed a solid tumor with elastic consistency and mahogany/brownish colour. Histologically, tumor was composed of cells with large, granular, deeply eosinophilic cytoplasm, and, instead, is objective, standardizable, and feasible even in samples from archival stained/immunostained slides.

Conclusions. Pediatric oncocytic ACTs are extremely rare in children and their diagnosis and prognosis are still matter of debate (3). Unlike conventional (non-oncocytic) ACTs, there is no general consensus about the pathologic scoring system that should be applied to these lesions (3). In this regard, the modified Weiss system, also known as “Lin-Weiss-Bisceglie score system,” actually applied for the adult oncocytic ACTs should be used. However if this system is accurate in predicting prognosis is still to be established in a large series of pediatric oncocytic ACTs.

References

PATOLOGIA PLEURO-POLMONARE

MIR-130A AS DIAGNOSTIC MARKER TO DIFFERENTIATE MALIGNANT MESOTHELIOMA FROM LUNG ADENOCARCINOMA IN PLEURAL EFFUSION CYTOLGY

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Introduction. Malignant mesothelioma is a rare tumor with dismal prognosis arising from the lining cells of the serosal surfaces, mainly the pleura. It is strongly correlated to the inhalation of asbestos fibers and usually presents with recurrent pleural effusion. However, a large number of malignant pleural effusions are due to lung adenocarcinoma. The distinction between these two tumors has considerable therapeutic and medicolegal implications and it can be very challenging in both the histologic and the cytological arena due to the morphologic overlapping. An appropriate immunohistochemical support is mandatory to achieve the correct diagnosis. MiRNA expression analysis could be a viable diagnostic tool in this field. The aim of this study was to explore the reliability of miRNAs as diagnostic markers to differentiate epithelioid malignant mesothelioma from lung adenocarcinoma, especially in pleural effusion cytology.

Methods. Bioinformatic analysis of publicly searchable datasets on miRNA expression profiling in malignant mesothelioma and lung adenocarcinoma was performed to select the most significant differentially expressed miRNAs. These were analyzed by quantitative PCR on histologic (41 malignant mesotheliomas and 40 adenocarcinomas) and cytological (26 malignant mesotheliomas and 27 adenocarcinomas) specimens. The diagnostic performances of the confirmed miRNAs were assessed.

Results. Bioinformatic analysis identified miR-130a, miR-193a, miR-675, miR-141, miR-205, and miR-375 as best diagnostic markers of malignant mesothelioma or lung adenocarcinoma. Of these, only miR-130a was significantly overexpressed in malignant mesothelioma compared with lung adenocarcinomas (p = 0.029 in histologic and p = 0.014 in cytological samples). Area under the receiver-operating characteristic curve proved that miR-130a is moderately accurate (70%) as diagnostic marker of malignant mesothelioma. By using the best cutoff, miR-130a showed a sensitivity of 77%, a specificity of 67%, a PPV of 69%, a NPV of 75%, and an accuracy of 72%.

Conclusions. Even though the diagnostic performances of miR-130a were similar but not superior to those of the currently used antibodies, miRNA quantification has some advantages compared to immunohistochemistry. Indeed, it is not affected by the same technical and interpretative limitations and, instead, is objective, standardizable, and feasible even in samples from archival stained/immunostained slides.
In conclusion, miR-130a expression analysis could be a reliable second level diagnostic tool to differentiate malignant mesothelioma from lung adenocarcinoma in pleural effusion cytology, mainly in those cases with ambiguous or negative immunohistochemistry.

**Conclusion.** In our organization the diagnostic accuracy was 97.3%. The inadequate cases rate would had been 23% if only Real Time or sequencing methods were available. Considering the generally mutual presence of EGFR/KRAS mutations and ALK rearrangements we avoid to 42 patients a new diagnostic bronchoscopic exam. This performance has been obtained by a multidisciplinary approach that tends to an efficient management of the sample coupling ROSE with sensitive molecular multitarget techniques. Cooperation between Pneumologist, Pathologist, Molecular Biologist and Oncologist is essential for the management of NSCLC patients.

**THE KEY ROLE OF MANAGEMENT OF NON SMALL CELL LUNG CANCER (NSCLC) SPECIMENS. A SINGLE INSTITUTION EXPERIENCE OF A MULTIDISCIPLINARY APPROACH**

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**Background.** The molecular profiling of non small cell lung cancer (NSCLC) has been deeply investigated in the last few years. While it is becoming essential to cross-examine multiple biomarkers even in very small samples, multigenes diagnostic platforms spread in clinical molecular laboratories for the advantage of multiplexes approaches with high analytical sensitivities. These multiplexed tests have the advantage of the very low amount of DNA input required, compared to conventional methods. However, the main goal for NSCLC sampling management remains the optimization of all available cytological and histological samples to guarantee both immunophenotyping of the tumour and genomic data for targeted therapies. A correct handling of the sample coupled with high analytical sensitivities methods and high expertise of people involved in these processes became essential especially in cases whose DNA extraction yields remain critical.

**Methods.** We retrospectively analysed the NSCLC cases observed in our institution in the period January 2014-March 2016. We offer ROSE (Rapid On Site Evaluation) upon request of Pneumologist, and in the same period we registered 263 procedures. We genotyped 438 NSCLC by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) assay with CE-IVD MiLyapid® Lung status kit (Diatech Pharmacogenetics, Italy) on Agena MassARRAY® System (Agena Bioscience, Inc. California). The system is a target method that investigates more than 230 mutations on 10 genes involved in NSCLC, with a DNA amount of at least 40 ng. In 35% cases, cytological samples were the only available material for molecular analysis (fine needle aspiration cytology, bronchoaspirate or pleural effusion liquid). For 65% cases we had histological samples available (surgical specimens, secondary lesions samples or small endoscopic biopsies).

**Results.** Among all cases, in 102 (23%) samples the amount of DNA extracted would have been insufficient for EGFR testing by mean of RealTime PCR or pyrosequencing, and they would had been classified as “not adequate”. These cases had been processed by mass spectrometry observing EGFR and KRAS mutations in 12 (11.8%) and 30 (29.4%) cases, respectively. Among all cases we had 12 (2.7%) tumours with neoplastic cell content lower than 30% that didn’t revealed any mutations in EGFR/KRAS genes or ALK rearrangements. For these patients we suggested a new molecular test on a more representative sampling of the lesion when possible.

**Conclusion.** We genotyped 438 NSCLC by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) assay with CE-IVD Myriapod® Lung status kit (Diatech Pharmacogenetics, Italy) on Agena MassARRAY® System (Agena Bioscience, Inc. California). The system is a target method that investigates more than 230 mutations on 10 genes involved in NSCLC, with a DNA amount of at least 40 ng. In 35% cases, cytological samples were the only available material for molecular analysis (fine needle aspiration cytology, bronchoaspirate or pleural effusion liquid). For 65% cases we had histological samples available (surgical specimens, secondary lesions samples or small endoscopic biopsies).

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**Conclusion.** In our organization the diagnostic accuracy was 97.3%. The inadequate cases rate would had been 23% if only Real Time or sequencing methods were available. Considering the generally mutual presence of EGFR/KRAS mutations and ALK rearrangements we avoid to 42 patients a new diagnostic bronchoscopic exam. This performance has been obtained by a multidisciplinary approach that tends to an efficient management of the sample coupling ROSE with sensitive molecular multitarget techniques. Cooperation between Pneumologist, Pathologist, Molecular Biologist and Oncologist is essential for the management of NSCLC patients.

**PLEURO-PULMONARY SYNOVIAL SARCOMA: CLINICO-PATHOLOGIC AND MOLECULAR CHARACTERISTICS FROM A MULTI-INSTITUTIONAL SERIES OF 48 CASES**

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**Introduction.** Synovial sarcoma is a high-grade malignant soft-tissue tumor that most commonly occurs in the extremities of young adults. Primary synovial sarcoma of the thoracic region is rare, but pleura and lung represent the most common visceral site. Synovial sarcoma may pose diagnostic difficulties at imaging studies, histology and immunohistochemical ground, but the finding of the specific chromosomal translocation t(X;18) (p11.2;q11.2) is highly specific in challenging cases. Surgery with margin-free margins is the only curative therapy, while chemotherapy and radiotherapy had little therapeutic effect and the prognosis is generally dismal. We collected a large series of primary pleuro-pulmonary synovial sarcoma (PPSS) in order to detail on the main clinico-pathologic, molecular and prognostic features.

**Materials and methods.** Forty-eight cases of primary PPSS were identified from routine practice and consultation files of 8 different institutions. Data on symptoms, as well as imaging, histologic, immunohistochemical and therapeutic features were collected. Molecular data of EGFR (exons 18-21), c-KIT (exons 9,11,13,17), BRAF (exon 15) and PDGFRs gene mutations by direct sequencing analysis were performed.

**Results.** The case series included 29 male and 19 female with a mean age of 54 years (range, 16-86 years). Eleven patients were smokers (23%) and none had asbestos exposure. Chest pain was the most common symptom (67%). Left thoracic side was involved in 29 cases (60%) and 16 cases showed multifocality (33%). At imaging, 33 cases (69%) appeared as lung tumors and 7 had a mesothelioma-like growth (14.5%). Median tumor size was 72 mm (range, 16-130 mm). The original diagnosis of synovial sarcoma was performed in 33 cases (69%), while 7 cases were recognized as sarcomas not otherwise specified 3 cases as sarcomatoid mesotheliomas, 2 cases each of sarcomatoid carcinoma and malignant solitary fibrous tumor and 1 fibrosarcoma. Seventy-nine percent (38 cases) showed monophasic histology and half of cases received chemotherapy and radiotherapy alone. Recurrences were recorded in 31 cases, 33 cases were died of disease and median follow up was 21 months (range, 1-110 months). Molecular evidence of t(X;18) involving SYT-SSX fusion gene was performed in
Genomic copy number alterations in 22 malignant peritoneal asbestos-related mesothelioma by comparative genomic hybridization-array (CGH-A)

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Introduction. Malignant mesotheliomas (MM) typically originate in the pleura or, less commonly, in the peritoneum; the latter accounts for approximately 5-20% of all malignant mesotheliomas. Risk factors for mesothelioma include occupational or industrial asbestos exposure, genetic predisposition and radiation. Although peritoneal mesothelioma is associated with asbestos exposure, this relationship is not as strong as in the pleural counterparts. MM of the peritoneum show an extremely poor prognosis and the cases are generally found at an advanced stage. The tumor results from the accumulation of a number of acquired genetic events at onset. The most frequent events observed in MM are deletions in 1p21-22, 3p21, 4p, 4q, 6q14-25, 9p21, 13q13-14, 14q, 15q15, 17p12-13, 22q12, and amplifications in 1p36.33, 5p, 7p and 8q shown at comparative genomic (CGH) analysis. Deletion of 9p21 is particularly frequent in MM, especially those with a sarcomatoid component. However, 9p21 deletions have been reported to be less frequent in peritoneal than in pleura mesothelioma. Gelsolin (GSN) is one of the most abundant actin-binding proteins (ABPs), and has been found to be a multifunctional regulator of physiological and pathological cellular processes including cancer. Previous studies have indicated that GSN may be a tumor suppressor gene that exerts a crucial role in carcinogenesis. The chromosomal localization of GSN is the distal end of the long arm of human chromosome 9 in bands q32-34.

Material and methods. Here we describe the use of CGH-array to characterize the genomic copy number alterations profile, that could be related to the tumorigenesis in a set of 22 peritoneal mesothelioma samples (14 epithelioid, 5 biphasic, 3 sarcomatoid types). All patients were exposed to asbestos and in this study we considered only four women with a known history of asbestos exposure. Primary tumor tissues were obtained with laparoscopic procedures and multiple biopsies were performed in each patient. The lesions were disseminated widely throughout the peritoneal surface of the abdomen and pelvis. Patients did not undergo cytoreduction, receiving only adjuvant chemotherapy after diagnosis.

Results. Mean survival was 10.9 months. Amplifications were more frequent than deletions. The recurrent minimal loss regions were at loci 9p12-p11 (6/22 cases), 9q12-q21.33 (11/22 cases), 9q21.33-q33.1 (8/22 cases), 8p23.1 (10/22 cases), 17p12-p11.2 (6/22 cases), 1q21 (9/22 cases). No loss at 9p21 was detected. Frequent gains were at 1p36.33-p36.32 (16/22 cases), 3q29 (11/22 cases), 5p15.33 (15/22 cases), 7p22.3-p22.1 (10/22 cases), 9q34.11-q34.3 (12/22 cases), 10q26.3 (13/22 cases), 11p15.5-p15.4 (13/22 cases), 12q44.33 (8/22 cases), 13q34 (9/22 cases), 16q24.1-q24.3 (8/22 cases), 17q24.3-q25.3 (10/22 cases), 20q13.3 (13/22 cases), 21q22.3 (9/22 cases), Xq28 (14/22 cases). The 1p36 and 5p15 are the minimal recurrent amplified regions more frequently observed. These regions contain putative target genes involved in many cancers and also in peritoneal mesothelioma. Moreover, our data indicate a strong association of asbestos exposure with DNA deletions. In particular, chromosome 9 is more frequently involved during carcinogenesis, and many loci sites of putative target genes of asbestos fibers are damaged in mesotheliomas affecting different sites.

Conclusions. The Gelsolin gene in 9q23-34 is a novel candidate for the role of tumor suppressor gene in malignant peritoneal mesothelioma. Our findings suggest that the frequent loss of gelsolin expression may be involved in the development of malignant peritoneal mesothelioma, as a potential molecular target of asbestos-induced carcinogenesis.

ALK gene amplification in non small cell lung cancer: A possible role in target therapy

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Introduction. Anaplastic Lymphoma Kinase (ALK) rearrangement is a therapeutic target for the treatment of Non Small Cell Lung Cancer (NSCLC). ALK–rearranged NSCLC patients constitute a new subgroup responsive to Crizotinib, a specific inhibitor. ALK gene could be involved by other genetic alterations besides the rearrangement, including the mutations and the amplification, that could lead to a constitutive activation of the receptor. ALK amplification (ALK-A) was described for the first time as an oncogenic mechanism in neuroblastoma, generally associated with a poorer outcome. In the last years, several studies have been described ALK gene extra copies in various cancer such as Inflammatory Breast Cancers, Anaplastic Large Cell Lymphoma, Rhabdomyosarcoma, Carcinoma of the Esophagus, Adult Renal Cell Carcinoma, Hepatocellular Carcinoma. In particular, ALK gene extra copies have been frequently described in lung cancer regardless the rearrangement. Furthermore, in vitro studies have demonstrated that neuroblastoma cell lines harboring ALK-A are sensitive to ALK specific inhibitors, such as TAE684 and Crizotinib. In the view of the dramatic clinical benefit of Crizotinib, it would be desirable to enlarge the subset of NSCLC patients that could benefit from ALK-targeted therapy. ALK-A could have a potential therapeutic implication, however few data are reported until now.

Materials and methods. The main aim of this study is to assess a possible role of ALK-A in the constitutive activation of the receptor and its signaling pathway in a large serie of NSCLC. 520 NSCLCs were collected: 395 were
Adenocarcinomas (ADC), 15 Adenosquamous Cell Carcinomas (AdSqLCs) and 110 Squamous Cell Carcinomas (SCC). Tissue samples were used for the tissue microarrays (TMAs) building, using the most representative areas tumoral and non-neoplastic from each single case. ALK-A were detected through FISH using ALK probe Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe and CEP2 probe (a centromeric alpha-satellite specific for chromosome 2). ALK protein expression was assessed by IHC (automated assay on the Ventana BenchMark XT processor Ab anti-ALK D5F3 Ventana). In addition, IHC analysis were performed in order to assess phospho-ALK and the activation of ALK Downstream Molecules, such as phospho -STAT3, phospho-ERK. Finally, ALK RT-PCR was performed in cases harboring ALK gene extra copies.

**Results.** 95 cases out of 520 (18.3%) showed ALK copy number aberrations through FISH analysis. (Fig.1). Among cases harboring ALK gene extra-copies 69/95 (72.6%) showed chromosome 2 polisomy. 26/95 showed ALK gene copy number increased: 14 out of 26 carrying ALK copy number gain and 12 out of 26 ALK-A. (Fig.1). All 12 ALK-amplified cases not were ALK-rearranged, only 1 case showed concomitantly ALK copy number gain and rearrangement. All cases with ALK gene extra copies were negative by ALK and phospho-ALK IHC. (Fig.2)

**Conclusion.** Our data suggesting that ALK-amplified NSCLCs showed a loss of the protein, unlike the close association between ALK-rearrangements and the protein levels. Moreover, the lack of ALK protein expression in ALK-amplified cases might be partially explained by transcriptional or post-transcriptional regulations mechanisms, or by technical critical issues. Finally, ALK gene extra-copies has not currently have a clear clinical significance, however this aberration could be an intriguing target in NSCLC therapy and further studies are required.

**References**

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**Mercoledì, 23 novembre 2016**

Aula Maestrale – 12:30 – 13:30

Moderatori: Filippo Crivelli (Busto Arsizio) – Federico Tallarigo (Genova)

**Temi di interesse generale**

**ROUTINE WORKLOAD AND MEASURES IN A CLINICAL-ORIENTED IMMUNOHISTOCHEMISTRY & IN SITU HYBRIDIZATION PATHOLOGY LAB**

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**Introduction.** There are several indicators to measure the workload of activities in Anatomic Pathology, the most used is the number of histological cases or number of sectioned tissue slides from formalin-fixed and paraffin embedded blocks. Recently, the Italian Society of Anatomic Pathology (SIAPEC) assigned a weight to the activities performed in a Pathology Lab. We sought to investigate the workload and the related-productivity of immunohistochemical and in situ hybridization activities, in a clinical-oriented Pathology Lab.

**Methods.** We numbered the immunohistochemical slides and slides with in situ hybridization analysis, evaluated along the year 2015 at the Azienda Ospedaliera Universitaria di Verona
THE IMPACT OF INFECTIOUS DISEASE DIAGNOSES IN THE PATHOLOGICAL DAILY PRACTICE

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Introduction. Histopathological diagnoses are often centered on neoplastic lesions. Nevertheless, infectious diseases can be encountered during daily histopathological routine diagnoses. The aim of the present study is to investigate how often the pathologist is involved in the diagnosis of infectious diseases and how accurate is the identification and recognition of the microorganisms he can offer with the knowledge and tools available at a histopathological service.

Materials and methods. All the histological diagnoses carried out at the Unit of Anatomic Pathology at Bellaria Hospital, (Department of Biomedical and Neuroromotor Sciences, University of Bologna, Department of Oncology, AUSL Bologna) in the period January 1992 – June 2015 were screened to retrieve all the cases having had a diagnosis of infectious disease. The search was carried out using the software in use using key words for each microorganism. The diagnostic reports were reviewed and categorized as follows:

1. positive (POS) when was possible identify a microorganism in the histological slide;
2. negative (NEG) when the histopathological features were consistent with infectious disease but no microorganisms were clearly visible;
3. borderline (BL) when histopathological features were consistent with infectious diagnosis, but the histological identification of microorganism was uncertain.

In borderline and negative cases, records were retracted at the Microbiology Unit of the University of Bologna, in order to evaluate when specific microbiological analyses were further carried out.

Cases were further subdivided on the base of the anatomic site of the sample and on the type of infectious diagnosis. All, but the Helicobacter pylorii infections, were retrieved and reviewed.

Results. During the period under study (January 1992-June 2015) at the Unit of Anatomic Pathology at Bellaria Hospital were carried out a total of 553118 cases. Among those cases 50687 (9,2%) cases having a diagnosis of positive or suspicious (borderline) infectious diseases were identified. Most of the infectious disease diagnoses were gastritis related to Helicobacter spp. (40390 cases, 79,7%) and pneumonitis due to Mycobacteria spp. (5558 cases, 11%).

The remaining cases were composed of Human papilloma virus (2977 – 5,9%), Candida spp.(583 – 1,1%), Actinomycetes spp. (467 – 0,9%), Cytomegalovirus (131 – 0,2%), Herpes simplex virus (120 – 0,2%), Molluscum contagiosum virus (120 – 0,2%), Hepatitis C virus (107 – 0,2%), Aspergillus spp. (106 – 0,2%), Toxoplasma Gondii (54 – 0,1%), Leishmania spp.(38 – 0,1%), Enterobius vermicularis (30 – 0,06%), Giardia spp.(25 – 0,05%), Bartonella henselae (25 – 0,05%), Pneumocystis jiroveci (24 – 0,05%), Echinococcus spp. (13 - 0,03%), Mucor mycosis (7 – 0,001%), Treponema pallidum (6 - 0,001%), Taenia solium (5 - 0,01%), Hepatitis B virus (4 - 0,01%), Sarcopset scabiei (2 - 0,004%), Strongyloides stercoralis (2 – 0,004%), Mycobacterium leprae (1 - 0,002%), Paragonimus westermani (1 - 0,002%).

As expected, Mycobacteria (5558 cases) and Helicobacter spp. (40390 cases) are the infectious agents most commonly investigated by the pathologist, and show a high degree of certainty of the diagnosis (18 suspected diagnosis in the first case and no borderline diagnosis in the second case).

Excluding the diagnoses of HP, we detected 10297 cases of infectious disease, that means 448 cases per year. Therefore it seems that the diagnosis of infectious disease is not rare, even in a general anatomic pathology unit.

According to the empirical data presented here, the pathologist plays a key role in the detection of infectious agents in tissue samples, and can provide a rapid morphological diagnosis which facilitates the clinical management. The cooperation with the microbiologists is crucial to achieve a definitive diagnosis. Histology and cytology, together with laboratory cultures, represent the most effective means for a precise diagnosis of the disease. In this retrospective study, the implementation of the digital archive proved to be an essential tool, which allowed us to easily identify the cases of interest. In order to facilitate the research of cases in digital archives, it is advisable to mention a fixed and standardized expression (as example, in the search for alcohol-acid resistant bacilli), it will be helpful to enter if the detection is [+] positive, [-] negative, [+/-] borderline. In this way an immediate feedback will be provided after a search is launched for a specific key-word.

Conclusion. As already suggested by several previous studies, also in the present review it has been highlighted the crucial work of the pathologist in the detection of infectious agents, in collaboration with clinicians, radiologists and microbiologists. All collected details must be available for quick reviewing, and a digital database will constitute an indispensable tool to achieve this aim, thus providing the material for epidemiologic researches and quality control studies. In other words it is clear that the histopathology and microbiological techniques are complementary, and both have an undeniable importance in the diagnosis and optimal treatment of infections.
RARE TUMOR BIOBANKING: CHALLENGES AND OPPORTUNITIES. THYMIC EPITHELIAL TUMORS AS A MODEL

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Introduzione. Tumour banks aim to identify biomarkers in a collections of tissue samples and their matched normal counterparts (blood, urine, other body fluids) and derivatives (DNA/RNA/proteins), associated to clinical data and/or clinical trials. The amount and quality of clinical data linked to the samples determine the biological value of the specific tumor system considered. Biobank organization is a complex tool involving logistic infrastructures as well as specifically developed informatic tools and ethical issues. Biobank implementation requires a complex storage and advanced technical organization. In our Oncological Institute, a multidisciplinary Team is now engaged in implementing our Tissue and Biological fluids-based biobank, both in the Pathology Department and in the Clinical pathology Laboratory. Moreover, the clinical relevant notes are subjected to workup in order to be available with the biological resources. In recent years, frequent tumor-specific types have addressed the frozen tissue biobanking activity. At the same time, some rare cancers traditionally constituting one of our main foci of interest such as Thymic Epithelial Tumors (TET) have been followed and collected with specific and/or broad purposes. However, a rare tumor biobanking activity requires first a committed multidisciplinary team. This is by no means an obvious observation. Heavy clinical health assistance problems favour the dispersion of precious biological material, at least as far as fresh frozen biological specimens are concerned. However the development of molecular techniques allows now to perform complex molecular investigations also in formalin-fixed, paraffin-embedded (FFPE) material.

Metodi. Along the years our interest in Thymic epithelial tumors allowed to organize a basic database provided with the essential dataset and a good collection of both FFPE material as well as fresh frozen material. We are contributing to the effort of a scientific society dealing with TET, the International thymic malignancy interest group (ITMIG), which was able to organize the most extended database of TET, collecting more than 10,000 TET cases.

Risultati. Basing on FFPE tissues and an Italian multicenter collaboration, we realized recently a 200 case Tissue Micro Array based study on angiogenesis, as well a pilot study on the EGFR microsatellite CA-SSR-1 in TET. This last study took advantage of a longstanding collaboration with several institutions providing long follow up of TET patients. Moreover, the first study on mature MicroRNA in TET as well as in peritumoral thymic tissue could be performed on 3 cohorts from different Institutions, showing the down-regulation of miR-145-5p in TET. A combined use of FFPE and frozen material provided material for a gene expression study providing evidence that the signature of miR-145-5p putative target gene mRNAs inversely correlated to miR-145-5p expression. Moreover, the Regina Elena National Cancer Institute is currently involved in the project TCGA of thymic epithelial tumors (TCGA-THYM) of the National Cancer Institute (NCI, US). The study includes thymoma and thymic carcinomas. 117 frozen cases collected from several Institutions all over the world constituted the core of the study, associated to matched blood samples. Few European centres, and among them our oncological Institute Regina Elena, through the Department of Pathology, joined this international study. Mostly North American institutions represent the majority of the participating groups. The study is near to be accomplished and published with also our contribute.

Conclusioni. Our objective is to improve and to implement the internal as well the external collaborations in order to characterize biomarkers of prognostic and predictive value in TET. The scarcity of human resources more than the availability of tumor tissues constitutes the major difficulty. Our next purpose is also to implement our TET registry by adopting a relational database. For us as Pathologists every single case is unique and worth of attention. However when dealing with the development of precision medicine, large cohorts of well documented cases and the informatic contribution to data mining is essential.

References


NUCLEAR BUBBLES (NUCLEAR PSEUDO-PSEUDOINCLUSIONS). AN EASILY AVOIDABLE ARTIFACT, THAT MAY SEVERELY IMPAIR THE INTERPRETATION OF MICROSCOPIC SECTIONS

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Introduction. An intriguing artifact in histopathology consists of the presence of single or multiple nuclear vacuoles referred herein as bubbles) resulting in a bubbly appearance of the nucleus. We have referred to them as “pseudo-pseudoinclusions” to distinguish them from the bona fide nuclear “pseudoinclusions”, resulting from an invagination of the nuclear membrane and regarded as a pretty reliable marker of papillary thyroid carcinoma (Santoianni, 1990; Rosai, 2006; Ip, 2010). The bubbles that are the subject of this communication are variously sized, colorless or pale grayish and sometimes coalescent and multiple, resulting in an empty-like appearance of the nucleus, with loss of chromatin texture and margination of nucleoli. When numerous and affecting most nuclei, they may severely interfere with the identification of cell types and grading assessment, especially in lymphoid neoplasms (Santoianni, 1990), but also in epithelial and mesenchymal tumors (Rosai, 2006; Buesa, 2007; Ip, 2010).

These nuclear bubbles have been previously thought to be caused by incomplete fixation, dehydration or clearing during tissue processing, or by an excessively high temperature during tissue processing, water bath or sections drying (Santoianni, 1990; Ip, 2010; Rosai, 2008). We retained that this artifact developed after formalin fixation and paraffin embedding, in particular we hypothesized that bubbles may be caused by water film residues trapped under the section that forcibly evaporated in the oven (60° for 10 minutes or longer is a standard method in histopathology laboratories).

Material and methods. To test this hypothesis we selected 100 paraffin inclusions from 60 different specimens representative of various normal tissues and pathological processes, all showing obvious bubbles on the original slides. We obtained new sections and dried them with a new method: at room temperature, either overnight on the open or with a fan for 45 minutes. On all pairs of slides three pathologists scored in a blinded fashion the presence of nuclear bubbles as numerous (N), rare (R) or absent (A).

Results. Slides scored N were respectively 196 (65,4%) and 4 (1,3%) on the original and on the fan dried slides. Furthermore, all observers agreed that the chromatic differentiation of the tissues into eosinophilic and basophilic was more pronounced and the overall quality of the sections substantially better upon ventilation as opposed to forced heating.

Conclusions. Common practice currently discourages forced drying of sections, but here we present evidence that introducing this step can significantly diminish the number of...
pseudo-pseudoinclusions artifacts present on slides, creating overall better quality sections and consequently more accurate diagnosis.

References

PATOLOGIA DELL’OSSO E TESSUTI MOLLI

CIC-DUX4 UNDIFFERENTIATED SMALL ROUND CELL SARCOMA: REPORT OF A CASE

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Introduction. CIC-DUX4 undifferentiated sarcomas are a group of EWSR1-negative primitive tumors or Ewing-like sarcomas with fusion of the human homologue of the Drosophila capicua (CIC) gene on chromosome 19 and the double-homebox 4 (DUX4) retrogene on chromosome 4 or 10. The vast majority of these sarcomas occur primarily in peripheral soft tissues, but rare bone and visceral cases have been reported1-3.

Materials and methods. Here we report a case of CIC-DUX4 undifferentiated small cell sarcoma (USCS) in a 26 year old man involving the soft tissues of left forefoot, between the IV and V finger.

Results. Macroscopically, the lesion was grey, with areas of necrosis, measured about 3 cm and was localized in the subcutis. On hematoxylin and eosin sections, a round cell component predominated, which was focally set in a myxoid stroma. Foci of hemangiopericytoma-like vascular growth pattern and mitoses were present. Immunoistochemically, tumor cells were weakly positive for CD99, diffusely positive for WT1 and cytokeratins (Cam 5.2 and AE1/AE3), while chromogranin, synaptophysin, S100, HMB45, Mart1, calponin, p63, CD45, TdT, myogenin, desmin and smooth muscle actin were negative. Upon fluorescent in situ hybridization (FISH) analysis, CIC gene rearrangement was identified, while SYT rearrangement was absent.

Discussion. Recently, a distinct CIC-DUX4 fusion derived from a novel translocation of t(4;19) (q35;q13) has been detected in some of the Ewing-like sarcomas, and CIC-DUX4 fusion sarcoma has been established as a new type of Ewing-like sarcoma. The tumor tends to affect children and young adults and involves mainly the soft tissues of the trunk and extremities. Clinicopathologic studies of large series of CIC-DUX4 sarcomas have not yet been performed because of its rarity. However, it has been reported that patients with CIC-DUX4 sarcoma tend to experience an aggressive clinical course and poor outcome. The diffuse immunohistochemical staining for cytokeratins observed in this case represents a potential a diagnostic pitfall.

References

UNUSUAL BENIGN SOFT TISSUE TUMORS WITHIN THE SPECTRUM OF 13q14 DELETION

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Introduction. Spindle cell lipoma, mammary-type myofibroblastoma and cellular angiofibroma are characteristically benign mesenchymal tumors with 13q14 deletion. In this regard F.I.S.H. analysis shows monoallelic loss of both RBL and FOXO1 in the majority of cases. We have collected two benign soft tis-
sue tumors composed of bland-looking round to ovoid cells that resulted to be difficult to classify. Immunohistochemical analysis showed that neoplastic cells were fibroblastic or myofibroblastic in nature. FISH analysis for the detection of the FOXI, located on 13q14.11, was performed. Interestingly both tumors showed the monoallelic loss of the FOXI. Based on these cytogenetic findings we re-evaluated tumor morphology but both cases did not fit within the spectrum of spindle cell lipoma, mammary-type myofibroblastoma and cellular angiofibroma. The present study contributes to widen the spectrum of the benign soft tissue tumors with 13q14 deletion.

**Materials and methods.** We collected two cases of benign soft tissue tumors with unusual morphology. Case n.1 was a 15-year old girl who presented with a 3-cm polypoid lesion arising from the posterior pillar of the palatine tonsil. Case n. 2 was a 56-year old man who presented a painless subcutaneous nodular mass (2 cm in greatest diameter). Immunohistochemical studies were performed with the labeled streptavidin–biotin peroxidase detection system using a large number of antibodies. FISH analysis for the detection of FOXI, located on 13q14.11, was performed. The Locus-Specific Identifier FOXI Break Apart Probe (Vysis-Abbott), which hybridizes to band 13q14, was used. FISH analysis was performed, as previously reported in detail, to search for 13q14 deletion in mammary and vaginal MFB. Briefly, in a normal cell, two fusion signals are present; in a cell that lacks one 13q14 region, only 1 fusion signal is observed. All non-overlapping interphase neoplastic nuclei with intact morphology were analyzed using H&E-stained sections as histotopographic reference. The number of nuclei counted ranged from 49 to 63 (mean ± SD, 54 ± 4.5). A specimen was interpreted as deleted if only 1 fusion signal was detected in more than 22% of the nuclei evaluated (N3 SDs above the average false-positive rate observed in control FISH experiments on normal paraffin-embedded tissue). One positive (spindle cell lipoma) and 1 negative control (normal tissue) were included in FISH analyses.

**Results.** Histologically examination revealed similar morphological features. Both tumors were predominantly composed of bland-looking small- to medium-sized, round- to oval-cells set in a variably edematous stroma. Only focally short spindle or epithelioid cells were seen. A striking feature was the presence of numerous round- to stellate-shaped keloidal-like-collagen mats interspersed among neoplastic cells. In addition numerous small- to medium- sized, thin-walled blood vessels were present in the tumor stroma. Mitotic activity ranged from 1 up to 6 mitoses per 10 HPF. Immunohistochemical analyses showed a diffuse vimentin immunoactivity in both tumors. In case n.1 about 30% of neoplastic cells were stained with desmin. A variable expression of CD99, bcl-2 protein and CD10 was obtained in both tumors. No staining was obtained with CD34, myogenin, alpha-smooth muscle actin, cytokeratins. FISH analysis showed monoallelic loss of FOXO1/13q14 loci as indicated by the presence of 1 fusion signal in more than 40% of cell populations in both tumors. Similar results (in more than 80% of cell population) were obtained in the positive control (one case of spindle cell lipoma). A normal pattern of signals (2 fusion signals) was detected in the negative control (normal tissue).

**Conclusions.** We report two unusual benign soft tissue tumors with 13q14 deletion. These tumors exhibit a different morphology when compared with the other well-known tumors with 13q14 deletion. The present study suggests that the spectrum of the benign soft tissue tumors with 13q14 deletion is wider than commonly believed.
enable better detailed tumour clustering when designing clinical trials using drugs targeting patient affected by small cell neuroendocrine phenotype of urothelial carcinoma.

References

THE PARIS SYSTEM CLASSIFICATION IN URINE CYTOLOGY IN PATIENTS TREATED WITH EMDA AND SYNERGO THERAPY

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Introduction. Bladder carcinoma is the most common tumour of the urinary tract. In 2012, it is estimated that bladder cancer will account for 429,793 new cases of cancer and 165,084 cancer-related deaths in the world. Among men, it is the fourth most common cancer and is the ninth leading cause of death from cancer. The ratio of men to women that develop bladder cancer is approximately 3:1 (1). Of newly diagnosed bladder cancer cases, approximately 70%-80% will present with non muscle-invasive bladder cancer (NMIBC), and despite endoscopic and intravesical treatments, 50%-70% will recur and 10%-30% will progress to muscle-invasive disease (2, 3). The effective treatment of NMIBC encompasses a range of different procedures and interventions. Electromotive drug administration (EMDA) represents a minimal-invasive method of intravesical instillation of therapeutic agents without the need of general anesthesia. EMDA has been used to deliver a variety of intravesical drugs across the urothelium like Gemcitabine, BCG, Doxorubicin, Epirubicin and Mytomycin C. In terms of tolerability, the use of EMDA-MMC in clinical practice to date has never resulted in haematological toxicity, severe allergic reactions, life-threatening adverse events or any treatment-related deaths. One of the developing treatments for high-risk NMIBC is the combination of intravesical chemotherapy and hyperthermia (HT), called chemo-hyperthermia (C-HT; Synergo). HT is also directly cytotoxic and is known to alter intracellular metabolism, to damage DNA, to impair cellular proliferation, and to increase tumor cell apoptosis. In our study we selected patients with high grade non-muscle-invasive bladder cancer, BCG non responder, treated with EMDA-MMC and Synergo system and we also examined the morphological changes associated with these treatments in urine cytology cases classified according to The Paris System for Reporting Urinary Cytology (TPSRUC).

Methods. During the period from January to December 2016 in the Division of Anatomic Pathology of the Catholic University of Rome (Italy) 35 patients, BCG refractory, with high grade non muscle invasive bladder cancer were selected: 13 patients underwent to EMDA treatment and 22 patients underwent to SYNERGO System treatment. The morphological changes were evaluated in urine cytology cases processed by Thin Prep methods and classified according to the Paris System. The results were correlated with bladder biopsies, obtained at the same time of urine cytology or within 6 months after urine cytology samples. In those cases resulting as negative both in cytology and in subsequent biopsy, no evidence of malignancy was observed in following clinical investigations (ureteral washing, CT scan for urothelial tract) and during follow-up. The mean of follow-up was 15 months. All cytological samples and histological biopsies were evaluated by two different pathologists (FP, GF) and finally the cases were compared between two methods.

Results. In 7 out 13 cases EMDA treated and in 10 out 22 cases Synergo treated the cytological features were represented as follow: increase of cellularity, nuclear size, N/C, with nuclear border thickened, chromatine fine and regular with thin nucleoli and cytoplasm with vacuolitation. In the background inflammatory cells were present with rare evidence of anisokariosis. The cytological diagnosis in accordance to Paris system was atypical urothelial cells (AUC) while the histological diagnosis was normal or low grade epithelial dysplasia. For these cases no evidence of malignancy was observed during follow-up.

In 6 out of 13 cases EMDA treated and 12 out of 22 cases treated with Synergo, the urine cytology showed single cells, increase of cellularity, nuclear size and N/C, with nuclear border thickened, thick-irregular chromatin without evidence of thin nucleoli. The cytoplasm was reduced without vacuolization and in the background inflammatory cells and numerous anisokariosis were present. The cytological diagnosis in accordance to Paris system was high grade urothelial carcinoma (HGUC) in 10 cases, suspicious urothelial high grade carcinoma (SHGUC) in 2 cases. In all of these cases the histological diagnosis was high grade urothelial carcinoma non muscle invasive or in situ urothelial carcinoma.

Conclusions. In the current study we examined the cytological alterations present in urine of patients BCG refractory, with high grade non muscle invasive bladder cancer, treated with EMDA or Synergo. The increase of cellularity, nuclear size and N/C with nuclear border thickened were common in patients treated with EMDA and Synergo, and they seem to not identify bladder carcinoma. The presence of isolated cells and the thick-irregular chromatin with or without macronucleoli and the evidence of numerous anisokariosis in the background are typical signs of malignancy in patients treated with EMDA or Synergo. The Atypical urothelial cells (AUC) category of the Paris system in patients treated with EMDA or Synergo seems to have a different significant than in patients not treated.

References
Bisulfite Sequencing and Methylation Specific PCR were performed on androgen deprivation therapy (ADT) treated patients. versus distinguishing low versus high Gleason grades (≤3 versus > 3), real-time RT-PCR in microdissected PC cell populations, analysed in PC samples by immunohistochemistry and by in vitro and human PC cell lines. The expression and androgen regulation of the core pluripotency genes SOX2, OCT4A and NANOG was functionally assessed in vitro in two AR+ PC cell lines.

Results. The mean level of SOX2mRNA was considerably down-regulated in the neoplastic epithelial cell populations from both low and high grade PC, with no appreciable differences between them, with respect to the normal epithelium, whereas expression levels of OCT4A and NANOGmRNA were significantly down-regulated only in the epithelium from high grade PCa. Gene promoter methylation was involved at least in the down-regulation of both SOX2 and OCT4.

SOX2, OCT4A and NANOG were visualized distinctly, by immunohistochemistry, in most of the basal cell layer of normal prostate glands, while their expression was largely lost in PC, thus confirming at protein level the molecular data. In particular, their expression was detected in a few cells forming low grade PCa foci and completely lost in high grade PC foci, with the exception of a few cancer cells bordering the invasion/expansion fronts or arranged in stromal infiltrating sheets and neuroendocrine differentiation foci.

SOX2, OCT4A and NANOG expression was increased in high grade PC from patients who underwent ADT versus PC from untreated patients and versus each of the patient before the treatment.

In vitro, the treatment of 22RV1 and LNCaP cell lines with dihydrotestosterone significantly down-regulated the expression levels of SOX2, OCT4, NANOG and other relevant stemness genes.

Conclusions. The core pluripotency genes are selectively and focally expressed within PC and epigenetic mechanisms are involved in their regulation. Androgen down-regulate their expression and ADT, which use is discouraged by the current guidelines, may favor their up-regulation thus leading to PC dedifferentiation and tumor progression.

SOX2 and SNAI2/Slug Genes Are Critical Drivers of Neuroendocrine Differentiation and Progression of Prostate Cancer

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Introduction. Prostate Cancer (PC) is a heterogeneous disease, which ranges from a smoldering to a rapidly fatal systemic malignancy with overt metastasis at presentation. Thus, critical issues in this research field are: a. identification of the molecular drivers of metastatization and, b. early assessment of the risk for disease progression, which may lead to a rational clinical decision making.

SNAI2/Slug is the major epithelial-to-mesenchimal transition regulator during cell migration and tumor metastatization, whereas the SRY(sex-determining-region-Y)-box-2-gene, SOX2, is an essential embryonic stem cell gene deeply involved in prostate tumorigenesis.

Thus, we investigated the expression profile of both transcription factors in microdissected PC foci with different grades of differentiation, established the mechanisms underlying their regulation and revealed novel aspects of their implication in the malignant evolution of PCa. Furthermore, we assessed their diagnostic and prognostic value in a cohort of 206 prostatectomized PC patients.

Methods. Laser Capture Microdissection, qRT-PCR, Quantitative Methylation Specific PCR and immunohistochemistry were used to analyze SNAI2 and SOX2 gene expression and regulation in PC samples. The results were examined according to the patient’s clinical-pathological profile and follow-ups. Functional in vitro studies were performed using human PC cells transfected to overexpress or silence SNAI2, SOX2 or both.

Results. The expression of both SOX2 and SNAI2 was down-regulated in most PCa epithelia, in association with gene promoter methylation, except for cell clusters forming: a) the expansion/invasion front of high-grade PCa, b) NED areas, or c) lymph node metastasis.

Knockdown of SNAI2 in PC cells down-regulated the expression of typical neuroendocrine differentiation (NED) genes such as of neural-tissue-associated adhesion molecules, N-Cad, N-Cadh 2, NcCAM and of the NED marker ENO2, whereas it abolished Chromogranin-A expression. SNAI2 silencing also lead to a dramatic down-regulation of the pluripotency genes SOX2, NOTCH1, CD44v6, WWTR1/TAZ and YAP1.

Interestingly, SOX2 silencing also down-regulated neuroendocrine differentiation genes and abolished SNAI2/Slug dependent NED, whereas its over-expression up-regulated NED genes along with neurotrophins/neurotrophin receptors, the majority of pluripotency and epithelial-mesenchimal transition transcription factors, relevant growth, angiogenic and lymphangiogenic factors and promoted in vitro PC cell invasiveness and motility.

Multivariate analysis revealed that SOX2mRNA expression in the primary tumor was significantly associated with LN metastasis. When SOX2mRNA levels were ≥1.00, relative to (XpressRef) Universal-Total-RNA, adjusted Odds Ratio was 24.4 (95%CI: 7.54-79.0), sensitivity 0.81 (95%CI: 0.61-0.93) and specificity 0.87 (95%CI: 0.81-0.91). Patients experiencing biochemical recurrence had high median levels of SOX2mRNA

Conclusions. This study discloses a network of novel SNAI2 and SOX2 target genes, which are critical drivers of PC aggressiveness and progression. Of clinical relevance are: I. the identification of SOX2 as a critical target to hinder the metastatic disease and II. the proposal to assess SOX2 expression in the prostate needle biopsy as a novel biomarker of nodal metastatization and a useful tool for tailoring the extent of lymphadenectomy at surgery.
**CLINICO-PATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF PENILE CARCINOMA AND ITS ASSOCIATION WITH LICHEN SCLEROSUS**

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**Introduction.** Squamous cell carcinoma (SCC) is the most frequent penile neoplasia. Its incidence is 1/100000 person-year in Europe, Italy included, and North America but it is about 2-4/100000 person-year in Africa and South America. This neoplasia is seldom reported in population in which circumcision at birth is an habit. It is usually an asymptomatic flat or exophytic lesion, which may be ulcerated; glans is the most frequent site of involvement.

Two cancerization ways have been described, each associated with specific histotypes: HPV-related SCCs have alteration in RB/p16 and p21/p53 cellular pathways with eventually hyperexpression of p16 molecule; HPV-independent SCCs have alteration in HER/PTEN/Akt and also in p53, this situation is associated to chronic inflammation and particularly with Lichen Sclerosisus (LS). This disease have a low incidence rate (about 0.07% in males); it has a remittent course with inflammation and subsequent fibrosis clinically evident as ivory-coloured macules on genital areas associated with itch, pain and urinary and sexual dysfunctions and it can eventually lead to phymosis. LS has been variably associated to SCC (4-50%) and it seems that a man with LS has a 4-5% risk of developing SCC in his lifetime.

This study evaluate the association between SCC and LS and the histopathological and immunohistochemical (hormonal receptors ERα, ERβ, AR, PgR; p53 and Ki67) features of both.

**Material and methods.** Twenty-one patients underwent to curative-intended excisional biopsies, partial and total penectomy for SCC in IRCCS AOU San Martino-IST between 2005 and 2014. Patients who had only incisional biopsies or inadequate biopsies were excluded.

Histological slides of each patient specimens were reviewed and specimens containing normal epithelium, preneoplastic lesion and SCC were selected. Each specimen was immunostained and evaluated for ERα, ERβ, AR, PgR, p53 and Ki67.

Results: The most frequent histotype was usual SCC (15 cases, 71.4%). LS was detected in 12 cases (57.1%). Ki67 proliferation index progressively increased (p < 0.001) from normal epithelium (5.619%) to preneoplasia (12.952%) to neoplasia (30.476%) and AR expression progressively decreased (p < 0.001) from normal epithelium (52.048%) to preneoplasia (28.095%) to neoplasia (95.2%). Immunohistochemical p53 expression increased (p < 0.001) in neoplasia in relation to preneoplasia and normal epithelium. No significant differences are observed for expression of ERα, ERβ and PgR.

**Conclusions.** Usual type SCC resulted the most frequent histotype. Association of LS with SCC appeared slightly higher (57.1%) than what has been previously reported in literature. The increasing of Ki67 evaluated proliferation index is biologically obvious; non-significant difference of p53 expression between preneoplasia (i.e. LS) and normal epithelium is not in line with literature.

Progressive loss of AR in cancerization is interesting and could be therapeutically useful with chemopreventive application of topic androgens in high-risk patients.

**SOCS3 IMMUNOHISTOCHEMICAL EXPRESSION SEEMS TO SUPPORT THE 2014 INTERNATIONAL SOCIETY OF UROLOGICAL PATHOLOGY (ISUP) GLEASON GRADING SYSTEM**

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**Introduction.** In the 2014 International Society of Urological Pathology (ISUP) Consensus Conference redefines Gleason grading system. [1] In a recent report for the first time we demonstrated that a hypermethylation of SOCS3 with a significant reduction of its mRNA and protein expression identifies a subgroup of prostate cancer with a more aggressive behavior. [2] In this study we evaluate the immunohistochemical protein expression of the suppressor of cytokine signaling (SOCS) proteins 3 (SOCS3) in different Gleason grade prostatic adenocarcinoma: classical Gleason grade 3, classical Gleason grade 3 upgraded to Gleason grade 4 according to the ISUP modified grading system and classical and modified Gleason grade 4.

**Methods and materials.** Two different pathologists (FP and GP) on the basis of strictly classical (1977) and modified (2014 ISUP) criteria, assigned Gleason pattern in 148 different areas of prostatic adenocarcinoma. [1] The Gleason grade were stratified into 3 different categories on the basis of histologic pattern: (1) 26 prostatic adenocarcinoma with classical and modified Gleason pattern 3, (2) 57 prostatic adenocarcinoma with classical Gleason pattern 3 upgraded to Gleason pattern 4 by 2014 ISUP Conference and (3) 65 prostatic adenocarcinoma with classical and modified Gleason pattern 4.

In particular we collected 15 neoplastic areas with cribriform pattern and 5 cases with glomeruloid features, assigning a Gleason grade 4. Immunohistochemical analysis for SOCS3 was performed and the SOCS3 staining intensity were evaluated in three different ways on the basis of the intensity of cytoplasmatic staining: positive (strong positivity in all neoplastic glands and in all the cells in the same neoplastic gland); negative (negativity in all neoplastic glands and in all the cells in the same neoplastic gland) and weakly positive (weak intensity staining in all neoplastic glands and in all the cells or with isolated negative cells in the same neoplastic gland). Colonic mucosa were used as positive control according to manufacturer’s instruction.

**Results.** Considering the immunohistochemical expression of SOCS3 we found that the SOCS3 pattern staining negative (-) or weakly positive (+/-) increase progressively in concurrence with the rise of Gleason grade and SOCS3 positivity (+) correlate with classical or modified Gleason grade 3. Moreover comparing the prostatic adenocarcinoma with classical and modified Gleason grade 3 and the prostatic cancer with classical Gleason grade 3, upgraded to Gleason grade 4, we found that the SOCS3 negative staining and SOCS3 with weak intensity staining correlate with the upgraded group suggesting a difference between two groups not only for histological aspects but also for a molecular background. Interestingly in cases with prostatic adenocarcinoma with glomeruloid features a weak staining for SOCS3 in glomerulation structures was observed. (Fig. 1)

**Conclusions.** In conclusion our data seem to support, from a molecular point of view, the modified criteria by 2005 International Society of Urological Pathology (ISUP) Consensus Conference and by 2014 International Society of
Urological Pathology (ISUP) as well as the hypothesis that the diagnosis of Gleason cribriform pattern 3 virtually does not exist and cribriform glands regardless of their size are nearly always considered pattern 4. The data concerning the SOCS3 immunohistochemical expression in glomeruloid pattern also for the limited number of cases, due to relative rarity of glomeruloid features, suggest additional studies to validate our observation.

**Figure 1.** Immunohistochemical analysis of SOCS3 protein expression. Adenocarcinoma Gleason grade 3 displays a stronger staining for SOCS3 (A, C, D) and cribriform pattern both with small round glands and with large, round spaced lumina with regular contour (C, D). In glomerulisation structures (B) weak staining for SOCS3 was observed (avidin-biotin-peroxidase complex method in paraffin section lightly counterstained with ematofloxin. Original magnification 200X A, B, C, D).

**References**


**HIGH FREQUENCY OF ALK COPY GAIN IN RENAL CELL CARCINOMA**

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**Introduction.** Anaplastic lymphoma kinase (ALK) translocation renal cell carcinomas (RCCs) have been reported by independent groups. ALK-inhibitors may be a therapeutical chance when progression of the disease does present in patients affected by RCCs with metastases. We sought to investigate ALK gene abnormalities and protein expression in a series of routinely clear cell RCC.

**Methods.** Two series of clear cell RCCs have been Recruited from the file of I.R.C.S. Fondazione Pascale in Naples and AOUI in Verona. ALK break-apart FISH kit was performed on formalin fixed and paraffin embedded tissues (chromosome 2p21-23). Gene copy number (gains and monosomy) and rearrangements were evaluated. Antibodies for ALK1 and D5F3 were used on all cases.

**Results.** 178 and 150 cases were respectively studied. Overall, 17% of clear cell RCCs showed gains of the ALK gene locus, spanning from 3 to 7 copies. One case evidenced ALK rearrangement (G3, solid-papillar architecture). No protein expression was visible at immunohistochemistry by using both ALK1 and D5F3 antibodies.

**Conclusion.** 1) ALK gains are observed in a minor subset of clear cell RCCs; 2) only isolated patients may harbor ALK gene rearrangement (< 1%); 3) further studies are needed on tissue metastases from clear cell RCC.

**References**


**GEOMETRICAL FEATURES OF THE EXTRACELLULAR MATRIX ASSOCIATED TO MUSCLE INVASIVE BLADDER CARCINOMA**

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**Introduction.** Urothelial bladder cancer (UBC) is the most common malignancy of the urinary tract, causing 145,000 patient deaths per year. Based on tumor stage classification, UBC is grouped into non-muscle-invasive bladder cancer and muscle-invasive bladder cancer (MIBC). Patients with MIBC are treated with radical cystectomy, lymph node dissection and urinary diversion, and these patients are at high risk of metastatic tumor progression and cancer-related death. Modifications of the extracellular environments are mandatory for tumor progression, both at the primary site and in the metastatic niches. As a major component of the microenvironment, the extracellular matrix (ECM) contributes to tissue homeostasis, whereas modifications of native features are associated with pathological conditions. Three-dimensional geometry is an important feature of synthetic scaffolds favoring cell stemness, maintenance and differentiation. We used scanning electron microscopy to assess the 3D structure and geometry of the human bladder ECM obtained from decellularized tissues validated by histological and immunohistochemical analysis. We assessed physical and geometrical measurements of the ECM from i) healthy bladder mucosa and muscularis propria to obtain information regarding the design of innovative bladder scaffolds, and ii) bladder carcinoma to better understand tumor scaffolds, useful for assessing pathways associated with tumor invasion and representing an effective model to test drugs against bladder cancer progression.
AIM of this study was to establishing ultrastructural features of tissue layers ECM of the human bladder, detailing those modifications occurring with MIBCs. A thorough characterization of the healthy and neoplastic human bladder ECM would pave the way to i) assessing pathways leading to modification of bladder ECM during tumor progression and invasion; ii) modeling a 3-D synthetic bladder wall able to create an environment more similar to the in vivo microenvironmental niche conditions to test innovative therapies; and, iii) for promoting bladder regenerative medicine in patients eventually needing bladder augmentation.

Methods. ECMs were isolated according to a protocol recently published and validated on the human colon (Genovese L. et al. 2014). Histological and immunohistochemical analysis. Cells and nuclei in the tissues and ECMs were evaluated by hematoxylin-eosin staining; cellular antigens and the presence of stromal components were evaluated by immunohistochemistry. Scanning electron microscopy (SEM). ECMs for SEM were fixed with 2.5% glutaraldehyde for 24 hours, dehydrated and dried overnight in hexamethyldisilazane. ECMs were coated with gold-palladium and examined with a Leica S420 scanning electron microscopy. Fibrils width and degree of organization of fibrils (anisotropy) were estimated by using the function “measure” and the plug-in FibrI Tool in the Image J software.

Results. In the healthy bladder we assessed features of basal lamina, lamina propria (fig. 1A) and muscularis propria (fig. 1B).

The basal lamina ECM showed a flat surface characterized by regular “footprint-like” depressions and empty spaces representative of capillaries. Lamina propria and muscularis propria ECMs were composed of fibrils of different widths and geometrical organization. Intertwisted bundles in the muscularis propria were organized in a structure of crests and clefts. ECM associated with bladder carcinoma was composed of thin and organized fibrils forming a compact layer enriched with vessels.

Conclusions. This study provided information regarding the ultrastructure and the geometry of a healthy human bladder ECM. Likewise, we compared these features with those observed in a set of urothelial MIBCs. Here we report novel evidence depicting differences and similarities in term of ECM ultrastructure between a non-neoplastic and a MIBC human tissue, along with a number of detailed measurements of the geometry of the healthy lamina propria and muscularis propria. Second, the study showed that MIBC ECM is characterized by an increased degree of fibrils organization, in agreement with a recent report on colorectal carcinoma. Findings of the present study are exploitable for the design of synthetic non-neoplastic bladder and MIBC scaffolds, and for assessing pathways associated with tumor invasion. Integrating microstructure and geometry with biochemical and mechanical factors could allow for the creation of an innovative synthetic bladder substitute or a tumor scaffold predictive of chemotherapy outcome.

References

Comunicazioni orali

275 mortem performed at Sacco Hospital, we need to limit the environmental pollution and occupational exposure during the execution of the post-mortem in such cases. For this reason we developed a method that, in our opinion, is generally useful in infectious diseases, particularly in Prion diseases which, due to a long-term collaboration with the Neuropathology Unit of Besta Hospital and with the National Registry of Creutzfeldt-Jakob disease and related disorders (1), constitute a prominent part (about 50%) of our post-mortem workload.

499 post-mortem for suspected Prion diseases have been performed at Sacco Hospital over a period of fifteen years. A firm diagnosis of prion disease was confirmed in 387 cases, including all the known forms of the human disease, except kuru (see table).

Methods. The post mortem and gross examination, histological and molecular tests are performed in accordance with international protocols (2, 3, 4) and national rules (1): the anatomical theater is provided with a forced aspiration method from below and a dedicated table that, just before post mortem, is coated with a waterproof yard equipped with an absorbent side. All the activities and sampling operations are carried out on the table, the saw and other equipment used (knives, scalpels, scissors, pliers) are constantly kept wet during idle phases (to avoid desiccation of biological material) after passage in 2% sodium chlorine, at the end of the operations the non-disposable equipment is decontaminated with 2% sodium chlorine and subsequently with enzyme solution (Aniosyme DD1), washed and then autoclaved in accordance with the current decontamination protocols; waste is destroyed by incineration.

Numerous methods have been proposed for the opening of the skull in cases of suspected or confirmed Prion disease and all share the need to limit the environmental contamination by aerosol, as a precautionary factor. Methods using the oscillating saw in conjunction with vacuum systems are to be excluded absolutely, since the vacuum will increase, rather than decrease, the production of aerosols and the decontamination of the device will be very difficult or even impossible, too. A number of more or less ingenious systems based on external more or less hermetic and more or less transparent covers have been proposed. A system which for some time has had some spread uses the oscillating saw under a trellis with clear plastic coating. Anyone who has tried this system has found that it is difficult to apply, and also lengthens the time, increasing operator exposure. The walls of the plastic cover, moreover, get quickly dirty, making it difficult to view the operative field and thus increasing the risk of injury. We use a waterproof oscillating saw (Medezine 5000) and broke down aerosols entering a small amount of water directly in the protection originally designed for the vacuum. The blade should not be sanked beyond diploe during the opening of the skull: the best result would be to limit the cut to the thickness of diploe without damaging the dura underneath, in order to avoid spreading of cerebrospinal fluid. The skull must be eventually

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<td><strong>Sporadic Creutzfeldt-Jakob disease</strong></td>
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Figure 1.
removed as usual with the help of a hammer and chisel. The same method is used for the removal of the ethmoid bone and the opening of the floor of the anterior cranial fossa, required to perform targeted samples of the ophthalmic nerve, the upper rectus muscle and retina (see image).

**Results.** In the numerous cases in which we have applied the method, starting from routine cases on which the tests have been carried out, it was sufficient to use a small amount of water directly in the cutting zone in order to obtain a total dust suppression with a minimum contamination of easily washable equipment (saw + plastic cap) or materials destined for incineration (absorbent cross). The procedure also allows us to perform the task without the help of a second operator.

**Conclusion.** To our best knowledge this is the safest method to avoid environmental pollution and occupational exposure, although it requires some experience by the operator.

**References**


**DESCRIPTIVE EPIDEMIOLOGY ON SUDDEN UNEXPECTED INFANT DEATH (SUID) AND SUDDEN INFANT DEATH SYNDROME (SIDS) OF VENETO REGIONAL CENTER: NEUROPATHOLOGICAL EVIDENCES**

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**Introduction.** Sudden infant death syndrome (SIDS) is characterized by the death of an infant that cannot be explained, despite a systematic case examination, including death scene investigation, autopsy and review of the clinical history (1). SIDS has been known since the beginning of recorded history, in fact it was the topic of the Old Testament’s description about the wisdom of Solomon (2). Solomon had to judge by the dispute between two mothers. One of their babies was found dead during the night. Solomon offered to cut the surviving infant in half with his sword but only the true mother, of course, supplicated him not to. Sudden infant death syndrome was originally defined in 1969 focusing interest on sudden death in infants without an recognized cause (3). Nowadays, sudden unexpected infant death (SUID) is a wide-ranging concept used to describe any sudden and unexpected death, whether explained or unexplained, including SIDS, which occurs during the first year of life (4, 5). Sudden unexpected death that remains unexplained after complete postmortem evaluation is considered to be equivalent to deaths classified as SIDS; in other words SIDS represents an unexplained SUID. A statement of reaffirmation for this definition and its rules was published by the Task Force on Sudden Infant Death Syndrome in October 2014 (6). Several differing and sometimes contradictory hypotheses of the underlying mechanisms of SIDS have been proposed. The most reliable seems to be the “triple risk hypothesis”. Based on this theory, proposed in 1994 by Kinney and Filiano, unexpected infant deaths might arise as a consequence of the combination of three factors coming together: a vulnerable infant, a vulnerable phase of development and a final insult occurring in this window of vulnerability. In the past, different causes were recognized as main pathogenetic conditions, in particular they were proposed or primary cardiac alterations, or primary respiratory anomalies or primary neurological predispositions. Recently, an unified neuropathological theory contributes to describe SIDS. According to this, serotonergic neurons play a crucial homeostatic function for the cardiorespiratory brainstem centers. Consistent with this assumption, many studies have reported a high incidence of morphological abnormalities and biochemical defects of neurotransmission, particularly serotonergic, in the brainstem of SIDS victims compared with control infants dying of other causes (7). This brain region includes the main nuclei and structures that coordinate the vital activities, such as cardiovascular function and breathing, before and after birth (8).

**Methods.** By the logical inference of Charles Sanders Peirce (1839-1914) we intend to apply the abductive reasoning on SUID of Padua Reference Centre of Veneto Region during the last two years. To abduce means to “guess” a hypothetical explanation from an observed circumstance. In abductive reasoning, unlike in the deductive one, premises do not guarantee the conclusion. One can comprehend abductive reasoning as “inference to the best explanation”. Diagnostic expert systems frequently employ abduction and that is why, in the scientific method, the causes of events are still unknown, although several even conflicting pathogenetic hypotheses of the underlying mechanisms have been proposed. It is thus possible to perform abductive analysis in the presence of missing or incomplete input evidence, which normally results in degrees of uncertainty in the output conclusions. Obviously, this method would will fit on SIDS pathogenesis because it is in the “niture and nurture” of unpredictable biological events, asking help to the “guessing”. According to our assumption, we intend to use the tool of neuropathological investigation to analyze all of SUID cases of Padua Centre from 2014 to 2016. By means of this approach, the neuropathological abduction becomes an inferential method to obtain some additional information for “bona fide SIDS”. Therefore, based on our abductive and descriptive epidemiology approach, we finally propose the “fourfold risk hypothesis” because we suggest to add into whole risk the “sine materia” condition too (9, 10, 11). Neurocardiac genes of susceptibility could explain brain-heart expression patterns and molecular phenotypes of neurocardiac genes linked with some of the “true unexplained SIDS” (12). In order to obtain this, we utilized the inferior olives of medulla oblongata as neuropathological biomarker of sudden cardiorespiratory arrest, to assess the full extent of time course of hypoxic-ischemic brain injury during unexpected sudden death in infancy. In fact, as reported in the literature (13, 14), neurons of inferior olive nucleus are particularly vulnerable during subacute hypoxic-ischemic insult, perhaps by retrograde trans-synaptic neurodegeneration from cerebellum connections. On the contrary, sparing of the inferior olivary nuclei, may be associated with cases of cardiac arrest encephalopathy (15, 16).

**Results.** We analyzed 14 cases of SUID, 5 of which were finally classified as SIDS. By double blind we counted the percentage of morphologically “normal” cells on apoptotic ones of medulla oblongata inferior olive nuclei. We obtained a high significant difference of alive cell percentage between 9 SUID cases (high level of apoptotic cells) and 4 SIDS cases (high level of alive cells), respectively. One “bona fide SIDS” case has been segregated from the other four SIDS, because it shown high level of apoptotic cells in inferior olive nuclei,
A HISTOPATHOLOGICAL AND CLINICAL ANALYZE OF THE GLIOSARCOMA CASES OF A SINGLE INSTITUTIONS

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Introduction. Gliosarcoma is a biphasic tumor that has both glial and mesenchymal component or the glial component gained subsequently a mesenchymal phenotype. Over the time these tumors were considered as two distinct entities, the first one has been considered as the real “gliosarcoma” and the latter as glioma with desmoplastic metaplasia(1, 2). The pathogenesis and the diagnosis of these tumors has been a topic of controversy.

Conclusions. Neuropathological analysis of bulb inferior olives represents a good tool to infer on the final events of SUID and in particular of “bona fide SIDS”. Among SIDS cases, only those with high rate of live cells in inferior olives nuclei could be considered the consequence of cardiorespiratory arrest and, therefore, part of “true SIDS” sine materia.

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PATOLOGIA CARDIOVASCOLARE

EOSINOPHILIC ALVEOLITIS IN A NEARLY-DROWNED YOUNG MAN AFFECTED BY PAPILLARY FIBROELASTOMA: A CASE REPORT

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Introduction. A pulmonary inflammatory infiltration rich in eosinophils can be present in many different conditions, therefore a differential diagnosis is needed. Among recog-
nized causes, infections and exposition to drugs are some of the most common; an idiopathic form has also been described. **Materials and methods.** We report a case of a 17-years old nearly-drowned man who deceased during intensive care. Autopsy was performed to determine the cause of death. **Results.** Histopathological slides of the lungs showed a spread inflammatory infiltration rich in eosinophils suggesting the diagnosis of eosinophilic alveolitis. Moreover, necroscopy revealed that the patient was unexpectedly affected by papillary fibroelastoma of heart, which is commonly considered as cardiovascular neoplasm, though its pathogenesis is still discussed. **Conclusions.** The presence of papillary fibroelastoma was thought to have dramatically contributed to death. The finding of eosinophilic alveolitis was put in relation to intensive care the patient underwent.

**EXOME AND WHOLE GENOME SEQUENCING FOR CHD DIAGNOSIS AND TREATMENT DURING PREGNANCY**

A. Capuani

**Introduction.** Many forms of Congenital Heart Disease (CHD) are nowadays detected by fetal echocardiography and with prenatal diagnosis of chromosomal abnormalities. Certain anomalies may be present in utero but have disappeared at the time of birth for the evolving plasticity of the myocardium through yet unknown triggering factors. Is there a human model to investigate not invasively the ongoing cardiac morphogenesis?

**Methods.** We anatomically assessed the hypothesis that the cardiac ventriculo-arterial malformations in any settings are sequential stages of the same embryogenetic and teratological process at ventricular level: the Malrotation of the Outlet Septum and the Trabecula Septomarginalis (TSM). (1,2) We reviewed the current investigation tools offered by the Molecular Biology with the enrichment strategies to amplify the targeted DNA.

**Results.**
1. The TSM on the normal V shape or variants follows the development of the right ventricle that we call Virtual Ventricle (3),
2. The sequential TSM malrotation is an anatomical teratological continuum (Shwalbe 1906) what we propose as Human Model for investigations on the developing heart,
3. Each cardiac phenotype has to be considered an independent molecular expression of the same pathological post transcriptional process.

**Conclusions.**
1. There is a molecular identity for each cardiac phenotype.
2. The TSM’S rotation model by Exome and Whole Genome Sequencing let targeting genetic and epigenetic variations and post transcriptional anomalies as well as the trigger factors.
3. These concepts open to the possibility of a not invasive early diagnosis of CHD and consequent molecular treatments during pregnancy: the Molecular Cardiac Surgery (4,5).
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BACK TO THE ORIGIN: EXPRESSION OF SEMAPHORIN 3C AND PLEXIN 2A IN A CASE OF CARDIAC PAPILLARY FIBROELASTOMA

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Background. Cardiac Papillary fibroelastomas (CPF) are rare benign tumors of the heart, constituting the second most common primary tumor of the heart after the myxomas. They are mostly found incidentally at the time of the usual cardiac investigations, like echocardiography or cardiac catheterization, and are mainly located on cardiac valves. Over 75% of the CPF develops on the valves, while the other ones are located on different endocardio surfaces. The aortic valve is the most involved site, followed by the mitral valve, while tricuspid and pulmonary valve involvement have been occasionally described. Clinical presentation, in symptomatic patients depends usually on the tumor location and is mainly due to fragmentation of the fronds with consequent cerebral, systemic or coronary embolization. Nevertheless, heart failure due to valvular obstruction, pulmonary embolism, pulmonary hypertension and sudden death are some rare clinical manifestations previously reported.

Methods. A 67 year-old lady was past medical history of diabetes, COPD (chronic obstructive pulmonary disease) was admitted to the emergency department after a cerebral transient ischemic attack. The routine echocardiogram showed an intracavitary right atrial mass, so the patient was prepared for surgery.

A spheroidal, 2.5 cm large, gelatinous mass, originating from the tricuspid annulus between the anterior ad the septal cusp, was resected and sent for histological evaluation. The operation was straight forward and the patient was discharged in sixth postoperative day. The first echographic follow up at six months was normal, with no evidence of new intracardiac masses.

Macroscopical examination showed a mass with a “sea anemone” appearance, 2,5x1,8x0,9 cm in size. Histologically a papillary lesion, constitute of avascular fronds of collagen and elastic fibres, lined endothelial cells. We cultured cells from CPF.

Immunohistochemistry and western blotting was performed in tissue and in cultured cells.

Results. Immunohistochemical and immunofluorescent examination showed positivity of the surface lining cells for CD31, S-100, Ferritin heavy chain, ferritin light chain, Tenascin, Plexin 2A, Semaphorin 3C; negativity for Cd1a. Immunohistochemical data were confirmed by Western blotting. Moreover we cultured cells from the lesion and analyzed them for protein expression by western blotting showing positivity for Plexin 2a, Semaphorin 3C, MITF. Immunocytochemical analysis of cultured cells demonstrated positivity for Calretinin, S-100.

Conclusions. The expression of Semaphorin 3C and Plexin 2A in CPF tissue and in Cultured cells could suggest the origin of this lesion from the neural crest.
**Introduction.** Digital Pathology represents one of the key factors in the future of organ transplantation. The association of pathologist’s skills with IT is emerging as a tool to support decision making processes in the diagnostic and surgical workflow related to transplants. Our aim was to review the reliability of digital pathology and telepathology services.

**Methods.** We searched scientific products in PubMed by coding key words as follows: transplantation, digital pathology and telepathology, during the last 10 years. To each article has been given a score on eight different categories concerning digital pathology aspects in transplantation: preparation, administrative, digital imaging creation, image analysis, image display, reliability study, general IT management and system integration.

**Results.** A total of 294 scientific articles have been found from 2006 to 2016. Twenty-five articles have been analytically detailed. The remaining articles were not included as they could not be categorized. Twenty-seven per cent of the articles comprised studies related to concordances and reliability in between digital versus not digital instruments or advanced digital systems (i.e. digital pathology versus standard microscopy) in order to assess digital image display systems or image analysis algorithms. 19% of the articles evaluated image analysis algorithms applications or digital images. 18% of the articles reported the use of digital pathology image display systems and 15% of the articles focused the digital image creation. 8% of the articles provided a focus on the pre-analytical phase of slide preparation. 5% of the articles included administrative aspects. 4% of the articles described the IT management and 4% of the articles developed issues on system integration between Healthcare Information Systems.

**Conclusions.** 1) review of the Literature provide all information to build safe digital pathology and telepathology services during organ transplantation; 2) most of studies verified the quality of image digital analysis, image display and digital imaging creation; 3) overall, reliability between digital systems versus traditional systems resulted in almost perfect concordances; 4) major investments has to be posed to system integration systems and IT management or administrative staff in order to overcome unmet needs when building digital pathology services during organ transplantation.

**References**


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**Giovedì, 24 novembre 2016**

Aula Maestrale – 18:00 – 19:10

Moderatori: Ezio Fulcheri (Genova) – Leonardo Resta (Bari)

**PATOLOGIA FETO-PLACENTARE**

**SISD AND SIUD: THE EXPERIENCE OF THE REGIONAL REFERENCE CENTER OF LIGURIA REGION. THE FIRST YEAR OF DATA AFTER THE APPLICATION OF LAW N. 31, 2 FEBBRAIO 2006**

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**Introduction.** Sudden infant death syndrome (SIDS) is a condition in which an infant, usually in the early postnatal period and nearly always before 6 months of age, dies usually during sleep for unexplained reasons. Sudden intrauterine unexplained death (SIUD) is defined as the death of a fetus during the third trimester of pregnancy. For both SIDS and SIUD has been proposed various physiological explanations of risk factors (include the prone sleeping position, overheating by excessive bundling, viral upper respiratory tract infections, parental smoking at home, and birthing injury resulting in an insult to the inner ear and central chemoreceptor zone, an immaturity that involves CO2 chemoreceptors that regulate respiratory control). Since 2006 in Italy there was a specific law for the management of SIDS and SIUD cases. The law became effective only in December 2014 (G.U. Serie Generale, n. 272 del 22 November 2014) and provide the institution of Regional Reference Centers and the application of precise protocols of intervention that are part of the law. The Regional Reference Center for prevention and study of SIDS (Sudden Infant Death Syndrome) and ALTE (Apparent Life Threatening Events) of Istituto Giannina Gaslini – Emergency Unit exist since December 2010 (regional resolution “D G R n° 1543”) and so Liguria Region was one of the few Regions in Italy that followed the previous law disposition and that in 2014 was ready to operate. In this report we want to submit the data of our experience as part of the Regional Reference Center for Liguria Region.

**Methods.** From 31 December 2014 to 31 May 2016 we collect 16 autopsy cases of SUID. Three cases came from the Gynecology and Obstetrics Unit of our Institution and the other 13 cases arrived from several hospitals all around our Region (2 cases fro0se from ASL 3 - Ospedale Villa Scassi Sampierdarena, 2 cases from E.O. Ospedali Galliera, 1 case from IRCCS -AUO San Martino -IST). Two cases were suspected SIDS and fourteen were suspected SIUD cases. In these latter cases we have collected and examined even the placenta as recommended in the law text. At the same time, as the pathologists performed the autopsy and the istological
investigation, the clinicians of the Regional Reference Center proceeded to preliminary interview with the parents in order to collect informations about the past and next pathological anamnesis. As soon as the histological examinations were completed we link all these data together and then, when we come to a diagnosis, we organized a multidisciplinary meeting to communicate the results to the parents.

**Results.** During the last seventeen months of activity of the Regional Reference Center for prevention and study of SIDS (Sudden Infant Death Syndrome) and ALTE (Apparent Life Threatening Events) we examined 16 cases of suspected SIUD with the new protocols required by law. There was another case in which we were not able to perform the autopsy because we lacked the father consent; the consent of both mother and father is mandatory for the execution of all the exams as it is specified in the first article of the new law. The two suspected SIDS cases were one of an infant boy of 3 months old and the other one of an infant girl of 2 months old. In the former case we found a brain growth restriction of the right emisfer and a severe pneumonia and in the latter one an important pneumonia and a nephritis as a cause of death. In the 14 cases of suspected SIUD the median gestational age at the time of death was 35 GW (with a range from 29 GW to 40 GW). In 13 of the 14 cases we find that the causes of death were due to several placental disorders and that often there was an association between multiple placental diseases or dysfunctions (infection diseases, placental malperfusions, unknown maternal diabetic disorder, fetal thrombotic vasculopathy, umbilical cord true knots, excessively long cord and anomalous cord insertion such as furcate insertion). Only in one cases at the time of the autopsy we find several fetus malformations consistent multiple congenital heart defects (transposition of the great arteries, ventricular septal defect, hypertrophy of right ventricle, stenosis of right ventricular outflow, stenosis of ductus arteriosus), left lung with three lobes and sindattilia. The placenta showed aspect of karyotype alterations. This was a non monitored pregnancy of an immigrant woman that was recently landed in Lampedusa. In none of the cases there was need for further tests (genetic, toxicological etc.).

**Conclusions.** Sudden infant death syndrome (SIDS) is a quite rare condition that in Italy has an 0.5% incidence on newborn every year, while sudden intrauterine unexplained death (SIUD) it has a much higher incidence (4-5%) and in our experience we confirm these data. In our experience we see the importance of the constitution of a Regional Reference Center in which different professional role (clinician, pathologist, forensic etc) can collaborate and work together to reach a diagnosis and to support the parents during an heartbreaking period. We have experienced that for us, as pathologist, one of the most difficult problems came from the management of the material for ancillary investigations. Especially we find that at this point it is difficult to identify those responsible to take care of all these biological materials. Once again we want to stress the importance of the placental examinations in the suspected SIUD cases. Placental examination, both gross and histological, must be performed in the same place and by the same pathologist that perform the autopsy. During this year the Regional Reference Center also worked in Istituto Giannina Gaslini to set up operational instructions to standardize procedures. These operative instructions will be the guide lines even for the other ligurian hospitals. Finally we believe that now it can be the time for an exchange of experience between the various Regional Reference Centers in order to understand if the provisions of the law are now operative and what can be improve for the future.

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**TEN YEARS OF AUTOPSIES OF MALFORMED FETUSES: TECHNICAL, EPIDEMIOLOGICAL AND PATHOLOGICAL CONSIDERATIONS FROM AN INSTITUTIONAL REGISTER**

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**Goal.** Clinical autopsies are declining worldwide, often wrongly considered as the ultimate insult to the deceased or a potential cause of medico-legal disputes. It is not the case of fetal and neonatal autopsies that remain stable or even increasing and that in any case must be made for legal obligation. Even in the case of therapeutic abortion for fetal pathology, the parents themselves often ask for autopsy of their unborn child. The recognition of genetic syndromes and relevant maternal diseases represent one of the cornerstones of future pregnancies programs. They also constitute an integration and verification of modern imaging and prenatal diagnostic techniques. The aim of the study was the critical review of a long autopsy series of malformed fetuses in a regional reference center.

**Methods and materials.** The study is based on the computerized archive of the Pathology Unit of IRCCS San Martino-IST. We reviewed all reports of fetal autopsy for the period 2004-2014 with gestational age > 12 weeks. Both spontaneous abortions and therapeutic abortions (ITG) are included, all cases with a reported abnormal karyotype, all cases with one or more malformations at autopsy and all cases with morphological changes compatible with karyotype abnormalities observed at histological examination of the placenta. Every case was autopsied according to institutional protocol: after a thorough external examination with annotation of the main anthropometric measures, all the organs have been dissected separately according to Virchow procedure then grossly described, appropriately sampled and histologically examined. The histological specimens were routinely processed, embedded in paraffin and cut to obtain 3-5μm-thick histological sections. For reporting the macro and microscopic findings an institutional, checklists-based protocol has been used. For all the cases also placenta underwent to pathological examination. Data obtained from the autopsy report were included on an Excell © database for appropriate statistical analyses.

**Results.** A total of 302 cases were considered eligible for purposes the study; about 30 cases per year and representing more than a third of the total institutional autopsy activity. The average age of the mother is rised fairly advanced, (34.5 ±
Discussion. Different patterns of interarterial anastomoses have been described in the Literature regarding cord vessels free segments of the cords or membranes. Significant histopathological findings in the chorionic plate, including a short common venous trunk, in the absence of other abnormalities within an abnormal cord fusion.

Conclusions. Fetal autopsy require a specific expertise and training, presented series represent a sort of compendium of human malformative pathology. The autopsy of the malformed fetus is a complex procedure: it requires multidisciplinary and, at the same time, super-specialist knowledge that can be guaranteed only in a regional reference center. The issuance of a comprehensive and effective autopsy report cannot be separated from the integration of different professional figures and may represent the starting point of the organization of mourning and planning of future pregnancies.

UNUSUAL VENO-VENOUS INTRA-CORD ANASTOMOSIS IN MONOCHORIONIC MONOAMNIOTIC TWIN PREGNANCY WITH UNEXPECTED PRETERM DOUBLE STILLBIRTH: CLINICAL DIFFICULTIES AND POST-MORTEM DIAGNOSIS

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Introduction. The authors present a case of unexpected preterm double stillbirth in monochorionic -monoamniotic (MOC-MOA) pregnancy, which revealed peculiar vascular abnormalities within an abnormal cord fusion.

Case report. Unexpected double stillbirth occurred in a 26 year-old woman at 35 weeks and 6 days of gestation in a previously normal pregnancy. Gross examination revealed only mild intrauterine growth restriction in both foetuses, while previously normal pregnancy. Gross examination revealed only mild intrauterine growth restriction in both foetuses, while only a small minority of the study population showed alterations in > 4 districts (n = 15; 5%). The most affected district resulted the skeleton (n = 80, 26.49%), followed by soft tissue and skin (n = 65, 21.52%), the heart and vessels (n = 55, 18.21%), digestive tract (n = 46, 15.23%), genitourinary tract (n = 39, 12.91%), CNS (n = 37, 12.25%), lung and thymus (n = 36, 11.92%) and umbilical cord (n = 12, 3.97%). In every district much of the individual observed alterations were very rare and sporadic and rarely showed a greater than 5% overall incidence even in the most common forms.

Conclusions. Fetal autopsy require a specific expertise and training, presented series represent a sort of compendium of human malformative pathology. The autopsy of the malformed fetus is a complex procedure: it requires multidisciplinary and, at the same time, super-specialist knowledge that can be guaranteed only in a regional reference center. The issuance of a comprehensive and effective autopsy report cannot be separated from the integration of different professional figures and may represent the starting point of the organization of mourning and planning of future pregnancies.

MASSIVE HEPATIC SUBCAPSULAR HEMATOMA AS AN UNEXPECTED HYPOXIC COMPLICATION: PREVENTABLE NEONATAL DEATH, NOT A IATROGENIC RUPTURE OF THE LIVER! A CASE SERIES

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Introduction. The physiopathology of massive subcapsular hematoma of the liver as a possible explanation of unexpected
and very rapid clinical deterioration of neonates showing anemia is too often ignored. However, neonatal death due to this complication may be avoided through timely diagnosis and appropriate therapy.

Subcapsular hematoma of the liver (SHL), consisting of a confined collection of a variable amount of blood which raises the hepatic capsule, rarely occurs in neonates, and is often undiagnosed or misdiagnosed, even in post-mortem examinations. SHL seems to be more frequent in premature neonates and associated with maternal, placental or fetal factors, that - in case of pressure surges - can suddenly increase its volume determining the rupture of the capsule. It has a non-specific presentation characterized by an unexplained progressive anemia and it can be very subtle especially in infants with very low birth weight, preterm delivery and/or suffering from intrauterine hypoxia. The diagnosis is frequently made on autopsy, the main finding being hemoperitoneum.

Materials and methods. The presented cases, which were studied at the Istituto Giannina Gaslini over the last 4 years of routine activity, were as follows:

Results. The first two cases underwent clinical autopsy, the third one a complete forensic approach. In all three cases, however, after clinical data collection, macro- and microscopic examination led to the conclusion that death had been caused by anemia due to hemoperitoneum, following the rupture of the hepatic capsule caused by the presence of a huge amount of sub-capsular blood.

Postmortem findings were regularly explained and delivered in programmed sessions of medical education to the entire pediatric hospital. In addition, the clinically acute and unexplained anemia together with a changed darkish color of hepatic area in the abdomen following difficulties in the extraction of the fetus despite caesarean section promoted an early sonographic diagnosis of SHL, promptly followed by the infusion of blood and fresh frozen plasma, by continuous inotropic support with dopamine. Hodge (1870) was probably the first to report a case of SHL. The recent literature reports a varying incidence of SHL from 1.2% to 9.6% in autopsy series, and the severity of the bleeding is described as potentially life-threatening within minutes, before treatment can be begun.

Conclusions. The authors would like once again to stress that regular exchange of information among clinical pathologists, neonatologists, obstetricians and gynecologists is the only effective method in order to achieve a widespread knowledge of this rare neonatal condition predisposing to sudden and unexpected death. Among these, SHL should be considered in cases of unexplained and very rapid clinical deterioration of neonates showing signs of anemia and hypovolemia, in our experience particularly in preterm babies with extremely low birth weight (birth weight less 1000 grams at birth) and in perinatal conditions associated to intrauterine hypoxia. Early diagnosis and treatment remain essential points to avoid fatal hemorrhagic shock.

Table I. Massive hepatic subcapsular hematoma as an unexpected hypoxic complication: preventable neonatal death, not a iatrogenic rupture of the liver! A case series.

<table>
<thead>
<tr>
<th>Case</th>
<th>Gestation (weeks)</th>
<th>Birth weight (grams)</th>
<th>Sex</th>
<th>Delivery</th>
<th>APGAR Score (1 min and 5 min)</th>
<th>Clinical presentation</th>
<th>Diagnosis of hemoperitoneum due to rupture of SHL</th>
<th>Outcome/Age at death (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24+2</td>
<td>782</td>
<td>F</td>
<td>Vaginal, breech presentation</td>
<td>5 and 5</td>
<td>IUGR, suspected perinatal asphyxia, leukopenia and low platelet count</td>
<td>Post-mortem</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>35+4</td>
<td>3065</td>
<td>F</td>
<td>Vaginal</td>
<td>0 and 0</td>
<td>Intrauterine fetal death</td>
<td>Post-mortem</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>39+4</td>
<td>3410</td>
<td>M</td>
<td>Vaginal, two Kristeller’s procedures</td>
<td>9 and 9</td>
<td>Severe respiratory distress with metabolic acidosis</td>
<td>Post-mortem</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>29+2</td>
<td>560</td>
<td>F</td>
<td>Caesarean section</td>
<td>8 and 9</td>
<td>IUGR, respiratory distress, anemia, pathologic Eco Doppler</td>
<td>Early postnatal</td>
<td>Surviving</td>
</tr>
</tbody>
</table>

Figure 1. Massive Hepatic Subcapsular Hematoma as an Unexpected Hypoxic Complication: Preventable Neonatal Death, not a iatrogenic Rupture of the Liver! A case series.

FATAL ACUTE FATTY LIVER OF PREGNANCY: A PARADIGMATIC CASE REPORT

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Introduction. Acute fatty liver of pregnancy (AFLP) is a rare
but life-threatening complication occurring in the third trimester. It is often fatal to both mother and fetus. The complicated clinical manifestations as well as an insufficient understanding of the disease make the precise diagnosis and effective treatment of AFLP challenging. A full understanding of the risk factors, clinical features, and test findings of AFLP is critical for its timely diagnosis and treatment.

**Materials and methods.** An unexpected death of a 22-year-old woman occurred 10 days after a regular delivery is described. She was discharged from the Obstetric Unit 48 hours after the birth of her child in apparent good conditions. Few hours later, she suddenly presented confusion, headache, arms pain and amnesia. She underwent many specialist consultations as well as a complete blood test together with microbiological and toxicological analysis resulting as inconclusive. Also cerebral CT was negative. Accordingly, a diagnosis of “dissociative amnesia related to pregnancy” was performed.

However, during the following days the clinical conditions got worse with a significant hyperammonemia (411 µmol/l), cerebral edema and coma. A clinical autopsy was requested in order to discover the cause of death under suspicion of a metabolic disorder involving the urea cycle. Gross examination was unremarkable. The weight of the uterus was 600 g, while its dimensions were 19x15x4 cm. Normal post-partum involutive phenomena were described both macroscopically and microscopically following an accurate dissection and sampling according to the specific protocols employed in a second level diagnostic Unit (Istituto Giannina-Gaslini). The weight of the liver was 2420 g, while its dimensions were 36x22x13 cm. The parenchyma showed a smooth capsule and a pale yellowbrown greasy cut surface. The microscopic evaluation revealed a diffuse and massive hepatic degeneration characterised by a steatosic microvesicular and intracytoplasmic fatty storage pattern. Based on such elements and the absence of other pathological findings at autopsy and microscopy, death was attributed to the fatal consequences of acute fatty liver of pregnancy.

**Results.** Fatty liver is characterised by accumulation of microvesicular fat that literally crowds out normal hepatocytic function. Grossly, the liver is small, soft, yellow and greasy. AFLP is an obstetric emergency with a prevalence of 1 in 7000–16,000 deliveries. Also known as acute fatty metamorphosis or acute yellow atrophy – it is the most common cause of acute liver failure during pregnancy. The clinical presentation generally consists of malaise, nausea, vomiting and epigastric pain followed by jaundice. From a pathological point of view it is crucial to perform a thorough differential diagnosis with other conditions such as HELLP Syndrome. The latter, e.g., is characterised by a cytokine- and neutrophil-mediated liver injury occurs that is not present in AFLP.

**Conclusions.** Early diagnosis and prompt progressive management, including early termination of pregnancy and comprehensive supportive care, are crucial for improving the prognoses of both mother and newborn.

**A PROPOSAL OF A NOVEL DIAGNOSTIC WORKFLOW FOR TRIAGE OF HYDATIFORM MOLE. A PILOT STUDY FOR SEQUENTIAL APPLICATION OF P57, MIB1 AND CH17 SISH**

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**Background.** Diagnosis of first trimester abortion lacks of specific, univocal and widely accepted morphologic parameters in order to identify a possible cause of abortion. Many specimens are collected after many days of intrauterine retention and suffer of widely regressive changes and/or karyosomal alterations and villi harbour morphologic features suggesting partial hydatidiform mole (PHM) or early complete hydatidiform mole (eCHM). Distinction of hydatidiform moles from nonmolar specimens and subclassification of hydatidiform moles as complete hydatidiform mole (CHM), PHM, or eCHM are important for both clinical practice and investigational studies. However, diagnosis based solely on morphology suffers from poor interobserver reproducibility and remains problematic even for experienced gynecologic pathologists. The risk of persistent gestational trophoblastic disease and hence, clinical management, differs for CHMs, PHMs, and nonmolar specimens. The unique genetic features of CHMs (androgenetic diploidy), PHMs (diandric triploidy), and nonmolar specimens (biparental diploidy) allow for certain molecular techniques, including immunohistochemical analysis of p57 expression (a paternally imprinted maternally expressed gene) and molecular genotyping, to refine the diagnosis of hydatidiform moles. Although p57 immunostaining alone can identify CHMs, which lack p57 expression because of the lack of maternal DNA, this analysis cannot distinguish PHMs from nonmolar specimens as both express p57 because of the presence of maternal DNA. Recently, a scoring method for ploidy analysis using silver in situ hybridisation (SISH) with a chromosome 17 centromere probe has been applied and validated to determine ploidy in first trimester abortion with morphological features of pos-

![Figure 1. Fatal Acute Fatty Liver of Pregnancy: a paradigmatic case report.](image-url)
sible HM. A chromosome 17 centromere probe SISH-based assay can reliably distinguish between diploid and triploid gestations. This test has diagnostic utility in distinguishing partial hydatidiform moles from histologic mimics.

**Aim and methods.** The aim of the study was to discriminate between non molar specimen, PHM and eCHM using a novel diagnostic approach. All those cases classified as possible chromosomal alteration but with morphology consistent with HM (either eCHM and PHM) were tested for immunoistochemical expression of p57 and MIB1. In cases negative for p57 and expressing extensive positivity for MIB1 (MIB1 > 95%) a final diagnosis of CHM was made (2 cases). If p57 expression was positive or discontinuous and MIB > 95%, SISH was applied in order to establish a definitive diagnosis of HM (PHM or eCHM) or non molar abortion. p57 and MIB1 expression was evaluated on trophoblastic cells, using Hofbauer cells as positive internal control 1-3. SISH evaluation was conducted on at least 50 trophoblastic and stromal cells, using a scoring method based on the average number of signals per nucleus and the percentage of nuclei with three signals, as reported in literature 4.

**Results.** Between January and December 2015, 171 consecutive specimen of first trimester abortion were evaluated. Thirty-one cases were elective abortion, 28 cases had extensive regressive changes, 27 specimen did not contain villi (inadequate for diagnosis of intrauterine pregnancy). Four cases were diagnosed as inadequate implantation/possible autoimmune disease. Two cases had characteristic morphology of CHM (large “cisternae”, atypical trophoblastic hyperplasia, trophoblastic inclusions), lack of p57 expression and MIB expression was very high (> 95%). Among 75 cases classified as possible chromosomal anomalies, 19 cases had morphologic features suggesting molar pregnancy, discontinuous or positive continuous p57 and MIB > 95% were tested for SISH. Four were classified as PHM, 15 cases as chromosomal anomalies. All the cases were tested for SISH. All 15 cases of non molar abortion (possible chromosomal anomalies) were diploid when tested for SISH. Among 4 cases classified as PHM on the basis of morphology and immunohistochemistry, 2 cases were diploid and was finally diagnosed as eCHM. Two cases out of 4 were confirmed as PHM (triploid status).

**Conclusions.** The application of this diagnostic workflow allowed the correct re-classification of 2 cases of eCHM (33% of molar pregnancies), erroneously diagnosed as PHM. The use of p57, MIB1 and SISH for Ch17 is easily applicable in routine diagnostic procedure and reliable in diagnostic workout of first trimester abortion specimens with morphologic features of molar pregnancies.

**References**


colposcopies. There were 2,175 abnormal colposcopy results (mostly grade 1 \( \text{ANTZ} \)). The colposcopic diagnoses were: acetowhite epithelium (1,002 cases), 603 keratosis, 279 punctate–280 mosaic patterns and 11 carcinoma. The cyto-histological diagnosis of the abnormal colposcopy results included 210 \( \text{L-SIL} \), 56 \( \text{H-SIL} \) and 11 carcinomas (277 cases). There were 170 abnormal cytology results (confirmed by histology), \( \text{(113 L-SIL, 54 H-SIL, 2 endocervical adenocarcinoma in situ (AIS) and 1 carcinoma) in patients with a normal colposcopy result (G 0). Whilst there were 1,898 abnormal colposcopy results associated to normal cytology and histology.}

**Conclusions.** It is well known that colposcopy has a low specificity (Barrasso, 1998), above all if used as a 1st level test. Indeed, should a positive Pap test be followed by a colposcopic grade 1 Abnormal Transformation Zone (ATZ), then, 79% of cases will be histologically positive. If there is no previous positive cytology, the positivity values of histology are very low i.e. in the range of 20% for grade 1 colposcopic ATZ.

Our data showed that 89,1% of the patients had both a negative colposcopy and Pap test and that both tests were positive in 1.3% of the cases. However, although 8.8% of patients, had a positive colposcopy, their cytology and histology were negative. Whilst, despite the fact that a 0.8% of the Pap tests and histology were positive, their colposcopies were negative. The discordance between the colposcopy and histo-cytology results indicates that colposcopy alone, i.e. without the association of cytology and histology, is not able to offer a definitive diagnosis. This is particularly true for abnormal colposcopy results (grade 1 \( \text{ANTZ} \) or higher) that should always have an anatomopathological confirmation.

In conclusion, the Pap test will not disappear due to the advent of the new technologies, just as the stone age did not finish due to the lack of stones and surely the Pap test will not become obsolete because physicians and patients no longer ask for it – but, as diagnostic cytology is no easy feat, it may well do so, due to a lack of cytologists.

**CLINICO-PATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF OVARIAN CLEAR CELL CARCINOMA: A TISSUE MICROARRAY STUDY**

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**Goal.** Ovarian clear cell carcinoma (CCC) represents 5–25% of all epithelial ovarian cancers. This histotype shows peculiar clinical and biological aspects, such as the association with endometriosis, the chemoresistance to Cisplatin and Paclitaxel and a poor prognosis, even if frequently diagnosed at an early stage.

Purpose of our study was to qualify on clinico-pathological and biomolecular point of view this rare type of tumors, in order to discover potential molecular targets and prognostic factors for the development of personalized therapies, which are the current trend of the future oncological treatment.

**Methods and materials.** Thirty-eight patients affected by CCC of the ovary were retrieved from the database of the Department of Pathology of the A.O.U. San Martino – IST of Genoa, from 2004 to 2014. All the patients were surgically treated from the Gynecology and Obstetrics Department of the same hospital.

We extracted from the histologic report the clinical and pathological data valuable for the study and reviewed the slides of all the cases. The most representative area of every case was selected and two contiguous Tissue Micro Arrays (TMA) were performed in the neoplastic area. From the arranged TMAs, immunohistochemical stains for Estrogen Receptor (ER), Progesteron Receptor (PR), p53, ki67, Cyclin D1, Bcl-2, Tubulin \( \beta 3 \), HER2/neu, PTEN and \( \beta \)-Catenin were performed.

**Results.** The mean age of our population study was 57.21±12.6. No clinical, radiological or echografic aspects appeared to be related with this specific histotype. At the moment of the surgical intervention, 36.84% of the tumors were located in the right ovary, 39.47% in the left ovary and in 23.68% of the cases the disease was bilateral. On histological examination, twelve cases revealed concurrent foci of endometriosis. The exclusive involvement of the ovaries was found in the pre-dominance of cases (26 patients; 63,13%), and the final sta-diation of the tumors revealed in nearly 70% of the population study a localized disease (stage I or II). Immunohistochemical profile of CCC on TMAs showed low expression of ER (16,88±27,96%), PR (8,05±19,25%), ki67 (31,98±27,78%) and p53 (25,88±26,93%). Molecules involved in apoptotic processes, such as Cyclin D1 and Bcl-2, revealed extremely variable results, while PTEN expression was lost in all cases and \( \beta \)-Catenin was strongly and diffusely expressed. HER2/neu was negative in the predominance of cases, but 4 patients presented a 2+ positivity, which would be worthy to further study with FISH technique. Fourteen patients revealed a strong expression in > 50% of neoplastic cells for Tubulin \( \beta 3 \), and in eight patients a weak expression in < 20% of neoplastic cells.

**Conclusion.** Ovarian clear cell carcinoma is a dreadful disease that strikes a discrete amount of women in their middle ages, in the apex of their social and life status. Our work confirmed the morphological and biomolecular characteristics reported in literature for this peculiar tumor. Noteworthy, Tubulin \( \beta 3 \) confirms to be a promising molecular marker for therapy response, since the hyperexpression of this protein is predictive of taxane resistance. The relevant positivity for HER2/neu in a subgroup of cases highlights the possibility of new therapeutic perspectives, such as the treatment with Trastuzumab.

**BRC A1 AND PAR P1 IMMUNOISTOCHEMISTRY IN OVARIAN CARCINOMAS: CORRELATION WITH HORMONE RECEPTORS AND BYOHUMORAL MARKERS**

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**Introduction.** Recent studies suggested a biological affinity between the anti-PARP1 sensitive ovarian carcinomas and the triple-negative breast cancers. In contrast to breast carcinomas, the ovarian serous high-grade carcinomas are positive for ER in 2/3 of the cases. There are no studies that analyze the correlation between ER, PR, Ki67, p53 and immunohistochemical expression of BRCA1 and PARP1 in ovarian cancer.

**Material and methods.** The study was conducted retrospectively on 111 patients with ovarian cancer: 77 cases diagnosed by University of Bari and 34 cases by the Catholic University of the Sacred Hearth Rome.
The follow-up was available for 97 patients out of 111 (87%) and it has been closed in May 2016. All patients were subjected to hysterectomy with or without lymphadenectomy, and peritoneal biopsies for staging. For all cases we have sought the opportunity to highlight the possible correlations of ER, PR, Ki67 and p53 with BRCA1 expression and PARP1 and survival, in order to draw operational conclusions for the therapy. The assessment of positivity has been given in relation to the threshold values and intensity of expression.

**Results.** The IIC for ER expression in > 10% of neoplastic nuclei was detected in 50 cases, for PR in 43 cases. In 35 cases the Ki67 was > 20%, a diffused positivity for P53 was present in 44 cases. Patients with higher PARP1 expression have higher PR expression too. P53, ER and Ki67 expression was not significant in relationship with BRCA1 and PARP1 IIC positivity. The overall survival curves for BRCA1+ / BRCA1- and PARP1+ / PARP- showed no significant differences in the group of patients with high value of the Estrogen Receptor (ER) than women with low value, even if the patients with increased expression of ER tend not to show a significant better survival. The expressions of BRCA1 and PARP1 show no difference in survival in women with low or high levels of Progesterone Receptor (PR).

**Conclusions.** There is a portion of patients BRCA1+ and PARP+ that express also positive for ER and PR; but in general there is no correlation between the expression of BRCA1 and PARP1 and hormone receptors. In 35 cases the Ki67 was > 20% a diffused positivity for p53.

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**RETROPERITONEAL LEIOMYOMA: A CASE REPORT**

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**Introduction.** Leiomyoma are common benign gynecological tumors and usually arise in the uterus and it is one of the most common benign conditions for which women undergo hysterectomy every year but it is very rare as a primary retroperitoneal neoplasm. Retroperitoneal leiomyoma has a great predominance of females, a marked morphologic resemblance to uterine leiomyoma by virtue of hyaline change end trabecular pattern of growth. But when encountering a tumor in this region with a leiomyomatous appearance, one should consider the alternative possibilities of uterine leiomyoma extending posteriorly, well-differentiated leiomyosarcoma, benign and malignant GIST.

**Methods.** We report the case of a 81-year-old woman who is hospitalized for diagnostic investigation after incidental finding of a pelvic mass on ultrasonography. CT imaging confirmed the presence of a wide solid mass of size 11 cm in the pelvic cavity.

**Results.** This mass had regular contours with isolated intraslesional calcifications, inhomogeneous vascularity and hypodense areas peripherally. Biopsy of the mass it was performed elsewhere and 3 frustules of size 5 mm each are taken. Those were stromal frustules immunoreactive for smooth muscle actin (SMA) and negative for S-100 protein, CKAEL/AE3 and CD34 so they were made of smooth muscle. Thus the entire mass was removed and it came to our attention. On the gross findings the tumor had irregular shape and showed a compact, swirling and whitish cut surface. Hematoxylin and eosin staining revealed the intersecting fascicles of spindle cells. There weren’t nor necrosis nor mitotic activity. The immunohistochemical staining was positive for SMA and desmin and negative for S-100 protein and CD117. Therefore our final diagnosis was retroperitoneal leiomyoma.
**Conclusion.** Our patient represents the rare case of retroperitoneal leiomyoma, which is hardly identified from internal examination and preoperative imaging. Surgical removal is essential for pathological diagnosis and treatment.

**References**

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**DERMATOPATOLOGIA**

**PRIMARY CUTANEOUS MELANOMA IN ELDERLY PATIENTS: POTENTIAL PROGNOSTIC MARKERS**

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**Introduction.** Cutaneous melanoma is an immunogenic cancer and its interaction with the aging immune system could have an impact on biologic behaviour of this disease. Compared with younger patients (pts), in elderly ones melanoma tend to present in a more advanced stage [1-3]. TILs (Tumor infiltrating lymphocytes), PD-L1 (Programmed Death-Ligand) and COX-2 (Cyclooxygenase-2) are potential markers of host immune response to the tumor, inflammation and carcinogenesis [4-7].

**Methods.** Our study analysed 113 consecutive cases of invasive melanoma (pT1 and over) occurring in patients aged ≥65 years at the time of diagnosis observed between Jan 2010 and Mar 2014. After microscopic review of haematoxylin-eosin stained slides, paraffin tumor sections were stained for PD-L1 and COX2. We tested the association of TILs and other pathological data (PD-L1 and COX-2 expression) with disease-free survival (DFI) and melanoma- specific survival (MSS).

**Results.** Median Breslow thickness in primary tumor was 3.3 mm (range min 0.4 mm - max 10.0 mm); ulceration was present in 43 pts (38%) of cases; mitoses were ≥1/mmq in 86%. A lower TILs grade was observed in 65% of cases, higher grade in 35%. PD-L1 expression on tumor cells, on immune infiltrate and COX-2 expression were positive in 28%, 47% and 41% of cases, respectively. With a median follow-up of 52 months, 36% of pts developed recurrence. Ulceration was associated with shorter DFI (HR 8.3, 95% CI 3.02-22.70, P < 0.0001) and MSS (HR 9.5, 95% CI 2.96-30.11, P < 0.0001). Lymph node involvement was associated with shorter DFI (HR 3.4, 95% CI 1.44-7.96, P < 0.0001) and MSS (HR 3.3, 95% CI 2.16-17.63, P < 0.0001). A higher TILs grade was associated with better DFI (HR 0.29, 95% 0.09-0.88, p = 0.011). Positive PD-L1 expression on immune infiltrate was associated with longer MSS (HR 0.32, 95% 0.11-0.93, p = 0.043), and was confirmed in multivariate analysis. No association was observed for COX-2 expression.

**Conclusions.** In our study, ulceration, lymph nodes involvement, TILs grade and PD-L1 expression on immune infiltrate...
were prognostic factors in elderly patients with a primary melanoma. Ulceration and lymph nodes involvement are unfavourable prognostic factors; on the contrary high TILs grade and PD-L1 expression on immune infiltrate were favourable prognostic factors.

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PRIMARY CUTANEOUS APOCRINE CARCINOMA: A CLINICO-PATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 12 CASES

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Background. Primary cutaneous apocrine carcinoma (PCAC) is an exceedingly rare malignancy, with poorly defined diagnostic features and scarce data concerning clinical behaviour and appropriate management. PCAC generally arises in adult age with no racial or sex differences in the axilla and the scalp; although it has also been described in the extremities, eyelids and anogenital areas. Tumors are initially locally invasive and systemic dissemination is often associated with regional lymph node metastases. Main differential diagnosis includes carcinomas with apocrine differentiation metastatic to the skin, particularly breast cancer. We herein describe the clinico-pathological features and immunohistochemical profile of a series of 12 PCAC.

Materials and methods. Twelve cases of PCAC were retrospectively retrieved from our pathological archives. Clinical information was extracted from medical records. Clinical investigations excluded the presence of a breast malignancy in all cases. Haematoxylin & eosin-stained sections were reviewed in all cases and additional serial sections were cut for immunohistochemical analyses with anti-gross cystic disease fluid protein-15 (GCDFP-15) (Ventana, clone EP1582Y), anti-estrogen receptor (ER) (Ventana, clone SP1), anti-progesterone receptor (PgR) (Ventana, clone 1E2) and androgen receptor (Ventana, clone SP107). All immunostains were performed on the Ventana BenchMark ULTRA immunostainer (Ventana Medical Systems, Tucson, AZ). The Ventana staining procedure included pretreatment with cell conditioner 1 followed by incubation with antibody. The signal, for all antibodies, was then developed with ultraView Universal DAB Detection Kit. After the staining run was complete, the tissue sections were counterstained with Mayer’s hematoxylin.

Results. Nine patients were males and three were females; mean age was 75.6 years (range 59-92 years). The maximum tumor dimension varied from 3 to 35 mm (median, 10 mm). No tumor was diagnosed clinically although malignancy was suspected in 9/10 cases in which a clinical diagnosis was proffered; the most common clinical impression was of basal cell carcinoma (7 cases). PCAC developed on the eyelids (4 cases), scalp (3 cases), retroauricular region (1 case), ear (2 cases); nose (1 case) and perianal region (1 case). Median follow-up was 39 months (9 to 72 months). Among patients for whom follow-up was available (9/12), 6 patients had no further evidence of disease. Three patients developed local recurrences of whom 1 patient also a subsequent regional cervical lymph node metastasis was demonstrated and no further metastatic dissemination after 6 months follow-up. Histopathologically, there was evidence of apocrine differentiation in all cases, showing tumor cells with large round nuclei and plump, eosinophilic, granular and sharp-bordered cytoplasm, occasionally associated with an apical decapitation secretion pattern (apical snouting). According to Robson et al., cases were classified in tubular neoplasms (3 cases); tubulo-papillary tumors (3 cases), solid tumors (4 cases) and combined (2 cases). Using the modified Bloom-Richardson criteria, tumors were classified as grade 1 (3 cases) and grade 2 (9 cases). Lymphovascular invasion was present in 2 cases and perineural invasion was detected in 2 cases. By immunohistochemistry, AR expression was observed in 9/11 (81%) cases, 6/11 (54%) expressed GCDFP-15 and 3/11 (27%) expressed both ER and PgR.

Conclusions. Our experience with PCAC shows that these rare tumors occur in elderly male patients in anatomical sites where apocrine glands or modified apocrine glands (Moll’s gland or ceruminous glands) are located, with similar morphological features (including decapitation secretion). Immunohistochemistry, of limited help in the definition of the primary versus metastatic origin, shows in most cases AR expression accompanied by loss of immunodetectable ER and PgR. The frequency of ER and PgR expression found in our series of PCAC was lower in comparison with that reported in previous studies and more in keeping with expected phenotype of most primary apocrine breast carcinomas. Clinical investigations excluded the presence of a breast malignancy in all cases. The surgical treatment and optimal management of PCAC have not be standardized yet, and, given their rar-
ity, the real prevalence of the cases with adverse outcome is difficult to be estimated. In our series, no fatal cases were demonstrated and we cannot rule out that discrepancies in clinical behaviour may reflect diversity in anatomical origin and complex histogenesis.

References

THE LONG NON-CODING RNA HOTAIR AS CIRCULATING MARKER IN THE MANAGEMENT OF PATIENTS WITH METASTATIC MELANOMA

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Introduction. The evolution of molecular techniques of “gene profiling” have allowed to identify several molecular pathways that underlie tumor progression. In recent years the role of lncRNA has steadily gained importance mainly because their aberrant expression was strongly associated with the development, progression and therapeutic response of numerous cancer types. We have recently shown that an important role in this process is certainly played by HOX genes, in particular we have put in light a progressive increase of posterior HOXC-genes expression, during all stages of neoplastic progression of melanocytes (nevi/primary tumors/metastases). Latest data have shown that the regulation of HOX genes expression is under control of particular long non-coding RNAs (lncRNAs). HOTAIR is the most studied among them, whose aberrant expression is associated with the metastatic progression of many malignancies.

Aim. The aim of this study was to verify the role played by HOTAIR in metastatic progression of melanoma and to evaluate the circulating levels of HOTAIR in the blood of patients with metastatic melanoma.

Material and methods. A series of melanocytic lesions were selected to evaluate the potential changes in the expression of HOTAIR during the evolution of the disease by in situ techniques and real-time PCR. We have also investigated the circulating levels of HOTAIR in the blood of patients with metastatic melanoma, comparing them with the levels observed in healthy people. HOTAIR expression was analyzed on metastatic tissues from the same patients.

Results. HOTAIR expression was identified in the cytoplasm of tumor cells as well as on their membrane where the expression of HOTAIR was prevalent. The data were also confirmed by RT-PCR after RNA extraction from tissues FFPEP. HOTAIR was identified, although with different expression levels in the blood of patients with metastatic melanoma. In selected metastatic tissues, trends of expression of HOTAIR appeared similar to those in the blood. We have also analysed 10 blood samples after immunotherapy. The levels of expression of HOTAIR was lower in patients who had responded to therapy.

Conclusion. The data obtained in this study have shown the fundamental role played by HOTAIR in the malignant transformation and progression of melanoma cells. Moreover, its identification in the blood, so its potential activity as circulating marker, might suggest its use in the management of melanoma patients, for example as a marker of relapses during the follow-up, or to monitor therapeutic response.

References

SCRAPING EXAMINATION OF SUPERFICIAL SKIN LESIONS: A CYTLOGICAL AND HISTOLOGICAL COMPARATIVE STUDY OF 400 CASES


Cytological scraping is a well-tolerated and economic procedure to quickly assessing superficial ulcerous lesions of the skin. In order to set the proper management of the patient, it is important to distinguish between benign and malignant lesions.

The accuracy of the cytological diagnosis of the scraping and its role in the differential diagnosis of ulcerated skin lesions have been assessed by carrying out a wide retrospective study, in which 832 scrapings collected at the National Institute for Cancer Research “Fondazione G. Pascale” were analysed. Cyto-histological correlation was available in 400 cases. Based on histological diagnosis, the series consists of 76% squamous lesions, classified as basal cell carcinomas (BCC), squamous cell carcinomas (SCC) and basal squamous cell carcinomas (BSC); 10% benign lesions and 14% of “other” lesions, which include actinic keratosis, skin metastases of melanoma and rare metastases.

A cyto-histological correlation was obtained in 309 cases (77%). Among the 91 discordant cases, only 16 were false positives; the remaining were misdiagnosed. The analysis of squamous lesions showed a sensitivity range of 68-94% and PPV values that varied between 46 and 85%, depending on the different histotypes (BSC < SCC < BCC). Benign lesions
TRPA1 IS EXPRESSED IN CUTANEOUS MELANOMA AND ITS DOWNREGULATION MIGHT CORRELATE WITH A MORE AGGRESSIVE CANCER PHENOTYPE

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Introduction. The Transient Receptor Potential Ankyrin 1 (TRPA1) belongs to the superfamily of TRP channels that are expressed in a large variety of cell types where they mediate a series of physiological functions and play major roles in pathophysiological conditions, including cancer. TRP ion channels by modulating intracellular calcium movements control cellular functions, such as cell growth, apoptosis and tissue invasion. Notably, different TRP channels are associated with cancer onset and/or progression, as either increase or reduction of the TRP protein expression have been reported depending on histological type and cancer stage. Recently it has been reported that UV A/B radiations activate TRPA1 in melanocytes, suggesting a channel role in phototransduction and melanin synthesis. The expression of TRPA1 protein has been previously demonstrated in vitro in different melanoma cell lines, such as A375, G361 and SK-mel-19. However, a functional role TRP channels, and in particular for TRPA1, in growth process and invasion in melanoma has not been clearly elucidated. Herein, we investigated the immunohistochemical expression of TRPA1 in a series of human cutaneous melanomas and assess its possible association with the onset of melanoma and its progression.

Materials and methods. Firstly, by calcium imaging experiments, we tested TRPA1 functional expression in primary Melanoma cell line (SKmel). To study the calcium response we used selective TRPA1 agonists (allylisothiocyanate) and antagonists (HC-030031). Secondly, by immunohistochemistry, we studied TRPA1 protein expression in 67 melanoma FFPE specimens representative of different stages: pT1 (n = 16), pT2 (n = 15), pT3 (n = 15) and pT4 (n = 21). Upon histopathological revision showed 42 SSM, 17 NM, 2 ALM, 1 Desmoplastic and 5 unclassifiable melanomas. Clinico-pathological data, including age, sex, anatomic tumor site, ulceration, Breslow thickness, Clark level, TILs and vascular invasion, were collected. Twenty-nine patients were females and 38 were males. FFPE (4 µm thick sections) were stained according to standard immunohistochemical procedures. Antigen retrieval was performed in citrate buffer (ph 6.0, 20 minutes 98°C). After blocking with UltraVblock, section were incubated with polyclonal antibody TRPA1 (1:800, 1h room temperature). Staining was visualized using Fast Red as chromogen. Nuclei were counterstained with Mayer’s haematoxylin.

Results. By calcium imaging experiments we confirmed the functional expression of TRPA1 in melanoma cells line (SKmel). The selective TRPA1 agonist, AITC, induced a concentration-dependent calcium response (EC50, 13µM). Responses to AITC (10 µM) were selectively abolished in the presence of the TRPA1 antagonist, HC-030031 (10 µM). Immunohistochemical analysis showed an inverse correlation between TRPA1 protein expression, tumor stage and Breslow thickness in the investigated melanoma. Specifically, in 75% of pT1 melanomas TRPA1 expression was found in >50% of the tumor cells while pT4 melanomas were prevalently TRPA1 negative or showed some weak (only 9% >50%) and localized positivity, confined to the in situ and RGP phase, with clear downregulation in the more invasive VGP. Intermediate TRPA1 expression was observed in pT2 (>50% of tumor cells were positive in 13.3% of cases) and in pT3 (>50% in 33.3%) melanomas.

Conclusions. The observation of a progressive reduction in TRPA1 expression from pT1 to pT4 stages indicates that channel downregulation is associated with a more aggressive cancer phenotype. Similar findings have been reported for the TRPM1 in melanoma and the TRPV1 in bladder cancer. It is possible that calcium homeostasis warranted by a still abundant TRPA1 expression in early stage melanoma contributes to inhibit the malignant burden of the tumor. However, the real significance and role of TRPA1 and its downregulation in melanoma cells remains to be established.

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PALEOPATOLOGIA

ANTHROPOLOGICAL COLLECTIONS, DENTAL INSTRUMENTS, PALEOPATHOLOGY: HOW DO THEY TALK TOGETHER IN THE MUSEUMS?

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In recent decades, the growing interest in scientific culture has given new value to the universities scientific collections, whose historical, scientific, documentary and educational role is now widely recognized. These collections are often notable for the numbers, heterogeneity and uniqueness of the samples collected for research and teaching. Over time, large amounts
of biological remains, scientific objects and instruments were assembled and passed on from one generation to the next. In order to preserve this important heritage, most of the collections are now stored in museums and some of them are on display to the public.

For efficacious scientific communication, common efforts are required to create a new dialogue among different scientific collections. In this perspective, it has started a successful collaboration between the Museum of Anthropology and Ethnography and the Museum of Dentistry at the University of Turin. The Museum of Anthropology and Ethnography was founded in early twentieth century by Giovanni Marro (1875-1952), medical doctor and anthropologist. The Museum includes anthropological and ethnographic specimens of different provenience, grouped into various collections. The anthropological collections are represented by a large number of archaeological ancient human skeletons and mummies dating back from the prehistoric to recent times. Other collections consist of medical specimens that include skulls and brains dating back to the beginning of the XXth century. Giovanni Marro collected human remains to provide anthropological data banks for the study of physical and cultural human evolution. The Museum is currently closed to the public but it is very active in the field of anthropological research.

The Museum of Dentistry, located within the University building “Dental School” of Turin, has a historical and educational character. The collection was gathered by Giulio Preti, Professor at University of Turin, who recovered a donation received in the ’30s, by Luigi Casotti, one of the major historian of Dentistry; the assemblage is represented by surgical instruments and antique scientific tests. Over the years, the collection has further developed with the addition of objects ranging from the end of the 19th century, thanks to the cooperation of the Amoretti family. The “Collection of Historical Dentistry”, inaugurated in 2008, today it is the only permanent exhibition in Italy dedicated to dentistry.

In 2014 the two Museums have put together their experiences and materials in order to create inside the Museum of Dentistry an exhibition about dental caries. There were selected and exposed some jaws from Middle Ages. With macroscopic analysis, it was demonstrated different stages of dental caries and some particularly serious features that are common for historical periods where medical knowledge was limited and dental care do not present or not accessible to the entire population (Fig.1). Moreover, dental caries can predispose to the development of dental abscess and ante-mortem tooth loss that were observed on ancient remains too. The observation of further disorders of dentitions, such as periodontitis, heavy dental wear, dental enamel hypoplasias, may offer us the opportunity to organize in the future new exhibitions on different topics.

References

Figure 1. Classification of caries observed on ancient human remains from medieval period.
THE PALEOPATHOLOGICAL EVIDENCES OF TREPANATION IN ITALY

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Introduction. Trepansation is considered the most ancient surgical procedure, consisting in the intentional removal of a bone portion of the neurocranium, practiced during life or post-mortem. This work is aimed at providing a review of the trepanations attested from the Italian territory.

Materials and methods. The cases of trepanations in ancient Italy are examined based on information provided by the literature and discussed. The surgical interventions have been examined considering a series of criteria, including archaeological context, sex and age distribution, involved bone, laterality, technique of trepanation, reasons of trepanation, and signs of healing.

Results. A total of 52 individuals from 42 different Italian archaeological sites were found to have evidence of trepanation. The analysis of the evidences of trepanation in Italy demonstrated that generally a difference between the trepanned individual and the social context of burial, leading to suppose a special role in the group, cannot be perceived. Trepansation in Italy covers a time span of approximately 7000 years, being the most ancient cases dated back to the V millennium BC and the most recent cases to the 17th-18th century AD. The geographic distribution appears quite homogeneous, with a prevalence of Central Italy, and an abundant evidence from prehistoric Sardinia. The majority of individuals shows a single trepanation, whereas some skulls present multiple holes. In Italy trepanation was reserved to adult individuals, except for some rare cases, and shows a relevant preponderance for the male sex. The most diffuse technique is scraping; cutting and drilling are rarely attested, in particular as a unique technique to obtain trepanation, whereas they were used more frequently in combination with scraping. As for the reasons of trepanation, in some cases a therapeutic intervention in order to treat a traumatic wound or other pathologies can be deduced, or a few evidences of trepanation performed as ritual intervention or as experimental surgery are attested; in the remaining cases reasons for trepanation remain unclear or not determinable.

Conclusions. Trepansation in Italy was diffused in all geographical regions and all periods, ranging from the V millennium BC to the 17th-18th century AD.

PALEOPATHOLOGY OF THE NATURAL MUMMIES FROM THE CHURCH OF SANT’ANNA IN MODICA (SOUTH-EASTERN SICILY)

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Introduction. The church of Sant’Anna in Modica (Ragusa province, south-eastern Sicily) was built at the end of XVII century, possibly in 1686, on the pre-existing chapel of S. Calogero, attached to a Franciscan Convent (1, 2). No significant structural damage was suffered by the church during the major earthquake occurred on January 11th 1693, that partially destroyed the friary (2). Since the end of the XIX century, the lack of planned maintenance resulted in a progressive decay of the building and in recent times the ceiling collapsed on the floor in the central part of the church (2). During the restoration works, many tombs and crypts were unveiled. In the crypt beneath the Crucifix altar, two mummified human bodies were found and recovered. In the year 2000 they were provisionally stored in a wooden case near the main altar, to be occasionally showed to visitors. A preliminary survey carried out by our group in 2010 allowed to recognize the poor conditions of the bodies, prompting us to advice a conservative intervention. Subsequently, we were entrusted to do a paleopathologic study of the mummies, that took place in June 2016.

Methods. The study was carried out by a conservative approach. Both mummies were recovered from the wood case and identified as MSA 1 and 2. A superficial cleaning allowed to remove dust and insects from the clothes. Sampling for laboratory investigations was limited to the surface of the clothes and to the exposed body regions, due to conservative reasons. Visual inspection of the clothes and the exposed parts of the bodies was performed. The mummies were then fixed to a wood sheet by wrapping them in clear plastic film to avoid shocks during the transportation from the hospital. Direct radiograms in different projections were obtained with the digital system Philips OmniDiagnost. Computed tomography (CT) scanning was performed by using a General Electric LightSpeed Pro 32 scanner with 0.625 mm thick sections, obtained with a 1:1 pitch at 50 mA and 120 kV, generating a total of 2819 scans for the first individual and 2761 for the second one. Tomodensitometric evaluations were made according to the Hounsfield scale and 3D reconstructions were carried out.

Results. The body of MSA1 was complete and in a very good state of preservation, belonging to a bearded man, dressed in clothes from the first half of XIX century (1810-1830). The age at death, according to dental wear and radiologic data, was 40±5 years. The mummy measured 170 cm in length and belonged to a slim subject, with no signs of anthropogenic manipulation. Amorphous material (remnants of encephalic tissues) was highlighted in the upper posterior cranial fossa, along with portions of the meningeal wrappings. Thoracic and abdomino-pelvic organs appeared extremely well-preserved and readily recognizable. These findings confirmed the occurrence of natural mummification, due to rapid dehydration, possibly related to hot dry climate. All dental elements were present, but two molars: left lower first and right upper third were lost ante mortem. Neither significant deposits of tartar nor dental wear were observed. Diffuse left pleural adhesions were observed, along with multiple, tiny calcifications of the lung and a peribronchial nodule measuring 14 mm in largest diameter. Such findings were consistent with primary pulmonary tuberculosis. The right lung appeared normally collapsed.

The body of MSA2 was complete and in a very good state of preservation, belonging to an old man, with wide remnants of clothes from the end of XVIII century. The age at death,
according to dental wear and radiologic data, was at least 75 years. The mummy measured 166 cm in length and belonged to a plump subject (as displayed by the abundant cutaneous folds) with no signs of anthropogenic manipulation. Amorphous material was evident in the posterior cranial fossa with a clearcut distinction of cerebellum. Thoracic and abdomino-pelvic organs appeared extremely well-preserved and readily recognizable. These findings confirmed the occurrence of natural mummification, due to rapid dehydration, possibly related to hot dry climate.

A 36 x 15 mm incision with smooth borders was observed in the fourth right intercostal space. A left fibrothorax with multiple calcifications of the lung, and a single right costal adhesion with partial lung retraction were observed. Such findings were consistent with primary pulmonary tuberculosis and suggested the possibility of a traumatic or iatrogenic pneumothorax. Additional radiologic findings include multiple gallbladder or right renal stones, multiple phleboliths in the pelvis, and severe osteoarthritis of the spine and the right hip. The presence of a bandage around the left knee, a leaf on the right ankle, and a chalky device on the right foot probably indicate analgesic medications.

**Conclusions.** The mummies found in the church of S. Anna represent a good example of natural mummification in the scenario of Sicily, characterized by huge numbers of bodies obtained by artificial or spontaneous-enhanced mummification (3). These uneviscerated mummified bodies allowed to identify different pathologic conditions and to understand social status and health conditions of these subjects. A point of great interest is that both subjects were affected by pulmonary tuberculosis, similarly to a XX century natural mummy found in the nearby town of Scici (4). These findings highlight the great impact of this disease on the island population during the last centuries. Moreover, the possibility of an iatrogenic pneumothorax, if confirmed, would antedate its introduction in medical practice of at least one century.

**References**
Introduction. Metaplastic breast carcinoma (MBC) is a rare tumor showing high histological grade and low or absence hormone receptor expression. It has pure epithelial and mixed (epithelial-mesenchymal) types. Herein we present clinico-histopathological characteristics and immunophenotype status of 5 cases of MBC.

Methods. Five cases of metaplastic carcinoma were reviewed for their clinico and histopathological features. Histopathologic features evaluated for each case included the age of the patient, tumor grade, tumor size, tumor growth pattern, histological subtypes, the extend of axillary lymph node involvement, estrogen/progesterone receptors, HER2 status, Ki-67 and other immunohistochemical markers expression. Clinical features included ultrasound and mammographic evaluations and oncological treatment.

Results. The mean patient age was 62.6 years and the mean tumor size was 3.6 cm. Histological grade was 3 for all patients. Average mitotic count was 25/10 HPF. Only one patient had axillary nodal metastases (1/14); the metastatic component was epithelial. Microscopically, 4 cases were mixed type (carcinosarcoma) contained high grade invasive ductal carcinoma components admixed with heterologous mesenchymal elements (osseous, chondroid and myoid/spindle cell); and one case was pure epithelial type (squamous cell carcinoma). Four of the 5 cases were triple-negative (ER, PR and HER2 negative) with and high Ki-67 proliferation index. All patients received adjuvant therapy.

Conclusion. MBC is a rare subtype of invasive breast cancer that accounts for less than 1% of all diagnoses. It is characterized by a larger tumor size at presentation, lower rates of axillary nodal involvement, higher rates of both local and distant recurrence, higher rates of ER, PR and HER2 negativity as well as a sub-optimal response to systemic therapies when compared to other invasive breast cancers. Further research studies will be required to develop targeted treatments with the goal of improving clinical outcomes.

References
patient experienced local recurrence and two patients died of metastatic disease (14.28% rate). None of these three patients had tumors with malignant heterologous component. Conversely, patients affected by borderline phyllodes tumors, including the subset with more than 10 mitoses per 10 HPF, didn’t develop distant metastases, although two patients showed local recurrences.

**Conclusions.** The present study confirms that the diagnosis of malignant phyllodes tumor is straightforward if all the histological parameters of malignancy (stromal hypercellularity, severe nuclear atypia and high mitotic rate) are satisfied. In this regard we suggest that diagnosis of malignancy should be restricted to those lesions with both severe nuclear atypia and > 10 mitoses per HPF (frankly sarcomatous tumors). On the contrary tumors with high mitotic activity (> 10 mitoses x 10 HPF) but with moderate nuclear atypia, regardless stromal cellularity, should be better classified as “borderline tumors”. This is supported by the evidence that none of these lesions recurred or metastasized. In our series only two patients (14.28%) with malignant phyllodes tumors died of metastatic disease. This metastatic rate is similar to that obtained by a meta-analysis study (16.71% of metastatic rate) based on 395 cases of malignant phyllodes tumors (1). Notably none of the patients with borderline phyllodes tumors from our series showed metastatic potential. In conclusion we suggest a conservative approach for borderline phyllodes tumors, whereas a wide local excision with negative surgical margins should be obtained for malignant phyllodes tumors.

**References**


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**STAT6: A SPECIFIC IMMUNOMARKER IN THE IDENTIFICATION OF SOLITARY FIBROUS TUMOR IN THE WIDE SPECTRUM OF THE SPINDLE CELL LESIONS OF THE BREAST**

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**Introduction.** Immunohistochemical nuclear detection of STAT6 is actually considered as a reliable surrogate for demonstrating the NAB2–STA6 fusion gene in paraffin-embedded tissues. Recently some studies have showed that this new fusion gene may be exploitable as specific diagnostic marker for solitary fibrous tumor (SFT), suggesting its potential diagnostic role in daily practice.

Although SFT is a spindle-cell fibroblastic tumor that usually occurs in the pleura, it is widely known that this tumor can arise virtually anywhere in the body, including the breast parenchyma. CD34 is usually considered as the most reliable SFT immunomarker, but it is neither sensitive nor specific to this tumor. As CD34 immunostaining can be shared by several spindle cell lesions of the breast, raising potential diagnostic problems in the differential diagnosis with SFT, there is the need to identify additional diagnostic immunomarkers for confirming the diagnosis of SFT.

The aim of the present study was to evaluate the immunohis-

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**Table I.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Nuclear Atypia</th>
<th>Stromal Cellularity</th>
<th>Mitotic Activity</th>
<th>Follow-up</th>
<th>Case</th>
<th>Age</th>
<th>Nuclear Atypia</th>
<th>Stromal Cellularity</th>
<th>Mitotic Activity/ Heterologous component</th>
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<td>5 x 10 HPF</td>
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<td>14</td>
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<td>4 x 10 HPF</td>
<td>6 years Alive</td>
</tr>
<tr>
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<td>69</td>
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<td>6</td>
<td>79</td>
<td>Severe</td>
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<td>19</td>
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<td>4 x 10 HPF</td>
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tochemical expression of STAT-6 in the differential diagnosis of a large series of spindle cell tumor and tumor-like lesions of the breast.

**Materials and methods.** We collected a large series of 35 spindle cell tumor and tumor-like lesions occurring primarily in the breast parenchyma. Based on morphological and immunohistochemical features, the following diagnoses were made: 13 cases of myofibroblastomas (MFB), 6 cases of desmoid-type fibromatosis (DF) 6 cases of spindle cell metastatic carcinomas (SCMC), 2 cases of dermatofibrosarcoma protuberans (DFSP), 2 cases of solitary fibrous tumor (SFT), 2 cases of “benign spindle cell tumor NOS”, 1 case of spindle cell lipoma, 1 case of leiomyoma, 1 case of nodular fascitis and 1 case of inflammatotory pseudo-tumor.

Immunohistochemical studies were performed with the labeled streptavidin–biotin peroxidase detection system using the polyclonal rabbit antibody (s20-S621, Santa Cruz Biotechnologies, Santa Cruz, CA, USA) against the STAT6 terminal was used at a dilution of 1:200, and after antigen unmasking (96 °C EDTA for 30 min). A pleural CD34-positive SFT with diffuse STAT6 nuclear immunoreactivity was used as external positive control. For negative control, the primary STAT6 antibody was omitted.

**Results.** Among all the spindle cell lesions tested, only the two cases of SFT showed a strong and diffuse (70% and 90% of neoplastic cells stained, respectively) nuclear immunoreactivity for STAT-6. Interestingly in one case, immunoreactivity was also obtained in the pre-operative core biopsy. None of the other spindle cell lesions showed immunostaining for STAT-6, with the exception of two cases of DF which exhibited a weak cytoplasmic staining interpreted as non specific immunoreactivity.

**Conclusions.** Although the histological diagnosis of SFT is usually straightforward for tumors occurring in the expected sites (pleura, soft tissues), it can be challenging if SFT arises in unusual locations, such as breast, kidney, prostate and uterus. The spectrum of spindle cell tumor and tumor like lesions occurring primarily in the breast is wide and includes benign and malignant lesions.

Our findings suggest that STAT-6 can be exploitable as a highly specific immunomarker in the confirmation of SFT diagnosis. Based on our findings we suggest STAT-6 be included routinely in the immunohistochemical panel used in the first diagnostic approach of the spindle cell lesions of the breast.

**SENTINEL NODE MICROMETASTASES IN DCIS DETECTED BY OSNA ESSAY: ARE THEY TRUE METASTASES?**

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**Introduction.** Sentinel node (SN) biopsy is generally considered the standard procedure for staging patients with clinically node-negative early breast cancer.

For about eight years the study of SN in many institutions in the world has also been performed by OSNA (One Step Nucleic acid Amplification), a molecular method which allows to evaluate the SN during the surgery with a highly reproducible technique, with a turnaround time of about 30’.

A large number of studies offered reasonable evidence that the CK19mRNA copy number, detected by the OSNA, can provide good estimates of macrometastases or metastases (CK19mRNA copy number > 5000) and micrometastases (CK19mRNA copy number < 5000 and > 250). With a CK19mRNA copy number < 250 the SN is considered metastasis-free. Ductal carcinoma in situ (DCIS), by definition, shouldn’t metastasise to the lymph nodes because cancer cells cannot reach the lymphatic vessels. Therefore, the examination of SN in patients with DCIS is only recommended for large or high grade lesions or when mastectomy has to be performed. Nevertheless, over the past few years, some studies highlighted the rare occurrence of SN metastases in patients harbouring DCIS. Possible explanations for such events could be the presence of occult foci of stromal micro-invasion or the possible occurrence of epithelial cells (benign or malignant) displacement, due to passive transport to the SN, following trauma by core needle biopsy (CNB) or fine needle aspiration cytology (FNAC). Furthermore, axillary nodes, though very rarely (0.1-0.2%), may harbour ectopic glandular breast structures and in such very rare cases ectopic breast glands may be responsible for false-positivity of SNs.

The current study was aimed at analysing the possible factors responsible for SN-positivity by OSNA assay in 12 cases of patients harbouring DCIS.

**Material and methods.** From January 2013 to December 2015, 1958 breast cancer patients from 3 different Apulian Hospitals (Policlinico and San Paolo Hospitals in Bari and Casa Sollievo della Sofferenza in S. Giovanni Rotondo) underwent SN analyses by the OSNA assay. Almost all the patients had received a preoperative diagnosis by CNB (trucut or mammotome) or FNAC, the remaining having had confirmed the diagnosis on frozen sections.

In all cases axillary lymph nodes were not palpable or ecgrophycally detectable, and SNs were identified by radioncinography (99Tc-labelled nanocolloid). At surgery, SNs were identified by handheld γ-ray detection probe and radioactive nodes were considered the SNs and excised.

OSNA ASSAY: Each excised SN was immediately sent on ice to the pathology laboratory where perinodal fat tissues were removed, each node was measured (3 dimension) and weighted and subsequently analyzed according to the manufacturer’s instructions (Sysmex, Japan). All patients in whom macrometastases were detected in the SN underwent immediate and complete axillary dissection (ALND), while only a few patients harbouring micrometastases in the SN were subjected to axillary clearance; the latter was not performed in patients with negative SN. All surgical samples (quadrantectomy or mastectomy) were formalin-fixed, paraffin-embbeded and stained with haematoxylin-eosin.

**Results.** From the 1958 patients enrolled from the 3 participating Apulian Hospitals in the period 2013-2015, a total number of 2,545 SNs was examined by OSNA (mean: 1.3 SNs/patient).

More than 75% of patients had had a preoperative diagnosis on core biopsy, 18% on FNAC and 8% on frozen sections.

Metastases were identified in 14.3% and micrometastases in 15.6% of SNs.

Overall, DCIS was postoperatively diagnosed in 132 patients and 12 (9%) of them (Tab.1) also harboured SN micrometastases (CK19mRNA copy number: 280-3000, mean value: 1.110). In one case only double SN-micrometastases were detected (CK19 mRNA copy number: 280 and 410, respectively). The vast majority (11/12) of such patients of the cases had been submitted to vacuum assisted core biopsy with a 11G needle (in 3 cases preceded from a FNAC), the remain-
...having undergone diagnostic quadrantectomy. In no case metastases to the non-SN were detected.

No differences could be shown among patients with DCIS as to the time interval between pre-operative biopsy/FNAC and surgery and to the needle gauge, irrespective of the status (micrometastatic vs. negative) of the SN.

Discussion. The presence of positive SN in DCIS has been repeatedly reported and it is generally considered a rare event. Positive SNs in patients harbouring DCIS have been detected both in SNs studied histologically by serial sections and by molecular whole-node analysis (OSNA assay) and, generally, this event has been referred to the presence of occult invasive foci in the primary tumour.

The increased accuracy of the examination of SNs (serial histological sections vs. whole-node molecular analysis) certainly facilitated the identification of such “metastatic foci”. Carter hypothesised the displacement of both normal or neoplastic cells following pre-surgical diagnostic procedures (FNAC or Core biopsy) as a possible cause of false-positive SNs. Moreover, epithelial cells can be hosted in SNs due to ectopic glandular tissue, or displacement of (benign or malignant) following vessel disruption after biopsy. The presence of epithelial cells in axillary nodes, mainly located in the marginal sinus, may be associated with inflammatory infiltration and, obviously, such mechanism can occur for both malignant and benign lesions, as well as for normal mammary tissue as a consequence of traumatic events; nevertheless, such mechanism is more common in case of papillary lesions in view of their friability.

Complete axillary clearance is becoming increasingly less frequent in patients harbouring micrometastases only to the SNs; therefore, the occurrence of "false positive" micrometastases to the SN is likely not to be followed by unnecessary axillary clearance. Nevertheless, the occurrence of micrometastases in the SN, even if sustained by low CK19 mRNA copy number, may lead the pathologist to obsessively search for occult invasive foci of the primary tumour, which could have been overlooked at first glance, and the oncologist to foresee a more aggressive tumour behaviour.

In all our cases an extremely accurate sampling of the primary tumour and the surrounding tissues was performed to rule out invasive or microinvasive foci of the DCIS but the search remained fruitless. Therefore, we are more prone to justify the presence of epithelial cells in the SNs revealed by OSNA assay as a passive transport through lymphatic vessels, traumatized by core biopsy or FNAC. This hypothesis is reinforced by similar pre-operative diagnostic procedures and time spans between the latter and surgical interventions in patients with DCIS and either positive or negative SNs. Finally, a conceptual issue still remains unsolved: is it correct to define “metastases” such very small loads of CK19mRNA detected in the SNs of patients with DCIS, benign lesions or even very small and well differentiated invasive tumours who have been subjected to preoperative diagnostic procedures? The concept of metastasis implies an active process of translocation of malignant cells at distance from the primary tumour whereas in the current study we give stronger support to the hypothesis that positive SNs in patients with DCIS are possibly due to passive dislocation of epithelial cells. In such instances, it seems more appropriate to sign out the SN as “positive” for epithelial cells of uncertain nature to avoid unnecessary additional surgical procedures and to refrain patients’ anxiety.

References

Table I.

<table>
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<tr>
<th>N°</th>
<th>Lesion</th>
<th>N. SN+</th>
<th>Copy Number</th>
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<th>Type of surgery</th>
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M= Mammotome. ALND= Axillary Lymph Node Dissection.

PROGNOSTIC IMPACT OF MULTIPLE MICROMETASTASES IN SENTINEL NODE OF BREAST CANCER

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Introduction. In recent years there is a great tendency to reduce axillary lymph-node dissection (ALND) following the diagnosis of micrometastases (MIC) in sentinel nodes (SN) for breast cancer. Data presently published indicate that the risk of further axillary involvement and axillary recurrence is very low in patients presenting micrometastases.

The same indications are followed also in cases showing micrometastases, in spite of the fact that no data have been published on the prognostic impact of multiple versus single mic deposits.

Aim of the present study was to review a series of SN biopsies, diagnosed with mic (metastatic lesions being comprised between 200 microns and 2 mm).

Materials and methods. All SN diagnosed with mic followed by ALND were retrieved from the files of Anatomic Pathology at Bellaria Hospital, University of Bologna, Bologna (Italy).

cases were reviewed and classified according to the number of mic deposits in: single mic (S-mics), when only one mic was present, or multiple mics (M-mics), when 2 or more deposits were observed in SN. In addition, the following data were recorded: tumor size, multifocality, histotype and grade,
presence of lymphovascular invasion. The data collected were compared with the number of non SN axillary lymphnodes presenting metastases.

**Results.** 119 cases constituted the basis of the present study, subdivided in 63 S-mic and 56 M-mic. Metastases in non-SN axillary lymph-nodes were detected in 6 (9%) S-mic and in 14 (25%) M-mic.

Conclusions. The data collected in this preliminary study indicate that M-mic show the same risk of involvement as small macrometastases in non SN axillary lymphnodes.

**References**


**Introduction.** In invasive breast cancer patients, both the eligibility to neoadjuvant therapy, and the assessment of the cancer risk prompt to anticipate the appraisal of the prognostic/predictive biological variables to the a pre-surgical phase (e.g. pre-surgical core biopsy sampling). Consistently, the Veneto oncology network recommends to immunohistochemically (IHC) assess the expression of steroid receptors (i.e.: E and Pg), Ki67, and Her2 on needle core biopsy samples as obtained from untreated patients. Moreover, recent guidelines (endorsed by the Italian Heath Care Minister) highlight that, compared to the surgical specimens, the core biopsy samples result into a better performance of IHC-testing (short cold ischemia, and optimal fixation). In 690 consecutive breast cancer patients, this study assessed the consistency of the IHC expression of Estrogen (E)/Progestosterone (Pg) receptors, Ki67, and Her2 in paired cancer tissue samples.
samples obtained before (690 core biopsy samples [CBS]) and after (690) the surgical resection (SR).

**Results.** Overall, the expression of E-receptors in CBS versus SR showed a high consistency (Scatter Plot correlation 0.87). When the cut off value of the E-expression was established at 5%, the Simple Kappa Coefficient of inter-samples concordance was 0.99 (Table I). A high correlation coefficient (> 0.97) was also obtained when E-expression was compared (CBS versus SR) in cancers stratified according with their largest diameter (< = 1 cm; 1-2 cm; > 2 cm), and their histology grade.

Also the Pg-expression showed a high consistency in CBS versus SR (Scatter Plot correlation 0.8376). When the positive cut off value of the Pg-expression was established at 5%, the Simple Kappa Coefficient of inter-samples concordance was 0.75 (Table I).

A high correlation coefficient ( > 0.97) was also obtained when Pg-expression (CBS versus SR) was compared in cancers stratified by both the size (0.64), and the histology grade (0.82).

Tested by Scatter Plot correlation, the inter-sample consistency of proliferative activity (Ki67), showed an “acceptable” level of concordance (0.7118). Conversely, poor results were obtained (Simple Kappa Coefficient = 0.36) when the positive Ki67 cut-off value was established as lower than 15%.

As for the inter-samples consistency of Her2-expression, the prevalence of Her2+ve cancers (IHC and FISH) was relatively low (8%), excluding any conclusive interpretation.

**Conclusions.** In breast cancer, the high level consistency of IHC E- and Pg-expression in CBS versus SR tissue samples allows to consider the pre-surgical assessment as representative of the receptors-status (e.g. the pre-surgical assessment may exclude any further testing on the surgically resected specimens). On the other hand, the results of the present study demonstrate that the tumor proliferative activity (e.g. Ki67 expression) has to be re-tested in the post-surgical specimens, also when a pre-surgical assessment has been already achieved (low representativity of core biopsy samples and/or tumor heterogeneity). In the present series, the low prevalence of Her2-expression does not allow any conclusive appraisal on the inter-sample (CBS versus SR) consistency of the protein expression. Bona fide, such a situation should result in suggesting to reassess Her2-status in surgical specimens, also when a pre-surgical IHC-score has been already achieved.

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**Table I.** Breast cancer: prognostic/predictive variables in paired tissue samples obtained before and after surgical treatment.

<table>
<thead>
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<th></th>
<th>Scatter Plot Correlation</th>
<th>K coefficient</th>
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<tr>
<td>Estrogen</td>
<td>0.87</td>
<td>0.99 (cut off 5%)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.84</td>
<td>0.75 (cut off 5%)</td>
</tr>
<tr>
<td>Ki67</td>
<td>0.71</td>
<td>0.36 (cut off 14%)</td>
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<tr>
<td>Her2</td>
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**ROLE OF PD-L1 IN PATHOGENESIS AND PROGRESSION OF TRIPLE NEGATIVE BREAST CANCER PHENOTYPE**

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**Background.** Triple-negative breast cancer is a particular phenotype of breast cancer that do not express estrogen receptor (ER), progesterone receptor (PR), or HER-2 genes. The only treatment available is surgery followed by chemotherapy or radiotherapy.

PD-1 is one of the most important co-expressed inhibitory receptors on the surface of T lymphocytes previously activated by interaction with an antigen but is also present on the surface of B lymphocytes, macrophages, dendritic cells and lymphocytes tumor infiltrating (TIL). (1, 2) PD-1 receptor binds two ligands, PD-L1 and PD-L2, and they play an important role in down-regulation of the immune system, preventing the T cell activation, reducing in turn the autoimmunity and promoting self-tolerance.

Programmed death-ligand1 (PD-L1) is a protein of 40kDa trans-membrane encoded by the CD274 gene. It’s is expressed on a wide variety of cells including natural killer, macrophages, dendritic cells, epithelial cells and vascular endothelial cells. (3) PD-L1 is the main ligand of PD-1 receptor and is over-expressed in most solid tumors (lung cancer non-small cell, colorectal, melanoma, renal carcinoma, breast cancer, etc) and on the tumor microenvironment cells in response to inflammatory stimuli (such as IFN-γ, TNF-α, LPS, GM-CSF, VEGF and IL-10 cytokines and IL-4). (4) PD-1/PD-L1 pathway signaling is an adaptive immune resistance mechanism enacted by the tumor cells to evade the immune response.

The data available in the literature about PD-L1 immunohistochemical expression in tumor tissues are not uniform, for the use of different antibodies clones and the absence of a standardized operative protocol.

**Aim.** The aim of this study was to evaluate the prognostic role of PDL-1 expression in a series of triple TNBC and to define a standardized protocol suggesting a “tumor score” for its evaluation.

**Methods.** The samples were included in a prognostic Tissue MicroArray and the proteins expression analysis was performed by immunohistochemistry. All the statistical analysis were carried out using the SPSS version 20.0 software (SPSS Inc., Chicago, USA). The association between PD-L1 expression and the clinico-pathological parameters was conducted using the χ² Test. Progression free survival (PFS) and Overall survival (OAS) was calculated using the Kaplan–Meier method.

**Results and conclusions.** PD-L1 expression directly correlated with proliferation index (Ki-67)(p = 0.009), glycemia (p = 0.043) and the presence of diabetes (p = 0.034) and indirectly correlated with menopause (0.006), presence of lymph node metastasis (p = 0.026) and relapse (p = 0.008). Moreover, an inverse trend of statistical association with status of patients (p = 0.068) was present. The analysis of Kaplan-Meier showed that an increased PD-L1 expression was strongly associated with better disease-free survival (DFS) (p = 0.020) but not correlated with overall survival (OS) (p = 0.626). For the definition and evaluation of score we used qualitative and quantitative parameters, and considered the percentage of positive tumor cells ≥ 10% (cut off). The combination of the two parameters has allowed to establish as:
Score 0: cases with absent immunoreactivity of membrane or mild / moderate cytoplasmic positivity.
Score 1: cases with incomplete membrane positivity with a moderate / intense immunoreactivity and with / without the cytoplasmic positivity in ≥ 10% of tumor cells. Score 2: cases with complete positivity of membrane, with a moderate / intense immunoreactivity, with / without the cytoplasmic positivity in ≥ 10% of tumor cells.

PD-L1 could be an important marker for prognostic stratification and for planning new therapies in patients with TNBC.

References
Background. Breast cancer is one of the most common cancer in women with more than 1,300,000 cases and 450,000 deaths each year worldwide. These tumors display an extraordinary inclination to grow in bone. About 10% of all breast cancer patients without evidence of bone metastases at the time of diagnosis will have a first relapse in bone within five years of their primary diagnosis. The recent finding of breast osteoblast-like-cells (BOLCs) in lesions with microcalcifications may explain the strong osteotropism of breast cancer cells. In particular, we previously demonstrated that breast epithelial cells that acquire mesenchymal characteristics through the EMT phenomenon can assume an osteoblast-like phenotype under “Bone Morphogenetic Proteins” induction. Moreover, these cells show the ability to produce calcifications made of hydroxyapatite through a process similar to that occurring during the physiological bone formation. The main aim of this study was therefore to test the hypothesis that the appearance of BOLCs in primary mammary lesions is a precursor (and hence an early predictor) of the formation of breast cancer metastases to bone. The main aim of this study was therefore to test the hypothesis that the appearance of osteoblast-like-cells (BOLCs) in primary mammary lesions is a precursor (and hence an early predictor) of the formation of breast cancer metastases to bone.

Material and methods. In this study, we collected 64 breast infiltrating carcinomas (IC), 50 breast benign lesions (BL) and 10 biopsies of bone metastasis selected from IC patients. Immunohistochemical, western blot and ultrastructural analysis allowed us to investigate the presence of BOLCs in breast cancer lesions and metastatic sites.

Results. We established the presence of a pool of “mesenchymal-like” cells in IC. In addition, our results demonstrated that the microenvironment of breast cancer is very similar to the microenvironment of bone. In particular, we noted a significantly higher expression of BMP-2/4 and PTX3 in breast IC compared to BL. Moreover, we also identified numerous BOLCs positive to RANKL and Vitamin D receptor (VDR). Thanks to ultrastructural analysis, we also revealed the presence of BOLCs at the metastatic site.

Discussion. In summary, our results support the interpretation of BOLCs as early markers of bone metastasis. Moreover, we can speculate about a mechanism of bone metastases from breast cancer. In our model, breast cells with bone osteotropism (BOLCs) detach from the primary tumor site (thanks to their mesenchymal characteristics) and colonize the bone surface triggering bone resorption by the interaction with resident osteoclasts via the RANK/RANKL pathway. In this context, the typical osteolytic lesions associated with bone metastases in breast cancer could appear thanks to the imbalance in the bone microenvironment due to the high amount of cells able to activate the osteoclasts (i.e. resident osteoblasts and the additional amount of BOLCs originating from breast metastases). The identification of breast cancer cells with high affinity for a bone environment opens new perspectives on prevention and therapy of bone metastases from breast.

References

THE EMERGING ROLE OF INTERLEUKIN-30 IN BREAST CANCER GROWTH AND PROGRESSION

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Introduction. Breast cancer (BC) is a leading cause of cancer-related death in women worldwide. Its molecular signature has profound clinical implications, but a critical role in disease progression has also been established for the tumor microenvironment. Microenvironmental signals and molecular alterations of cancer may condition each other and have an impact on tumor behavior. We recently reported evidences that the endogenous Interleukin (IL)-27 cytokine subunit p28, also known as IL-30, exerts a tumor-promoting function, favoring cancer cell proliferation and gene expression alterations, specifically in prostate cancer. Therefore, we wondered whether IL-30 had a role in BC biology and if it might have useful clinical implications.

Methods. Human (h) BC cell lines were stimulated with recombinant(r)IL-30 and then analyzed for proliferation, apoptosis and gene expression profile by RT-qPCR. In a preclinical setting, BC cells were orthotopically implanted in NSG mice and locally treated with hrIL-30 to assess its effects on tumor growth. Expression of IL-30, in breast tissue and axillary nodes from a cohort of 156 patients with different molecular classes of BCs, was investigated by real-time RT-qPCR and immunohistochemistry and the molecular data obtained was correlated with clinical-pathological parameters and follow-ups.

Results. In vitro, hrIL-30 up-regulated the expression of MYC, MUC1, EGF, VEGF-A, and particularly of IL-6, in both triple-negative and HER2+BC cells. In triple-negative BC cells, hrIL-30 boosted proliferation, migration and invasiveness, up-regulated CSF1, PTGS2/COX2, CXCL10, Leptin, TMYP, MMP14, SONIC-HEDGEHOG and markedly increased CXCL1, IL-1-, IL-8, and PECAM1 expression, whereas it down-modulated PTEN, TP73 and the metastasis-suppressor gene KISS1. Knockdown of STAT1/STAT3 signaling hindered hrIL-30-dependent induction of the main tumor growth and progression factors. In vivo, hrIL-30 fostered triple-negative BC growth, by promoting cancer cell proliferation and vascular dissemination and intra-tumoral CD11b+/Gr1+ myeloid cell infiltrates. Analyses of clinical samples revealed that IL-30 was absent in normal mammary ducts, ductules and acini of histologically normal breast and scanty in the few stromal infiltrating leukocytes (ILK). Its expression was frequent.
in BC and was associated with triple-negative and HER2+ molecular subtypes. In BC- and draining lymph node (LN)-ILK, mainly CD14+ monocytes, CD68+ macrophages and CD33+CD11b+ myeloid cells, IL-30 increased with the stage of disease and correlated with recurrence. Cox proportional hazard model demonstrated a negative correlation (p = 0.042) between IL-30 expression by LN-ILK and overall survival (adjusted HR: 2.52; 95% CI: 1.03-6.13).

Conclusions. This study reveals for the first time IL-30’s implications in breast carcinogenesis, showing that: I. IL-30, directly and/or by subverting multiple oncogenes and tumor suppressor genes, favors cancer cell proliferation, migration and dissemination; II. it may boost cancer cell expression of soluble mediators, which may promote myeloid cell recruitment and tumor progression; III. IL-30 is expressed in most human BCs, and is associated with triple-negative and HER2+ subtypes; IV. IL-30’s expression by leukocytes infiltrating tumor and draining LNs correlates with BC stage and, more importantly, that a high level of IL-30 in BC draining LNs is an independent predictor of poor clinical outcome.

**BREAST TUMOURS RESEMBLING PAPILLARY THYROID CARCINOMAS (BPTC): REPORT OF 15 CASES WITH LONG TERM FOLLOW UP**

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**Introduction.** Breast tumours resembling papillary thyroid carcinomas (BPTC) has been described in 2003. Here, 15 cases are reported with long term follow up.

**Materials and methods.** Thirteen cases are from the consultation files of two of us (MPF & VE). Two cases had been previously included in the original series. Representative blocks were available in 7 cases and the selected sections were stained for ER; PR; mitochondrion; CD 31; CD 34; keratin 7; keratin 14; p63; GCDFP-15; smooth muscle actin; calponin; TTF1; thyroglobulin; collagen IV, AR, Ki67 and HER2 using an automated slide processing platform. The cut-off value for ER, PR and AR positivity was set at >1%. The percentage of Ki67-positive cells was recorded, and the cut-off for dichotomizing tumours with low and high proliferative fractions was established at 10% of positive cells. Clinical data of the 15 patients including age, type of surgery, type of treatment, occurrence and type of relapse and current status were obtained. Pathological data included morphological features, grade, size of the tumours, peritumoural vascular invasion and stage of tumours. The study was conducted in compliance with the ethical regulatory issues.

**Results.** Patients were female, mean age 64 years (range 48-85 years). Cases were discovered at mammography, except for case 3 that had a lump present for ten years. All cases initially underwent conservative surgery. Sentinel nodes obtained in six cases were negative. Tumour size was in mean 1.6 cm (range 0.6-2.5 cm). Thirteen cases were scored G1 and two were G2. Vascular invasion was focal in one case. The majority of the patients (66.7%) had a pT1 and the remaining had pT2 tumours (33.3%). The lymph node status was pN0 in 4 and pN1a in 2 out of 6 cases. All cases displayed neoplastic cells arranged in solid to papillary structures as well as follicles of different size filled by eosinophilic colloid-like material. These structures were closely reminiscent of those seen in thyroid and were observed in all cases. Follicles varied in number being scanty in some cases, numerous in other and were the majority as in case 15. Neoplastic cells evidenced abundant eosinophilic granular cytoplasm polarized mostly towards the basal pole. Nuclei were optically clear with a small nucleolus and a well evident nuclear membrane. Numerous grooves and occasional eosinophilic nuclear pseudo-inclusions were visible. All cases studied for mitochondria in >50% of the neoplastic elements. ER, PgR, AR, HER2, TTF1, Thyroglobulin were all negative. Ki-67 scored less than 10% in all cases. Myoepithelial cells were absent in the 10 cases tested (p63 and smooth muscle actin negative). Laminin and collagen IV antibodies evidenced abundant material surrounding the neoplastic solid clumps as well as within the papillary fronds. This material contained within it numerous small vessels as shown by CD 31 and CD 34 and superficially gave a fallacious feature of basal lamina. Case 2 recurred after 36 months from initial surgery. The patients underwent mastectomy and axillary dissection. One axillary node evidenced a metastatic deposit with similar histological features of the primary tumour. Case 3 had an intramammary metastatic lymph node. All patients are alive and well after 77 months (mean) of follow up (range 10-192 months). No fewer than 3 patients had 10 years of follow up.

**Conclusions.** Breast tumours resembling papillary thyroid carcinomas have to be recognized to avoid misdiagnosis as metastatic PTC from thyroid. BPTC are triple negative carcinomas with indolent clinical behavior. They rarely recur and metastasize. No case of death has been described. This is the reason to retain for these lesions the term ‘tumour’ and avoid that of carcinoma for neoplasms that rarely recur and metastasize. All the present cases were invasive, paralleling the features seen in solid papillary carcinomas of the breast that also have indolent behaviour. Studies are needed to elucidate the reason why such cases in spite of extensive invasion show a very low grade of malignancy.

**Acknowledgments.** The following colleagues are thanked for the generous offer of single cases: J.Wellings, G.Rindi; M. Brisigotti; M. Ricci; M. De Nicotis, P.Della Palma, R. Zenklusen, C. Gerber.

**References**

PATOLOGIA MOLECOLARE

SIRE NEXT GENERATION SEQUENCING PANEL: DIAGNOSTIC TOOL FOR CIRCULATING FREE DNA ANALYSIS


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Background. Tissue availability is a crucial point in NSCLC. The introduction of Liquid Biopsies allows to determine circulant biomarkers, specifically using free DNA. To simultaneously analyze multiple patients sample at high sensitivity, Next Generation Sequencing (NGS) can be narrowed to target a limited number of actionable genes. Here we prospectively applied a lab-developed narrowed gene panel (SiRe) to produce a DNA library covering 568 actionable mutations in six gene (EGFR, KRAS, NRAS, BRAF, cKIT and PDGFRα) applied a lab-developed narrowed gene panel (SiRe) to produce a DNA library covering 568 actionable mutations in six gene (EGFR, KRAS, NRAS, BRAF, cKIT and PDGFRα) 3.

Methods. This daily clinical practice study was performed on cfDNA obtained from Non Small Cell Lung Cancer blood samples (serum and plasma) prospectively collected either prior to treatment administration in patients without tissue availability (n = 46) or after a progressive disease (n = 19) from a first line gefitinib (n = 14) or afatinib (n = 5) therapy. SiRe detected an activating EGFR mutation in 4/46 (8.9%) cases and in T790M in 9/19 (47.4%) at the time of tumor progression. Using tissue data as gold standard, the SiRe panel showed a sensibility of 90.5% and specificity of 100%

Conclusion. The SiRe panel is an effective tool enabling the implementation of NGS for cfDNA mutational profiling in molecular pathology practice.

References

ULTRA DEEP NEXT GENERATION SEQUENCING IS AN USEFUL TOOL FOR EGFR TESTING ON PAUCICELLULAR SAMPLES REFERRED TO A CENTRALIZED LABORATORY

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Background. In previous studies we showed that Non Small Cell Lung cancer (NSCLC) cytological material collected in house, following rapid on site evaluation by our pathologist, is usually adequate for testing; conversely, samples outsourced from referral laboratories are often paucicellular and not always adequate for analysis by standard molecular techniques. In this setting, next-generation sequencing (NGS), narrowed to target a limited number of genes (ultra-deep sequencing), may enable testing of paucicellular samples, thanks to an increased sensitivity.

Methods. A narrowed NGS panel was designed in order to produce a DNA library covering actionable mutations in genes (EGFR, KRAS, NRAS and BRAF) involved in NSCLC. This custom panel was validated in vitro studies on HCC827 (EGFR, p.E746-A750 del) and A549 (KRAS, p.G12S) cell lines to assess the sensitivity of point mutations and indels. Then, two groups of cytological samples composed either by in house collected highly cellular smears (n = 30) or outsourced...
paucicellular (n = 30) were analysed by NGS on a Ion Torrent personal genomic machine.

Results. The designed panel was able to detect EGFR p.E746-A750del and KRAS p.G12S mutation until the last dilution tested (1:10000) thanks to the use of optimized parameters set in variant caller plug-in (v.5.0.2.1) which enabled the detection of low abundant mutations with a specificity of 100%. As far as the clinical samples are concerned the median DNA concentration was 32.69 ng/ul for in house cases, 12.02 ng/ul for outside referred cases. Median "mean target coverage" and run metric parameters overlapped between the two groups. A similar rate of mutation was detected regardless of starting DNA input.

Conclusions. By using a NGS panel enriched for amplicons relevant to NSCLC, paucicellular cytological specimens sourced from referral laboratories generate DNA sequencing data of comparable quality and quantity to that obtained from highly cellular in house collected cases.

References

NEXT-GENERATION SEQUENCING OF THYROID FNA SAMPLES USING THE ION AMPLISEQ™ CANCER HOTSPOT PANEL V2

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Background. Fine needle aspiration (FNA) cytology is accurate and cost-effective in the evaluation of thyroid nodules. However, molecular techniques may contribute to risk-stratification in indeterminate cases 1. Although next generation sequencing (NGS) is a promising technique for the molecular testing of thyroid FNAs, thyroid-specific cancer gene panels are not commercially available. Conversely, the Ion AmpliSeq Cancer Hotspot Panel v2 (CHPv2), which includes the genes most frequently mutated in thyroid cancer, is commercially available and may represent an alternative to thyroid-specific panels. To date, CHPv2 has performed only on “ideal” cytological samples featuring > 10 ng DNA input and satisfactory post-sequencing metrics 3.5. The aim of this study was to extend NGS to less than ideal samples, which represent a large portion of routine clinical specimens.

Methods. To this end, we retrospectively analyzed 37 thyroid smears using CHPv2, regardless of any pre-analytical and post-sequencing metrics thresholds. Specifically, we evaluated the performance of CHPv2 on the BRAF, NRAS, HRAS, KRAS and RET genes. Results were verified by pyrosequencing.

Results. Thirty-four of the 37 (91.8%) thyroid FNAs were successfully processed. BRAF, NRAS and RET somatic variants were detected in 22/34 (64.7%) samples. Post-sequencing metrics are reported in Table 1. Next-generation sequencing had a high sensitivity (94.4%), specificity (85.7%) and accuracy (88.4%).

Conclusion. CHPv2 is a valid option for the molecular evaluation of thyroid FNAs by NGS. Notably, this approach is accurate and effective even when applied to routine cytology samples that usually do not have optimal pre-analytical and post-sequencing requirements.

References

Table I. Mean and ranges of post-sequencing metrics in cytology samples successfully processed by NGS.

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<td>Uniformity (%)</td>
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</table>

ION TORRENT NEXT-GENERATION SEQUENCING FOR ROUTINE IDENTIFICATION OF CLINICALLY RELEVANT MUTATIONS IN BIOPSY AND CYTOLOGY SPECIMENS OF NON- Small Cell Lung Cancer Patients

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Introduction. The number of biomarkers that will be need to be assessed in clinical daily practice in molecular pathology is rapidly increasing. This calls for the implementation of methods probing the mutational status of multiple genes. Resected
tumors are the most informative samples for molecular tests and have been indicated as preferred sample type for EGFR analysis in non-small cell lung cancer (NSCLC). Unfortunately, only a minority of patients with NSCLCs, less than 30%, are eligible for surgical resection as advanced staging precludes the surgical approach, thus limiting the availability of neoplastic tissue for molecular analysis. Because minimally invasive diagnostic procedures are most often used in a diagnostic workup of lung cancer, small tissue specimens and cytological samples are often the material available for analysis. Because the increase in the number of genes to test is associated with a decrease in the sample size, the pathologist is facing a new challenge: optimization of available tumor tissue.

In recent years, Next Generation Sequencing (NGS) has begun to supplant other technologies for gene mutation testing. Targeted, amplicon based NGS offers simultaneous sequencing of thousands of short DNA sequence in a massively parallel way and may offer a cost effective approach for detecting multiple genetic alterations with a minimum amount of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue blocks. However, transfer of NGS technology to clinical daily practice requires validation. This study aimed to evaluate the clinical applicability of the Oncomine Solid Tumor DNA kit (CE-IVD), using the Ion Torrent Personal Genome Machine, to biopsy and cytology specimens of NSCLC cancer patients with real time PCR as reference.

Methods. Prospectively, 106 consecutive and unselected routine samples were simultaneously processed by our current technology, based on real time PCR and by Ion Torrent PGM sequencing. The primary sample types were either lung, transbronchial and pleural needle biopsies or EBUS-TBNA and EUS-FNA cytology specimens. The aspirated material obtained by EBUS-TBNA and EUS-FNA were mixed in Thin prep medium for cytological analysis meanwhile the tissue fragments present in solution were submitted to formaline fixation and paraffin embedding. In this way all the molecular analyses were performed from FFPE tumor samples. Unstained 10mm paraffin sections were prepared for DNA isolation performed with the QIAmp FFPE DNA kit (Qiagen) according to the manufacturer’s instructions. The H&E stained slide from the same block, previously reviewed by a pathologist was used as guide for the macrodissection. The percentage of tumor cells of the samples ranged from 5 to 90%. NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, USA) was used to assess DNA sample quality and DNA quantity was evaluated using the Qubit photometer (Life Technologies) and the Qubit dsDNA HS (High Sensitivity) Assay Kit according to the manufacturer’s instructions. Detection of EGFR and KRAS mutations were performed in the context of clinical daily practice by real time PCR in a 7500 Real-Time PCR System (Applied Biosystems) using a CE-IVD kit (Entrogen) for detecting EGFR (exons 18, 19, 20, 21) and KRAS (exons 2, 3, 4) mutations.

According to the manufacturer’s protocols, 10 ng of DNA for each sample was used for library preparation with the Ion Oncomine Solid Tumor DNA kit (CE-IVD) (Thermo Fisher Scientific). This panel is composed by 92 amplicons covering > 1800 Hotspot mutations in 22 genes (AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, KRAS, MAP2K1, MET, NOTCH1, NRAS, PIK3CA, PTEN, SMAD4, STK11, TP53). Template preparation by emulsion PCR was performed on the Ion Chef system (Thermo Fisher). Sequencing was carried out on the PGM (Thermo Fisher). Data analysis was carried out with Torrent Suite Software V.5.0.4 (Thermo Fisher). The Ion Reporter 5.0 (Thermo Fisher) was used for variant analysis and annotation.

Results. Quantity of extracted DNA ranging from 1.9 ng/ l to 181 ng/ l. For 2 cases, the amount of DNA obtained after extraction was too low to reach the required 10 ng for targeted sequencing (DNA concentration ranging from 0.19 to 0.9 ng/ l). Among the 104 cases, 102 (98%) were successful sequenced (number of mapped reads > 100000 or average base coverage > 500x). The unsuccessful cases were considered non informative because of a number of mapped reads < 100000 and average base coverage < 500x. Ten cases were considered suboptimal, however the quality of sequencing was considered good enough for further analysis. All the other cases (n = 92, 90%) had a number of reads > 100000 and an average base coverage > 1000X. The sequencing performances were summarized in the table below. We then considered the influence of different primary sample types on the sequencing performance. Tissue fragments (FFPE) obtained by EBUS-TBNA and EUS-FNA showed a significantly higher number of reads and base coverage depth compared to biopsies (Kruskal-Wallis test p = 0.01).

Sixty-four of the 104 samples (61.5%) have successful testing both by NGS and the real-time PCR method (EGFR and KRAS analysis), allowing study of concordance. We obtained an overall 100% concordance in EGFR and KRAS. We found 12/102 (11.7%) EGFR mutations and 27/102 (27%) KRAS mutations both by NGS and real-time PCR. EGFR and KRAS mutations were mutually exclusive. In 77/102 (75.5%) samples at least one mutation was observed among the 22 cancer-related genes. Thirty-eight mutations were identified in the TP53 gene (38.5%), eight in STK11 (7.8%), seven in BRAF (6.8%), six in PIK3CA (5.8%), two in PTEN (1.9%), and one each in CTNNB1 (0.9%), FBXW7 (0.9%) and FGFR1 (0.9%). Germline variants in MET (T1010I) were observed in four cases (3.9%) and in FGFR3 (F384L) in three cases (2.9%).

Conclusions. in conclusion, the present study validated the clinical applicability of the Oncomine Solid Tumor DNA kit for screening lung cancers. We show that targeted NGS using the Ion Torrent technology provides information about multiple genes starting from a very limited amount of DNA. Overall, The Oncomine Solid Tumor DNA kit is specific and sensitive enough for mutation analysis of gene panels and can be incorporated into clinical daily practice.

| Mapped Reads | 247033 SD=226930 CI 95%=201638 to 292427 |
| Uniformity of Coverage | 95,75% SD=6,67% CI 95%=92,62% to 95,43% |
| % reads covered at 500X | 90,09% SD=16,16% CI 95%=84,46 to 95,54% |
| Mean read depth | 2497 SD=2252 CI 95%=2047 to 2947 |
OPTIMIZATION OF MICROSATELLITE INSTABILITY ANALYSIS IN CASES OF LYNCH SYNDROME-RELATED ENDOMETRIAL Cancers

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Introduction. High exposure to oestrogens, obesity, and not least a family history, are considered the main risk factors for endometrial cancer (EC), one of the most common tumours among women in Western countries and the most common form of extra-colonic neoplasm in patients with Lynch Syndrome (LS). This tumour, often defined as “hereditary non-polyposis colorectal cancer”, is caused by germline mutations in DNA mismatch repair (MMR) genes that lead to the accumulation of sequence errors, represented by point mutations, and micro- or macro-insertions/deletions. These can induce neoplastic transformation when they occur in tumour-suppressor genes or oncogenes. Functional inactivation of MMR genes by mutations or epigenetic changes is detectable through the analysis of short DNA tandem-repeat sequences (microsatellites) in highlighting the high microsatellite instability (MSI-H) phenotype. These are more prone to DNA polymerase replication errors.

To date, over 160 target genes have been identified in MMR-deficient tumours of several tissues. The profiles of instability target genes differ between EC and gastrointestinal tumours with MMR deficiencies, both qualitatively and quantitatively. While instabilities characterizing colorectal tumours mainly occur at BAT loci (90% of tumours) and the TGFβRII locus (73%), ECs are characterized by a more heterogeneous pattern of instability with smaller allelic shifts. Given the high frequency of LS-related ECs, there is great clinical interest in their characterization, and in the processes leading to gene instability in LS extra-colonic malignancies. Therefore, our aim was to test the performance of microsatellite markers NRIP1, SPRP, JAK1, PTEN, NR27, and BAT40, which were selected after a literature review focusing on MSI-H-associated endometrial carcinogenesis. We compared these with the Pentaplex Panel, which is typically used to perform MSI-H analysis in our laboratory, to optimize the screening process for accurately selecting EC candidate genes for a second level of MMR analysis.

Methods. Microsatellite instability (MSI) analysis was performed on DNA extracted from tissues comprising at least 80% of tumour cells using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). The microsatellite markers used were NR21, NR22, NR24, BAT25, and BAT26 (Pentaplex Panel); NRIP1 (mononucleotide repeat in exon 3); SPRP (mononucleotide repeat in exon 3); JAK1 (mononucleotide repeat in exon 4); PTEN (mononucleotide repeats in exons 7 and 8); NR27 (BIRC3 gene); and BAT40 (poly-T sequence, Chromosome 1). The amplified tumour DNA and the matching normal DNA sequences were analysed using capillary electrophoresis and GeneMapper software (Applied Biosystems; Thermo Fisher Scientific). Immunohistochemistry (IHC) staining of MMR protein was performed on formalin-fixed, paraffin-embedded tissues using the EnVision FLEX+ Visualization System and the following primary antibody: MLH1 (clone ES05), PMS2 (clone EP51), MSH2 (clone FE11), and MSH6 (clone EP49) (Dako Carpinteria, CA, USA).

Results. MSI analysis was conducted on 74 cases of EC, previously diagnosed at the Anatomic Pathology Unit ULSS2 Veneto, Italy, first using the Pentaplex Panel, proposed by Suraweera et al., consisting of the five mononucleotide markers listed above. Of these 74 cases, 63 (85%) were judged MSI-stable (MSI-S) and 11 (15%) highly MSI-unstable (MSI-H). The most frequently mutated markers were BAT25 and BAT26, while NR22 was less frequently mutated. NRIP1, SPRP, and JAK1 markers were used according to Ferreira et al., and these were used to identify the most frequently mutated genes after we had performed genome-wide research on EC-expressed genes characterized by repeated coding sequences similar to microsatellites. Among the 74 cases analysed, three had an NRIP1 mutation, one had an SPRP mutation, and none had a JAK1 allelic shift. The cases with NRIP1 and SPRP mutations were previously classified as MSI-H using the Pentaplex Panel discussed above. Based on results published by Kuismanen et al. identifying phosphatase and tensin homolog (PTEN) tumour suppressor gene sequence alterations as typical of EC, MSI analysis was extended to exons 7 and 8 of this gene. None of the 74 cases presented with mutations involving the regions mentioned above. The NR27 marker, located inside the BIRC3 gene, was selected based on a modified Pentaplex Panel proposed by Buhard et al. Ten cases out of the 74 analysed, previously defined as MSI-H using the Pentaplex Panel, showed NR27 instability. The BAT40 marker, a poly-T sequence localized on chromosome 1, was selected because of its documented sensitivity for documenting extra-colonic LS-related tumours. None of the 74 cases were unstable for this marker. Given the polymorphic nature of BAT40, analysis was conducted on both tumours and matching normal tissues, so it was possible to exclude a polymorphic origin of the allelic shifts found. It is important to note that four of these nine BAT 40 mutated cases would have been classified MSI-S using the Pentaplex Panel, thereby excluding them from a diagnostic algorithm for the selection of candidate patients for second-level MMR gene analysis. One of them had loss of the MSH6 protein shown by IHC, a frequent event in LS-related EC. The exclusive use of the Pentaplex Panel would have led to the loss of one patient as a candidate for MSH6 sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis.

Discussion. Although the Pentaplex Panel proposed by Suraweera et al. has proven to be more sensitive and specific than the Bethesda Panel, and its use has been recommended strongly in the international literature, a lack of performance emerged from our analysis of LS-related extra-colonic tumours, particularly MSH6-deficient ones. Based on our findings, the use of NRIP1, SPRP, JAK1 and PTEN markers, which allow the identification of single base allelic shifts, did not increase the sensitivity of the screening process previously conducted by the use of the Pentaplex Panel, so their use is not recommended. In contrast to the report by Goel et al., who found poor sensitivity for the NR24 marker, our data highlight the poor performance of the NR22 marker. Therefore, we propose a change in the original Pentaplex Panel for EC screening, replacing NR24 with the NR27 marker, which has proven to be more sensitive. Furthermore, the introduction of the BAT40 marker seems to have been very useful, especially for identifying cases of low gene instability often associated with isolated loss of MSH6 protein expression in cases of EC, otherwise not detectable by the exclusive use of Pentaplex Panel. We conclude that use of the BAT25, BAT26, NR21, NR24, NR27, and BAT40 markers guarantees optimization of microsatellite instability analysis, especially for tumours associated with extra-colonic LS. Together with IHC evaluation of MMR protein expression and methylation analysis of the MLH1 promoter, these provide valuable tools to select
patients with ECs for a second-level molecular analysis of genes involved in DNA MMR.

References

MOLECULAR PROFILE OF 25 STAGE I COLORECTAL CANCERS (Dukes’ A) WITH UNFAVORABLE PROGNOSIS
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Background. Although most patients with Stage I (Dukes’ A) colorectal cancers (CRC) show an excellent prognosis, a few fraction of them (5%) die from metastatic disease. Different histological unfavorable prognostic markers have been identified as responsible for the progression of these tumors such as high grade WHO tumor, the occurrence of tumor budding, poorly differentiated clusters of tumor cells at the invasive edge (PDC), lymph-vascular invasion and lymph node micrometastases. Recently, several studies have reported that mutations in KRAS, BRAF or PIK3CA oncogenes are involved in tumor genesis and in the development of metastasis in CRC from the early stages.

Materials and methods. The aim of the study was to investigate the biomolecular profile of 25 Dukes’A cases with unfavorable prognosis, selected from the Specialized Colorectal Cancer Registry in the District of Modena, who died of metastatic disease during the follow up period (4.8% of the Stage I tumors diagnosed from 1989 to 2004; average disease-free survival of 2.9 ± 2.4 years). Hotspot mutations were detected in amplified genome DNA by using Mass Spectrometry based on single base extension technique (Mass Array Sequenom Platform - Sequenom, San Diego, CA; Myriapod Colon Panel). The occurrence of mutations in KRAS, NRAS, BRAF and PIK3CA genes was evaluated in surgically removed primary tumors. 22 cases were pT2 and 3 were pT1 whereas 15 cases were located in the rectum, 7 in the left colon and 3 in the right colon; 24 tumors were low grade and 1 was a high grade tumor.

Results. Mutated status was detected in 18 of 25 (72%) cases. KRAS mutations were evidenced in 16 tumors while BRAF mutations were observed in 2 tumors. No NRAS mutations were found. PIK3CA mutations were observed in 4 cases as additional mutations coexistent with KRAS mutation. A single KRAS mutation was detected in 11 cases [exon 2, codon 12 in 8 cases (4 G12D mut, 3 G12V mut and 1 G12F mut), exon 2, codon 13 in 2 cases (G13D mut in both cases) and exon 4, codon 146 in 1 case (A146T mut)]. Among the remaining KRAS-mutated cases, an additional PIK3CA mutation was observed respectively in exon 1, codon 108 in 2 cases (R108H mut, respectively associated with G12D and A146T) and in exon 9, codon 545 in other 2 cases (E545K mut, respectively associated with G12R and G13D). One case showed a triple mutation in KRAS: exon 2 (G12D mut and G13D mut) and exon 4 (K117N mut). BRAF mutations were observed in exon 15, codon 600 (V600E mut). All tumors showed several unfavorable histological features including tumor budding, PDC, lymph-vascular invasion and lymph node micrometastases. These features were found to be correlated with the mutated status of the tumor and with the presence of multiple mutations (double and triple mutations). No correlation was observed with the tumor site and the WHO tumor grading.

Conclusion. Our results suggest the existence of a strong correlation between the occurrence of KRAS, BRAF and PIK3CA mutations and progression of CRC in early stage (Dukes’ A). Mutations in these genes could become a fundamental additional parameter for the definition of the degree of malignancy in this subset of CRC.

EVALUATION OF EGFR MUTATIONS IN THE PLASMA OF NSCLC PATIENTS IN ROUTINE SETTING: CORRELATION WITH PATIENT MATCHED TISSUE
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Introduction. Non Small Cell Lung Cancer patients with activating somatic mutations in EGFR show a significant benefit in progression free survival upon treatment with Tirosin Kinase Inhibitors (TKI). So far, EGFR tissue/cytologic testing has been considered as the gold standard to provide the molecular features of the tumor. However, in some patients the availability of the biological material is limited because of its insufficient amount and/or of its low quality. Recently, the circulating cell free tumor DNA is emerging as a specific and sensitive biomarker for tumor genotyping. CfdNDA is released into circulation with not yet clarified mechanisms and it is becoming in the clinical and diagnostic practice a reliable source of tumor genetic material. From one side, analysis of cfd DNA represent a valid and alternative option to the classical tissue testing, on the other side it’s reaching a more important role in the managing of the disease during time
especially in the monitoring setting. In particular, in those patients who develop a resistance to the TKI therapy through the emergence of the secondary mutation T790M (about 60% of patients), the chance of testing the cfDNA for search of such mutation in the plasma has the objective advantage to lower the risk and discomfort of making a new biopsy of the tumor lesions necessary for ascertain the resistance mechanism.

Identification of T790M positive patients is relevant for the choice of the third generation TKI, which are capable of overcoming the T790M-associated resistance. However, the molecular genotyping by using cfDNA as genetic material is complicated by the presence in the plasma of elevated background noise from the non neoplastic normal DNA, which strongly dilute the tumor DNA. To this respect, the molecular techniques employed for the search of EGFR mutations should be enough robust, sensitive and standardized.

In order to assess the ability of different methods to detect EGFR mutations, including T790M, in cfDNA, we compared the results obtained by three different realtime pcr platforms in two cohort of NSCLC patients, one at diagnosis and one after TKI-treatment at the progression of the disease. In selected cases the results obtained on plasma have been correlated with those obtained in patient matched tissue.

**Materials and methods.** Blood samples have been collected in K3 EDTA tubes and processed within 30 min by two consecutive centrifugations (1600g for 10 min e 16000 rpm for 10 min). The plasmas have been immediately stored after preparation at -20°C. CfDNA has been isolated from 3ml of plasma by using the QIAamp DNA circulating Nucleic Acid kit (Qiagen). Tumor DNA from 29 available matched tissues has been purified by using the FFPE DNAeasy kit (Qiagen). Analysis of exon 19 deletion, L858R and T790M mutations has been performed in all plasma samples with the PCR Real Time therascreen EGFR plasma kit (Qiagen) (sensitivity range from to mutated DNA/ wild type DNA as indicated in manufacture instruction). In selected cases, cfDNA has been ri-tested by using a higher sensitive kit (PNA-muthy BioClarma kit, sensitivity1% as indicated in manufacture instruction) and an homemade LNA-clamp Real time PCR specific for the detection of T790M (sensitivity ranges between 0.5 and 1%, determined by using different mixture of mutated DNA/wild type DNA. EGFR mutations in tissues have been detected by therascreen RGQ PCR kit (Qiagen).

**Results.** Plasma samples of 70 NSCLC patients at diagnosis (pre-treatment) have been tested by using PCR Real Time therascreen EGFR plasma kit and 7/70 cases were positive for sensitizing mutations of EGFR. The patient-matched tumor tissue was available for 29 patients and the results of EGFR testing showed an high level of concordance between plasma and tissues (28/29 cases; 96.5%) with a unique patient negative for EGFR mutation in the plasma and mutated in the corresponding tissue.

The plasma of 29 patients, already defined as EGFR mutated at the diagnosis, were collected at progression of the diseases during TKI treatment and tested with the PCR Real Time therascreen EGFR plasma kit.

Original sensitizing EGFR mutations were detected in 21/29 of the cases (72.14%). Four out of the eight EGFR mutation negative cases have been re-tested with higher sensitivity PNA-muthy kit and were confirmed to be negative, a finding that indicates that the absence of the original sensitizing EGFR mutation was not due to the sensitivity of the technique but probably to a bias of the blood samplings. In the cohort of patients tested at the progression after TKI, 21 plasma samples were tested for the presence of resistance T790M mutation by using three different realtime pcr approach. 8 cases out of 21 acquired T790M positive as assessed by the PCR Real Time therascreen EGFR plasma kit Qiagen (38%); while using both the PNA-muthy kit and the homemade PCR Real Time, the number of T790M resistance mutation positive samples reached 12 cases (57%).

In 9 cases out of the 21 plasma studied tested at the progression, a repeat biopsy has been performed in the two weeks after the plasma collection. In all the tissues tested the original activating EGFR mutations could be detected while the presence of the T790M resistance mutation was observed in five out of 9 cases (55% of the cases). Thus, three T790M positive in tissue resulted T790M negative in corresponding plasma while the remaining 6 samples of tissue showed concordance with the plasma (6/9 cases, 70%). Indeed, the four concordant T790M negative samples can be considered “truly” T790M negative cases (44%) and this finding indicates that in these cases the progression was probably due to other mechanism rather than the T790M development.

**Conclusion.** In our cohort of NSCLC patients at diagnosis there is an high concordance for EGFR mutations between plasma and matched tissues (96.5%), in contrast in the cohort of NSCLC patients at progression during TKI treatment, concordance is around 55% (5/9 cases).

The cases can be discordant for both the activating mutations and the T790M resistance mutation. However, the percentage of T790M positive cases detectable by molecular genotyping of the cfDNA is not so different from the one reported in the literature for the T790M testing in the lesion tissues (12/21 cases, 55% vs 60%). However, in some instances more sensitive molecular technique such as PNA- or LNA- based Real Time PCR, should be employed for the search of the T790M in the plasma in order to rescue those cases which can be misclassified as negative by using technique not sufficiently sensible.

Detection of T790M by using DNA from the repeat biopsy of the new tissue lesions in patients at progression added additional 3 T790M positive cases to the 12 identified in the plasma. However, the fact that the majority of T790M positive cases may be already identifiable by the T790M genotyping in the plasma, indicates that the NSCLC patients who need to undergo to a challenging new biopsy, are few and that the search of T790M should be approached at first instance in the plasma.

Finally, EGFR plasma testing is feasible in the routine settings of molecular diagnostic labs but each lab should be aware of the pitfalls due to the low level of tumor DNA in the plasma and also to the heterogeneity of T790M expression in the neoplastic cells which renders more difficult avoiding false negative in the plasma.

**References**

GENE EXPRESSION ANALYSIS OF LONG-TERM RESPONDER HER2-POSITIVE METASTATIC BREAST CANCER PATIENTS

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Background. HER2-positive breast cancer is an aggressive phenotype characterized by high-grade histology and high proliferation rate usually associated with poor prognosis. The introduction of HER2-targeted therapies drastically changes clinical outcome of these patients, so that median overall survival (OS) is now estimated to be about 3-3.5 years. Interestingly, some of these patients remain alive and disease-free many years after the diagnosis of metastases. Nowadays, neither clinical nor biological factors have been clearly associated with long-term response. The aim of the present study was to investigate the molecular characteristics of Long-Responder (LR) patients in order to identify biomarkers able to differentiate LR patients from patients who experienced early progression during anti-HER2 treatments (Poor-Responders, PR). We investigated the molecular mechanisms underlying this "exceptional response".

Materials and methods. A total of 34 patients with confirmed diagnosis of HER2-positive metastatic BC were included in the study. HER2 positivity was evaluated by immunohistochemistry (score 3+) or Fluorescence In Situ Hybridization (FISH). Patients were selected according to clinical outcomes and divided into two groups: 20 patients with at least 36 months of PFS (LR group) and 14 patients with disease progression within 12 months of the start of anti-HER2 therapy (PR group). Median PFS in the LR group was 54.8 vs. 4.0 months in the PR group. All patients received first line treatment (chemotherapy or hormonal therapy) as treatment of the start of anti-HER2 therapy (PR group). Median PFS in the LR group was 54.8 vs. 4.0 months in the PR group. All patients received first line treatment (chemotherapy or hormonal therapy) as treatment of the start of anti-HER2 therapy (PR group).

Whole Genome Expression analysis comparing LR vs PR HER2-positive patients identified interesting genes expression differences. Mainly, up-regulation of the MAPK pathway was found to be significantly different in the two cohorts (p value < 0.05).

Conclusion. Whole Genome Expression analysis comparing LR vs PR HER2-positive patients identified interesting genes expression differences. Mainly, up-regulation of the MAPK pathway was found to be significantly different in the two cohorts (p value < 0.05).

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Introduction. Cyclophilin A (CypA) is an 18 kDa protein detected in epithelial cells. It is one of the most common aminopeptidase N (APN) forms,
Materials and methods. This preliminary investigation includes 15 patients with RC treated with CRT followed by surgery in our Institute between 2013 and 2014. Four patients had a TRG of 1 or 2 (grouped together), 6 patients had a TRG score 3 and five patients had a TRG score 4. Proteins from pre-treatment tumor biopsies were screened by comparative proteomic approach by using 2-D difference gel electrophoresis (2D-DIGE). After normalization of data, for every single patient, the spots corresponding to the identified CypA were measured for protein abundance (Decyder software).

2.5-micron sections were cut from formalin-fixed paraffin embedded tissue from pre-treatment biopsies and post-treatment surgical resection. Immunohistochemical analysis was performed in an automated system (Benchmark XT and Ultra, Ventana, Tucson, AZ, US) using monoclonal antibody against CypA (1:400; Abcam, Cambridge, UK). An irrelevant rabbit antiserum served as a negative control. RC cells with cytoplasmic and/or nuclei immunoreactivity were considered positive. The percentage of positive cells and the intensity of staining were semiquantitatively evaluated by two pathologists who were blinded to clinical data, as follows: the percentage of positive cells in 0%-5% was counted 0; the percentage of positive cells in 5%-25% was counted 1; 26%-50% was counted 2; 51%-75% was counted 3; ≥ 76% was counted 4. Staining intensity was scored as 1 = yellow-brown; 2 = light brown; 3 = dark brown. The staining index score was the sum of the percentage of positive cells and the intensity of staining.

We used ANOVA test to compare data form proteomics analysis, immunohistochemical evaluation and clinicopathological parameters.

Results. Proteomic analysis on pre-treatment biopsies showed an average lower level of CypA expression [log (-0.34)] in cases with TRG 1-2, when compared with cases with TRG3 [log(-0.12)]. No conclusive results were evident in the TRG 4 group, maybe due to actually unknown factors that could affect CypA level in these patients or to the low number of cases studied. A significant relation between decrease of CypA proteomic levels and the presence of lymphatic (p = 0.043) and vascular (p = 0.004) neoplastic invasion was evident. Immunohistochemical CypA expression on pre-treatment biopsies was able to discriminate between TRG1-2 vs TRG 4 patients (p = 0.0403), being higher in TRG4 cases, whereas no differences emerged when TRG3 and TRG4 cases were grouped together. Immunohistochemistry on endoscopic biopsies and on surgical samples showed no relation between CypA expression scores and clinicopathological parameters. On surgical specimens, both immunohistochemical and proteomic analyses were unable to confirm pretreatment data. Qualitative immunohistochemical evaluation showed CypA expression also in endothelial and smooth muscle cells of vessels and in the peritumoral stroma in surgical samples.

Conclusion. These preliminary data suggest the potential utility of CypA protein as predictive biomarker for CRT response in locally advanced RC. When tested on pre-treatment biopsies, CypA could help to discriminate between patients with complete response (TRG1) (reducing invasive surgical approaches) and patients who will benefit (TRG3-4) from intensified neoadjuvant treatment. The relation between vascular/lymphatic neoplastic invasion and lower CypA levels is still unclear. CypA exists in an intracellular form and can also be secreted by tumor cells in response to inflammatory stimuli. By proteomic analysis it is not possible to discriminate between intra and extracellular proteins. However, the secreted form of the protein is thought to act in an autocrine/paracrine manner, stimulating vascular growth and modulating endothelial function. We speculate that the inverse association between CypA expression and vascular/lymphatic invasion, which is partially in contrast with literature data, could be due to still unknown interactions between the protein and the microenvironment and CypA low level may eventually facilitate neoplastic vascular permeation. Interestingly, CypA immunohistochemical expression was found in endothelial and smooth muscle cells of peritumoral vessels.

In conclusion, CypA seems to be a promising prognostic/therapeutic biomarker in RC; however, these preliminary data need to be further validated in larger case series.

References
estimated on objective endoscopic and histological parameters, in the form of Mucosal Healing (MH). There is no universally shared definition of MH; it can be interpreted as a total remission of damage of the intestinal mucosa, or as a reversion to healer conditions. Assessment of MH by histology can be contributing to the definition of remission, to the evaluation of the efficacy of the therapy, consequently to the choice between different agents and dosages; reaching MH could indicate longer time of release from disease. It can give to the histopathological examinations of biopsies during follow up of patients affected by IBD new usefulness than the only diagnosis, disease extension and activity index.

A histological definition of MH may not be universal and could change by clinical stage, patient’s disease, differentiating IB between ulcerative colitis (UC) and Crohn disease (CD), and by the kind of drug used. Moreover, it should agree with clinical and endoscopic remission.

We investigated a population of 51 children, affected by IB (both UC and CD), studying colonic mucosal biopsies before and one year after Azathioprine therapy.

Goal of our research was to find a correlation between histopathological MH and clinical and endoscopic MH, in a pediatric population of patients.

Methods. Fifty-one patients in pediatric age affected by IB (27 UC and 24 CD) were selected by pediatric colleagues of the University Hospital Federico II of Naples. All patients underwent colonoscopy with mucosal biopsies from every colonic segment, including terminal ileum, before starting Azathioprine therapy for 1 year. Thereafter, a new colonoscopy was performed and biopsies were taken in the same places of the first examination. All biopsies were submitted to Pathology Section and processed according to diagnostic purposes. For all patients, each biopsy was evaluated by two pediatric expert pathologists according to a semi-quantitative count producing 2 different scores for architectural distortion and inflammatory activity. Both scores ranged from 0 to 3, where 0 translated no alterations, 1 mild, 2 moderate and 3 severe alterations. Architecture score was given combining crypt branching, distortion, atrophy and mucin depletion. Inflammatory score was given combining the intensity of lymphoid infiltrate in the lamina propria with the presence of additional findings, such as basal lymphoplasmacytosis, increase of eosinophils in the lamina propria, presence of granulomas (MC) and activity. The presence of activity made it always a score of 2 or 3. So each patient had for each biopsy an architectural and inflammatory score, that was compared with the biopsy taken from the same place in the following colonoscopy.

Results. Results are given comparing the scores between first and second colonoscopy.

Among 24 CD patients, 8 children showed in both architectural and inflammatory score a decrease in almost all biopsies; 5 showed a decrease only in the inflammatory score, 2 did not show any significant variation; in 4 children data were contrasting; in some biopsies the score decreases, in other it increased. Three cases showed increase in both scores, while 2 showed only inflammatory increase.

Among 27 UC patients, 8 children showed in both architectural and inflammatory score a decrease in almost all biopsies; 5 showed a decrease only in the inflammatory score, 1 only in the architectural score, 1 did not show any significant variation; in 10 children data were contrasting; in some biopsies the score decreases, in other it increased. Two cases showed increase in both scores.

Conclusions. Interpretation of results was problematic; pediatric colleagues interpreted data together with clinical points and endoscopy. They made a sum of the scores from each biopsy comparing it between the two colonoscopies. No significant association with clinical data and endoscopy was found, concluding that in this setting, histological evaluation was not useful in finding a MH.

However, some data could be reached also by this experience. First, the score should be as simplest as possible. Two scores, both ranging from 0 to 3, are difficult to reproduce between pathologists and too complex to analyze for clinicians. We hope in a much better agree and clinical usefulness having a two-tiered score. Second, while a healing was easily obtained in children undergoing a therapy, a complete remission, given by architectural remission, was difficult reached. Probably, a better definition of MH is simply a decrease of the score and not the reaching of a complete healing.

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TUMOR BUDDING AS RISK FACTOR FOR NODAL METASTASIS IN PT1 COLORECTAL CANCERS: A META-ANALYSIS
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Introduction. Worldwide, the screening programs for colorectal cancer (CRC) have significantly increased the prevalence of the endoscopically removed of pT1 CRC; among these early malignancies, the prevalence of metastatic regional disease ranges from 10% to 20%. Different histological parameters are currently considered in the prediction of the metastatic risk associated to pT1 CRC. However, their reliability and implementation in the clinical practice are debated. Tumor budding (TB) is included among the variables that may consistently prognostic the metastatic risk. However, due to the variability of its definition and method of assessment, the TB evaluation is not consistently recommended in the histology reporting of pT1 CRC.

This meta-analysis investigates the prognostic role of TB in the assessment of the nodal metastatic risk in pT1 CRC.

Methods. An electronic literature search was performed using PubMed and Scopus without language restrictions, from their inception until 05/31/16 seeking studies that considered TB (presence versus absence) in pT1 CRC and nodal metastasis (presence versus absence). The following data were extracted: i) study population (e.g. sample size, demographic); ii) number of patients with/without nodal metastasis; iii) number of patients with/without TB; iv) pathology criteria in the TB assessment; v) microscopic magnification applied in TB assessment; vi) implementation of immunohistochemistry...
expression (MLH1, MSH2, MSH6 and PMS2). Clinical and Mismatch Repair status was determined by MSI analysis and/or completion surgery after the complete endoscopic resection of a pT1 CRC. The results of this meta-analysis do support the priority of including TB assessment as mandatory histologic parameters to identify those patients that require completion surgery after the complete endoscopic resection of a pT1 CRC.

**PATHOLOGIC AND MOLECULAR FEATURES OF BRAF MUTATED COLORECTAL ADENOCARCINOMAS**

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**Introduction.** There is growing evidence of the diagnostic, prognostic and predictive role of BRAF mutation in colorectal cancer. BRAF mutation, together with MSI and CIMP, is also a key feature of large bowel tumors that develop through the serrated pathway. Aim of this study was to examine the clinical and pathological features associated with BRAF mutant colorectal cancers in relation to microsatellite instability status and to compare in the group of Mismatch Repair proficient (MMR-P) carcinomas the characteristics of BRAF-mutated, KRAS-mutated and KRAS/BRAF-wild-type tumors.

**Materials and methods.** Direct Sanger sequencing was used to detect mutations of the BRAF (exon 15) and KRAS (exon 2) genes in the DNA from 1147 colorectal cancer samples, either frozen or paraffin embedded, of patients who underwent surgical resection at our institution between the years 1990 and 2015. Mismatch Repair status was determined by MSI analysis and/or immunohistochemical analysis of Mismatch Repair protein expression (MLH1, MSH2, MSH6 and PMS2). Clinical and pathologic parameters evaluated were: age and sex of patients, tumor site (proximal or distal colon), histological type (WHO classification), TNM stage and grade of differentiation (well/moderate vs. poor). Statistical analyses were performed using the chi-square test or exact Fisher test. The results were considered statistically significant when \( P < 0.05 \).

**Results.** BRAF mutation was detected in 29.6% of the 1147 cases examined and was significantly associated with female sex (\( P = 0.005 \)), age > 69 years, proximal location, high histological grade, and mucinous or medullary histology (all \( P < 0.001 \)). A strong relation was observed between BRAF mutation and MMR status. BRAF mutation was detected in 59.2% (277/468) of Mismatch Repair deficient (MMR-D) and in 9.1% (62/679) of MMR-P carcinomas (\( P < 0.001 \)). All the BRAF-mutated MMR-D tumors displayed loss of MLH1 protein expression. Stratifying by MMR status, a significant relation between BRAF status and tumor site and grade was observed in both groups. In the group of MMR-P cancers, BRAF mutation was also more frequently found in tumors with a mucinous component with respect to common adenocarcinomas (\( P < 0.001 \)).

526 MMR-P carcinomas were analysed for both BRAF and KRAS exon 2 mutations and were subdivided in three groups: BRAF-mutated (62 cases), KRAS-mutated (287 cases) and KRAS/BRAF-wild-type (177 cases). With respect to the other two categories, BRAF-mutated tumors were characterized by more frequent proximal location (85.7%, \( P < 0.001 \)), poor differentiation (45.7%, \( P = 0.004 \)), and mucinous histology (61.4%, \( P < 0.001 \)). KRAS-mutated carcinomas differed from KRAS/BRAF-wild-type carcinomas for a more frequent location in the proximal colon (65.5% vs. 52.1%) and mucinous differentiation (37.0% vs. 20.2%).

**Conclusions.** BRAF mutation occurs frequently in MMR-D colorectal cancers and only in a small fraction (about 10%) of MMR-P tumours. As a whole, the clinical and pathologic features of BRAF mutated colorectal cancers are quite superimposable with those of MMR-D tumors. In this study we evaluated the characteristics of BRAF mutated tumors in relation to MMR status. Among MMR-D cancers, only small differences were found between BRAF-mutated and BRAF-wild-type cases, probably related to their sporadic or hereditary origin. Conversely, the pathologic features of MMR-P BRAF-mutated and BRAF-wild-type carcinomas were quite different. MMR-P BRAF-mutated adenocarcinomas are mainly characterized by proximal location, mucinous phenotype and high histological grade and therefore they seem to represent also pathologically a distinctive subset of tumors.

BRAF and KRAS mutation were confirmed to be mutually exclusive by our study. KRAS mutations are much more frequent than BRAF mutations in colon cancer, but the pathologic features of KRAS-mutated carcinomas are not clearly defined. We found that MMR-P KRAS-mutated tumors occur more frequently than KRAS/BRAF-wild-type tumors in the proximal colon. Furthermore, among MMR-P carcinomas KRAS mutation rate was significantly higher in mucinous carcinomas. Therefore, mutations in the KRAS gene seem to contribute to the genetic differences between right and left colon cancer and to play a relevant role in the pathogenesis of mucinous adenocarcinomas.

**References**


THE PREDICTIVE VALUE OF THE INCREASE IN EOSINOPHILS AS A MAJOR FACTOR OF AGGRESSION IN PATIENTS WITH ULCERATIVE COLITIS

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Introduction. The clinical history of Crohn’s disease (CD) and Ulcerative Colitis (UC) (1) is characterized by alternating periods of remission and exacerbation. In the pathogenesis of these entities eosinophils probably play a role in the inflammatory microenvironment of the lamina propria in the course of IBD through the release of pro-inflammatory cytokines and proteins to cytotoxic action. (2,3) The therapeutic first-line treatment of UC, which involves the use of amino-salicylates, steroids and immuno-modulatory agents, is intended to promote the clinical-endoscopic and histological remission of the disease. The first-line treatment differs in patients with UC. In fact, added to the group of patients in stable remission after therapy, there is a second group of patients with a more aggressive course of the disease, who require second-line treatments (biological C.D. drugs) in a short time. The aim of our work was the retrospective histological evaluation of UC cases characterized by aggressive clinical course and not responding to first-line drugs, as opposed to cases of favorable course, with the intention of identifying morphological prognostic factors useful in differentiating between the two groups.

Materials and methods. We reviewed retrospectively 28 first diagnosis of UC, of which 21 cases characterized by an aggressive course of the disease and soon candidate to second-line treatment and 7 controls in stable remission after first-line treatment. Among the cases, 12 were males and 9 females, aged, at diagnosis, between 15 and 65 years (mean = 31.57 years). The disease duration ranged from 2 years to 13 years. Among the controls, 5 were males and 2 females, aged, at diagnosis, between 26 and 66 years (mean = 46.85 years). The disease duration ranged from 2 years to 5 years. Both cases and controls had a history of using drugs (NSAIDs species), parasitosis, CMV, allergic states, immune disorders, malignant neoplasm. In both groups, full colonoscopy was performed with exhaustive biopsy sampling, (from) of 2 to 4 cores representative mucous, in all segments (ileum, ascending colon, transverse, descending, sigmoid colon and rectum). The level of endoscopic severity was assessed according to the MAYO SCORE. All biopsy samples were evaluated by two experienced pathologists (VV, GL). The level of histological severity was evaluated according to the following parameters: presence of crypt abscesses, erosions, ulcerations.

Results. At first diagnosis, for the endoscopic evaluation of the illness severity, a MAYO score from 2 to 3 points was measured both in the cases and in the controls. At histological evaluation the group of increased aggression presented, in 100% of cases, samples with an increased proportion of eosinophils ( > 60 / 10HPF) in each of the colonic segments examined. Furthermore some features of eosiophysilic granulocytic inflammatory infiltrate correlated with increased aggressiveness of the disease. This could be seen for example in the formation of interstitial aggregates of eosinophils, defined by the presence of at least 4-5 contiguous eosinophils, the presence of degranulation of cytoplasms, as activation expression of eosinophils, the presence of eosinophils in the intra-epithelial and submucosa. Histological evaluation of the specimens obtained by the control group showed no increase in interstitial eosinophils. Our preliminary study found a correlation between the increase of eosinophils in the colorectal mucosa of patients with UC and disease aggressiveness.

Conclusions. In patients with ulcerative colitis who do not respond to first-line therapy, the increase of eosinophils in the lamina propria is a tissue marker with a negative prognostic value, that is predictive of increased aggressiveness of the disease and reduced response to drug first-line therapy.

References


β-CATENIN INTERACTION WITH NHERF1 AND RASSF1A METHYLATION IN METASTATIC COLORECTAL CANCER PATIENTS

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Introduction. Colorectal cancer (CRC) is the third most frequent cancer type and its incidence continues to increase worldwide. There is therefore a need to identify new biomarkers to further characterize this malignancy. β-catenin plays a central role in the Wnt signaling pathway which is altered in 90% of CRC. It also binds Na+/H+ exchanger 3 regulating factor 1 (NHERF1) through its PDZ domain and interacts with the RAS-association domain family 1 isoform A (RASSF1A) which is able to bind RAS involved in the mitogen-activated protein kinase (MAPK) pathways. Both pathways have been studied discretely, and are involved in the tumorigenesis of CRC but the mechanisms of their possible crosstalk are still not fully understood.

In this study, we examined the expression and sub-localization of β-catenin, NHERF1 and RASSF1A proteins, in addition to the methylation status of RASSF1A, in tumor adjacent normal tissue (ANT), primary tumors (T), and paired liver metastases (LM) of metastatic CRC. The aim was to compare the levels of protein immunoreactivity and RASSF1A methylation in the progression of CRC. We studied, for the first time, the interactions and relationships among the three proteins and RASSF1A methylation in order to investigate their biological meaning in metastatic CRC.

Methods. We assessed immunohistochemical expression of β-catenin, NHERF1 and RASSF1A proteins and RASSF1A methylation in 51 patients with stage IV CRC. Biomarker expression analysis was carried out counting the percentage of...
Figure 1. Membrane and cytoplasmic localization of β-catenin in ANT (A), membrane, cytoplasmic and nuclear expression in T (B), and membrane, cytoplasmic and nuclear β-catenin staining in LM (C). NhERF1 immunoreactivity is present at the apical membrane, in cytoplasm and nucleus in ANT (D), while in T (E) and LM (F) it becomes mostly cytoplasmic and nuclear. Heterogeneous cytoplasmic staining intensity of RASSF1A in ANT (G), granular cytoplasmic staining in T (H) and cytoplasmic and nuclear immunoreactivity in LM (I) (original magnification x200i).

Figure 2. Correlation analysis among β-catenin, RASSF1A and NhERF1 in metastatic CRC. In T samples cytoplasmic β-catenin correlated significantly with membranous (A) and nuclear (B) NhERF1, while in LM RASSF1A methylation correlated significantly with cytoplasmic (C) and nuclear (D) β-catenin. Abbreviation: T, primary tumor; LM, liver metastasis.
immunoreactive cells for ANT, T, and paired LM. Representative images of the immunostaining of β-catenin, NHERF1 and RASSF1A proteins in each type of sample are shown in Figure 1 (A-I).

Methylation analysis was performed on T and paired LM tissues. The level of methylation was determined as a ratio of the quantity mean of the target gene to the quantity mean of the reference gene. The correlation between β-catenin, NHERF1 and RASSF1A expression and RASSF1A methylation was tested using Pearson’s Correlation Coefficient (r). Statistical significance was achieved at p-value < 0.05.

Results. Regarding the T compartment, the Pearson rank test showed that cytoplasmic β-catenin expression was positively correlated to membranous NHERF1 and nuclear RASSF1A expression (r = 0.3002, p = 0.0323; r = 0.293, p = 0.0368; respectively) (Figure 2A and Figure 2B). Concerning LM, instead, the Pearson rank test showed a positive correlation between cytoplasmic β-catenin expression and RASSF1A methylation (r = 0.4019, p = 0.0068) (Figure 2C). Moreover, also nuclear β-catenin expression was positively correlated to RASSF1A methylation (r = 0.3194, p = 0.0345) (Figure 2D).

Conclusions. In conclusion, our results showed a heterogeneous distribution of β-catenin and NHERF1, confirming a dynamic role of these two proteins in metastatic CRC. In T, β-catenin functioned as an active protagonist, and it was predominantly associated with NHERF1. In paired LM, interestingly, we noted an increase of the oncogenic role of β-catenin through RASSF1A inactivation. Thus β-catenin confirmed its crucial function in CRC progression through different effector proteins involved in this dynamic process.

ENDOSCOPIC SUBMUCOSAL DISSECTION (ESD) TECHNIQUE IN PRECANCEROUS CONDITIONS OF THE GASTROINTESTINAL TRACT. THE ROLE OF THE PATHOLOGIST

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Background. In recent years, improvement in the quality of endoscopic imaging and better awareness of the early signs and symptoms of gastrointestinal tract neoplasms have led to an increased recognition and detection of these forms. This, in turn, has stimulated researchers and clinicians to find alternative approaches to surgery, approaches that introduced in clinical practice (after extensive investigation) both endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). The latter in particular has been used, in the upper and lower gastrointestinal tract, to treat relatively large mucosal lesions. An important point is the appropriate handling of the excised specimens, substantially larger than the common endoscopic biopsies, for a proper pathological evaluation, in particular the histological evaluation, of the surgical margins (lateral and deep surgical margins). According to specific guidelines, the specimens should be stretched and pinned on a board in order to be immersed in formalin for fixation. However, such handling may be time-consuming and in addition leads to suboptimal flattening of specimens and alterations in the evaluation of margins due to the multiple pins needed to keep the specimen firmly stretched. Aim of this study is to report our experience with the histological assessment of colorectal and upper gastro-intestinal tract en bloc ESD.

Material and methods. A total of 46 en bloc ESD (24 from the Institute of Pathology of Modena and 22 from the Institute of Pathology of Brescia), from 12 female patients and 28 male patients (mean age 71 years±12.5; range 33-88 years) were included in the study. ESD were performed for lateral spreading tumors (LST) in 12 cases, performed for sessile polyps with greater diameter > 1cm (15 cases) and performed for flat non-polyoid lesions (3 cases). ESD followed a non-complete poly resection in 16 cases. Patient’s age, site, size and endoscopic appearance (classified according to the Paris and Kudo classification) of the lesions were noted. Pathological examination included histological type of the lesion (adenomatous or carcinomatous), growth architecture (villous, tubular, mixed or serrated), grade of dysplasia (low-moderate/high-grade dysplasia) and the presence of early invasive carcinoma in submucosa (SM measurement). Specimens were gently placed on a board which was put into a special fenestrated box (Biocassette for mucosectomy, Biopitca, Milano, Italy) covered with a thin sponge and immersed in 10% neutral buffered formalin for fixation. The box cover provides gentle pressure on the specimen, allowing flat fixation without distortion, and avoids the use of pins that may alter a correct evaluation of the margins. Then, the specimens were photographed, sectioned thinly along the minor axis, and paraffin embedded. After cutting a first section of the internal part of the specimen, lateral parts (resection margins) were re-embedded following a 180° rotation and then cut again, in order to better visualize the margins and improve the diagnostic yield. Histology slides were prepared using conventional hematoxylin & eosin staining, and immunohistochemistry was performed when needed with Pan cytokeratin, Desmin, Smooth Muscle Actin, CD31, using a streptavidin-biotin peroxidase method running in a Roche XT immunostainer.

Results. Among the 46 en bloc ESD, 17 were obtained from the upper gastrointestinal tract (10 from the stomach, 5 from the esophagus, 1 from the cardias, 1 from the duodenum) and 29 from the colorectal tract (15 from the right colon, 3 from the transverse colon, 1 from the sigmoid colon and 10 from the rectum). The mean dimension of the ESD was 2.2cm x 1.8cm for LST, 2.2cmx1.8cm for sessile polyps, 2.1cmx1.6cm for flat non-polyoid lesions and 2.5x1.9 for ESD performed after non-complete poly resection. An average of 3 histological blocks was prepared for each case, with no difference for each group. Malignant neoplasia was diagnosed in 6/46 ESD (13%): three of the 15 sessile polyps and 3 of the 16 ESD following non-complete poly resection. Tumors were represented by one G2 pT1 neuroendocrine tumor - NET and 5 low-grade conventional adenocarcinoma, (4 pT1 and 1 pT2 tumors). In pT1 tumors, the depth of submucosal invasion was estimated as sm1 in three cases and sm2 in one. Pre-invasive adenomatous lesions were observed in 29/46 cases (63%) and were represented by 15 cases of villous and tubulovillous adenoma with high-grade dysplasia and 14 cases of tubulovillous adenoma with low-grade dysplasia. In the remaining 11 cases, serrated architecture of the glands or normal mucosa with inflammation were observed. In 8/46 (17%) ESD, a positive deep surgical margin was observed in 2 cases with adenocarcinoma, 1 with NET, probably due to the deep extension of the lesion, and 5 with low-grade dysplasia. In particular, positive deep surgical margins were observed in 33% of ESD performed for LST, in 13% of ESD performed for sessile...
polyps and in 14% of ESD following non-complete polyp resection. In 12/46 (26%) ESD, low-grade dysplasia was observed in lateral surgical margins. Dysplasia was observed in ESD following non-complete polyp resection (37%), in ESD for flat non-polypoid lesions (33%), in ESD performed for LST (25%) and in ESD performed for sessile polyps (13%).

**Conclusion.** The en bloc ESD allows the full histological evaluation of the resected mucosal samples maintaining their correct positioning during pathological examination. It allows to evaluate the surgical margins completely and, whereas foci of submucosal invasion due to early cancer occur, en bloc ESD permits the accurate measure of the SM depth, improving the accuracy of the diagnosis.

**SUCCINATE-DEHYDROGENASE-DEFICIENT GASTROINTESTINAL STROMAL TUMORS ARE ENRICHED IN O6-METHYLGUANINE-DNA ALKYLTRANSFERASE-METHYLATED CASES: A PREMISE TO A POSSIBLE USE OF ALKYLATED AGENTS IN THESE HITHERTO CHEMOREFRACTORY TUMORS?**

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**Introduction.** Succinate-dehydrogenase (SDH)-deficient gastrointestinal stromal tumors (GISTs) constitute a small KIT/PDGFRA-WT subgroup featuring relevant DNA methylation. These tumors, although often indolent, have revealed mostly chemorefractory in aggressive cases. DNA methylation, namely promoter methylation-induced O6-methylguanine-DNA alkyltransferase (MGMT) inactivation, improves the efficacy of carmustine and temozolomide in gliomas. DNA methylation has been found to affect MGMT in some GISTs; however, investigated cases were not assessed for SDH.

Some GISTs were recruited in past trials testing temozolomide and carmustine on sarcomas, with negative results. However, a possible effect of these agents on MGMT-methylated GISTs could have escaped detection, since: 1) tested GISTs were neither selected by genotype nor selected or investigated for SDH; 2) MGMT was studied in two cases only, revealing baseline activity; 3) these trials were performed prior to the adoption of Choi criteria, proved to be the only reliably recognizing GIST response to therapy. We investigated whether SDH-deficient GISTs are preferentially MGMT-methylated, as a premise for possible investigations on alkylating drugs in these tumors.

**Methods.** MGMT methylation in 29 pathogenetically heterogeneous GISTs (14 KIT/PDGFRA-WT, 2 of which arisen in the context of NF1) from our institution was investigsted by MS-PCR. SDHB immunohistochemistry identified SDH-deficient cases.

**Results.** 4 GISTs were SDH-deficient (all KIT/PDGFRA-WT, NF1-unrelated, gastric and at-least-in-part epithelioid). MGMT-methylated cases tended to prevail in the SDH-deficient group (3/4, 75%, vs. 6/25, 24%; p = 0.08, Fisher exact test).

**Conclusions.** SDH-deficient GISTs tend to be MGMT-methylated. Studies on MGMT-methylated GISTs could justify a reappraisal of alkylating agents in GIST treatment, with possible implications for the malignant SDH-deficient cases.
Introduction. Colorectal cancer (CRC) screening programmes exclude from invitation the patients who have been diagnosed with a CRC, under the assumption that they receive an intensive care according to protocols of follow up. In order to test such assumption, we evaluated the compliance of CRC patients recorded by the Veneto Tumour Registry (VTR) with the protocol for follow up of AIOM (Associazione Italiana Oncologia Medica), which prescribes two clinical examinations per year for at least 5 years and one colonoscopy in the first year, to be repeated, if negative, after 3 years and again after 5 more years.

Methods. We considered the patients with a CRC recorded by the VTR during 2006-2009. We set the beginning of the follow up according to the date of the surgical intervention, as reported in the Hospital Discharge Records (HDR); if no surgery was reported, the date of incidence was used. Information about follow up (clinical examination and/or colonoscopy) was collected from HDR and Outpatients Records, that were available up to December 31, 2014. Vital status was evaluated up to the end of 2014 too. We evaluated compliance with follow up according to year of incidence, gender, age, Country of birth (as a proxy of citizenship), setting of diagnosis (screening, non-screening), Local Health Unit of residence.

We considered as compliant with follow up those patients who, during a given year of follow up, underwent a clinical examination and/or a colonoscopy.

Results. We included in the study 4,416 patients, who were resident in 7 different Local Health Units (median follow up: 5 years). The proportion of compliers with follow up was 65.3% in the 2nd year of follow up and then decreased to 51.8% (6th year) and 38.7% (8th year) (test for trend: p < 0.0001).

Compliance was higher among patients diagnosed more recently: 2nd year 62.7% vs. 64.3%; 5th year 54.7% vs. 60.3% among patients diagnosed in 2006 and 2009, respectively. Compliance was higher among 50-59 year old patients (2nd year: 75.4% vs. 75.7% in 80+ year old patients; 8th year: 52.6% vs. 7.5%) and among males (2nd year 66.8% vs. 63.2% in females; 8th year: 40.5% vs. 36.4%).

Among 50-69 year old patients, compliance was higher among screen detected cases (2nd year 81.4% vs. 72.6%; 8th year: 49.1% vs. 46.7%).

Finally, compliance among subjects who were born abroad was higher at 2nd year (70.1% vs. 65.2% in Italians), but lower at 8th year (29.6% vs. 45.2%).

Multivariate analysis confirmed the lower compliance among 80+ year old patients and a trend of increasing compliance in subjects diagnosed in the most recent years; we finally observed a significant association between compliance and the Local Health Unit of residence.

Conclusions. Compliance with follow up protocols is highest in the first years after diagnosis and progressively declines. The lowest compliance rates were recorded among oldest patients. The local organisation of pathways for follow up services influences compliance. These results represent useful indicators for planning and evaluation of services; in detail, colorectal cancer screening programmes should assess the individual compliance of each patient diagnosed with CRC before excluding him/her from invitation to screening.
### EMOLINFOPATOLOGIA

**ANAPLASTIC LARGE T CELL LYMPHOMA (ALCL) IS CHARACTERIZED BY HIGH EXPRESSION OF P-SELECTIN GLYCOPROTEIN LIGAND 1 (SELPLG) THAT POSITIVELY CORRELATES WITH CD30 EXPRESSION AND TCR SIGNALING PATHWAY**

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**Introduction.** P-selectin glycoprotein ligand-1 (PSGL-1), coded by the SELPLG gene, is one of the major ligands of selectins, it is normally expressed in microvilli of polymorphonuclear leukocytes, monocytes, activated T-lymphocytes, plasma cells and activated platelets, where it has a major role in regulating the tethering, rolling and extravasation. It may bind also von Willebrand factor (vWF) and chemokines such as CCL21 and CCL19 to facilitate T-cell lymph node entry. PSGL-1 is a homodimeric disulfide-linked glycoprotein, which is highly post-translationally modified. It may bind with variable affinity to its different ligands depending on the status of its modifications.

PSGL-1 cytoplasmic domain transduces signals upon activation via spleen tyrosine kinase (Syk) after clustering within lipid rafts; the signaling leads to the secretion of cytokines or the activation of membrane integrins to promote extravasation. Noteworthy, PSGL-1 is involved in core molecular programs such as Syk, PLC_δ2, PI3K_ or MAPK pathways that let envisage a role for this molecule beyond cell adhesion and extravasation. The cross-linking of PSGL-1 in activated T cells is able to induce apoptosis, and PSGL-1 acts as an immune checkpoint regulator that promoting T cell exhaustion also through Programmed-cell death-protein-1 (PD-1) upregulation, and IL-2 signaling quencing.

PSGL-1 expression in pathological conditions has been reported in lymphoproliferative disorders showing plasmacytic differentiation and in multiple myeloma, (MM) where it has been suggested as a potential target for humoral immune therapy.

In T-cell lymphomas, the expression of PSGL-1 and its molecular correlates have been not investigated so far. Most T-cell lymphomas are classified as peripheral T-cell lymphomas (PTCL) a heterogeneous group of aggressive non-Hodgkin lymphomas (NHLs). The most common subtypes of PTCL, are PTCL not otherwise specified (PTCL-NOS), anaplastic large-cell lymphoma (ALCL), and angioimmunoblastic T-cell lymphoma (AITL). Up to date, no effective therapeutic targets have been identified, except for the TNF receptor CD30, targeted by the anti-CD30 drug conjugated antibody Brentuximab-Vedotin.

In this study we investigated the expression of PSGL-1 and its molecular co-relates in T-cell lymphomas and lymphomatous samples. SELPLG was first investigated in B-cell setting, being plasma cells neoplasms and naïve B-cells positive and negative controls respectively. Among B-cell lymphomas, Classical Hodgkin Lymphoma (CHL) and T-cell/histiocyte-rich diffuse large B-cell Lymphoma (TCRBL) showed the highest expression of SELPLG. Notably, these histotypes are characterized by a very rich environment comprising T cells and myeloid cells. In the T cell setting consistent expression of SELPLG was detected, with ALCL showing the highest expression (Fig. 1). ALCL encompasses different clinical entities that histologically share the presence of large pleomorphic T-cells expressing CD30. ALCLs can be classified according to the expression of Anaplastic Lymphoma Kinase (ALK). Surprisingly, no statistically significant difference was detected in SELPLG expression between ALK+ and ALK- ALCL specimens. Based on data from GEP analysis, we evaluated the expression of PSGL-1 in TMAs of 150 PTCL, confirming that PSGL-1 was consistently highly expressed in ALCL (Fig. 2). Promoted by GEP and IHC analyses, where CD30+ ALCL and CHL resulted highly expressing SELPLG, we hypothesized that a positive correlation between SELPLG and TNFRSF8 (CD30 coding gene) might occur. Our data confirmed this hypothesis. The correlation was observed both in T and B lymphoma settings, suggesting that it is not cell-type intrinsic. Network analysis by Genemania pointed to six genes potentially correlated with both SELPLG and TNFRSF8, namely MSC, SNX20, TNFRSF4, TNFRSF25, TNIP1 and TRAF1. We next evaluated the genes that showed a positive Pearson’s product-moment correlation (coefficient 0.75 to 1) with SELPLG in different settings. Strikingly, ALCL showed the highest number of genes significantly correlated with SELPLG, which do not overlap with the other settings analyzed, suggesting that SELPLG activity might be peculiar in ALCL. GSEA of such genes pointed to specific pathways and cellular functions, many of which previously associated with cancer, including RNA splicing and RNA processing functions. Subsequently, we tried to classify ALCLs according to SELPLG expression in order to identify relevant differences in the transcriptional program related with SELPLG. Almost 4000 genes differentially clustered together with high or low cases (Fig. 3), TNIP1 (from Genemania) and T cell receptor (TCR) signaling genes were among them. Among TCR
signaling genes, LCK, LAT, ZAP70, SYK proved to be correlated with SELPLG. These data suggest that PSGL-1 signaling correlates with TCR signaling in ALCL. In order to functionally characterize PSGL-1 in ALCL cells, we first investigated its expression on cells with two different antibodies: KPL1 (a known blocking antibody against PSGL-1) and TB5 (with non-blocking functions). Coherently, KPL1 was able to interfere with ALCL adhesion in vitro, while TB5 was not effective. As PSGL-1 required raft formation during signaling, we evaluated the capability of two antibodies to induce capping and found that only TB5 was able to do so. Consistently, since ALCL has an activated phenotype, TB5 was effective.
in induce apoptosis though variably in the different ALCI

cell lines. Finally, we evaluated via CIBERSORT method if

SELPLG expression might influence the microenvironment
characterizing the enrichment of specific bystander immune

elements. ALCIs result enriched in Mast cells and neutrophils

and in comparison with other PTCLs and normal controls, and

this enrichment was particularly relevant in highly-expressing

SELPLG ALCIs.

Conclusions. SELPLG expression characterize T-cell lymphoma,
in particular ALCI and it correlates with CD30 expression.

SELPLG expression correlates with relevant pathways such as

TCR signaling.

In ALCI cell lines, PSGL-1 has a functionally expression that
can be used to modulate adhesion, signaling and death.

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FINE NEEDLE CYTOLOGY AND ANCILLARY TECHNIQUES IN PLASMA CELLS TUMORS. A TEN-YEARS EXPERIENCE AND REVIEW OF THE LITERATURE

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Introduction. Plasma cell neoplasms (PCN) are clonal prolif-
erations of plasma cells generally arising in the bone marrow
and leading to single or multiple lytic bone lesions. PCN may
also arise in extramedullary sites as primary localization or as
extramedullary diffusion of PCN.

METHODS. Fifty-three consecutive PCN, diagnosed by fine-
needle cytology (FNC), have been collected in two Institu-
tions in 10 years. Anatomical sites were osteolitic lesions in
17 cases (3 sacrum, 3 vertebrae, 2 ribs, 3 jaws, 4 clavicle/ster-
num and 2 hips) and 36 extramedullary different sites, includ-
ing soft-tissue (15), breast (3), lymph node (11), liver (1), oral
cavity (1), pleural effusion (3) and CSF (2). The patients age
ranged from 45 to 82 yrs, (median 65 yrs); clinical data and

electrophoretic profiles, suggesting a PCN, were present in 35
patients. In 19/53 cases PCN was first diagnosed by FNC; in
3/53 cases during PCN staging, in 9/53 and 22/53 cases dur-
ing the treatment or the follow-up. FNC were first evaluated
by rapid on-site evaluation (ROSE); additional alcohol-fixed
smears, cell-block (CB) and cell suspensions were prepared in
38 cases and used for immunocytochemical (ICC) assessment
of EMA, CD138 and CD56 and flow-cytometry (FC) using
CD38/CD56,CD19 and light chain.

Results. FNC diagnoses of PCN were obtained in all the
cases, either in osseous and extramedullary localizations
combining cytological features and ICC/FC data. ICC and FC
were effective in 28 cases (12 FC and 16 ICC). In 10 cases
ICC and FC were not effective because of insufficient mate-
rial. In 15/53 cases, in which FNC was performed during PCN
staging or follow-up, ICC and FC were not performed and the
diagnosis was cytopathological only.

Conclusion. FNC, combining cytological and phenotypical
data, produces a rapid, reliable and accurate diagnosis of PCN
in all its presentations and allowing a specific and timely

treatments.

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RICHTER’S SYNDROME WITH PLASMABLASTIC LYMPHOMA AT PRIMARY DIAGNOSIS: A CASE REPORT WITH REVIEW OF LITERATURE

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Introduction. Richter’s syndrome is considered as the rare
development of an aggressive lymphoid malignancy in a
pre-existing small lymphocytic lymphoma/chronic lympho-
cytic leukemia. The most common aggressive lymphoma
developing in this setting is diffuse large B cell lymphoma,
but classical Hodgkin lymphoma and much rarer other enti-
ties such as prolymphocytic lymphoma and dendritic cells
sarcoma are also described, most frequently in the progression
of the disease over the time. A clonally relation between the
two neoplastic proliferations can be frequently found, while
clonally-unrelated cases are commonly considered indepen-
dent tumors, probably due to variable combination of multiple
causes, responsible independently of the two neoplasms.

Richter’s syndrome with plasmablastic lymphoma is very
rarely reported, during the clinical course of the small lympho-
cytic lymphoma/chronic lymphocytic leukemia.

Material and methods. Herein an unusual case of Richter’s
syndrome with the co-existence of plasmablastic lymphoma
and B-small lymphocytic lymphoma in the same lymph-node
at time of first diagnosis is described. We report a case of
61-years-old man with multiple enlarged axillary, thoracic
and abdominal lymph-nodes. A complete supraclavicular
node was excised. Completely effaced lymph-node parenchy-
ma included two clearly distinct components. One population
showed diffuse small monomorphic lymphoid cells popula-
tion with round nucleus and clumped chromatin, the other
completely different consisting of sheets of large-sized cells
with moderate eosinophilic amount of cytoplasm and round
nuclei with prominent nucleoli. A complete immunohisto-
chemical study was performed. The small cells component
was diagnosed as SLL/CLL, while the large cells component
was interpped as plasmablastic lymphoma, characterized in
particular by aberrant expression of cyclin D1 and co-expres-
sion of both surface immunoglobulin light chains. The two
populations showed the same clonal origin.

Conclusion. The term Richter’s syndrome indicates the rare
transition of small lymphocytic lymphoma/chronic lympho-
cytic leukemia into an aggressive lymphoma. Although both
clonally related and unrelated cases have been reported, real Richter transformation implies demonstration of a genetic correlation between the two neoplasms. In the majority of cases, the high grade lymphoma consists of a diffuse large B cell lymphoma, while the occurrence of plasmablastic lymphoma is very rare.

References

TARGETED NEXT GENERATION SEQUENCING OF BREAST IMPLANT ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA REVEALS MUTATIONS IN JAK/STAT SIGNALING, TP53 AND DNMT3A

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Introduction. Breast implant-associated anaplastic large cell lymphoma (BI-ALCL) is an uncommon form of ALK-negative ALCL occurring in women with either cosmetic or reconstructive breast implants, which has been recently included as a new provisional entity into the revised World Health Organization classification of haematological malignancies. BI-ALCL usually presents as a neoplastic peri-implant effusion. Nevertheless, solid infiltrating masses and cases with unfavourable disease course have been described. The molecular alterations underlining the natural course of the disease still remain unknown.

Methods. Herein, we report the results of targeted next generation sequencing performed in 7 BI-ALCL identified in the archives of 3 institutions (Sant’Andrea Hospital, Rome, Italy; Spedali Civili, Brescia, Italy; Columbia University Medical Center, New York, USA) over 7 years. DNA extracted from microdissected tumor cells on formalin-fixed paraffin-embedded tissues was sequenced on an Illumina HiSeq2500 using a panel covering mutations in the coding and non-coding regions of 465 cancer-associated genes. Variants identified using NextGENe were compared to those obtained from matched constitutional DNA to identify somatic mutations. Common variants present in the 1000 genomes database, and the database of Columbia University were removed. Somatic mutations were classified using the prior literature, and two different prediction algorithms (SIFT and Polyphen-2), and confirmed by bidirectional Sanger sequencing.

Results and discussion. Informative results were obtained in 5 of 7 cases; analysis failed in 2 cases due to the poor quality of the DNA. In 2 of the BI-ALCL cases we identified 5 somatic variants affecting 4 genes. A STAT3 variant (p.S614R), affecting the SH2 domain which mediates STAT3 dimerization, was detected in one of the 2 BI-ALCL. Notably, the S614R STAT3 mutation has recently described in one BI-ALCL case, as well as in angioimmunoblastic T cell lymphoma, chronic lymphoproliferative disorders of natural killer cells, and T-cell large granular lymphocyte leukemia. Moreover, gain-of-function mutations in STAT3 have also been reported in ~18% of systemic ALK-negative ALCL and ~5% of cutaneous ALCL. An in vitro study using BI-ALCL-derived cell lines showed activation of JAK/STAT3 pathway through autocrine production of IL-6, suggesting a possible pathogenic mechanism. The second BI-ALCL case, a frameshift deletion causing premature stop codon in SOCS1 (p.P83Rfs*20) was detected. SOCS1 exerts the function of a negative feedback regulator of the JAK/STAT3 pathway. The p.P83Rfs*20 mutation partially deletes the SH2 domain, which downregulates the kinase activity of JAK. Loss-of-function mutations of SOCS1, leading to constitutive activation of JAK/STAT signaling, have been described in B-cell lymphomas and in classical Hodgkin’s Lymphoma. Moreover, SOCS1 was found to be silenced by miR-155 in ALK-negative ALCL. The finding of gain-of-function mutations in STAT3 and loss-of-function mutation in SOCS1 supports the hypothesis that the deregulated activation of JAK/STAT3 signaling pathway may contribute to BI-ALCL pathogenesis. A missense mutation of TP53 (p.D259Y), affecting the DNA binding domain of p53, was also observed in the aforementioned case. Although TP53 mutations are uncommon in PTCL, p.D259Y has been reported in several solid tumors. The second BI-ALCL case with somatic variants showed a truncating mutation in DNMT3A (p.W176X). DNMT3A is a DNA methyltransferase required for genome-wide de novo methylation. Mutations in DNMT3A have been reported in ~8-22% of myeloid neoplasms and ~33% of PTCLs. Recent studies have identified DNMT3A mutations in preleukemic hematopoietic stem cells (HSCs) of patients with acute myeloid leukemia, and within CD34+ progenitors in patients with T-cell lymphomas. We can speculate that DNMT3A-mutant HSCs are potentially predisposed to develop neoplasms further acquiring mutations in other genes, which can cooperate with DNMT3A mutations to drive the type of hematological malignancy. Our data suggest that DNMT3A mutations in BI-ALCL might contribute to the deregulation of the DNA methylation landscape of transforming T cells.
No somatic mutations were identified in 3 out of 5 cases. Nevertheless, it remains plausible that other alterations, such as the generation of fusion transcripts, might be responsible to the deregulation of the JAK/STAT pathway in the non-mutated BI-ALCL cases, similarly to what observed in systemic and cutaneous ALCL.  

Altogether our results indicate that STAT pathway mutations occur in BI-ALCL, and identify for the first time mutations in SOCS1, TP53 and DNM3Ta as additional somatic events in this rare lymphoma. Our study contributes to the knowledge of the molecular alterations that characterize BI-ALCL, and warrants further investigation of the involved molecular pathways in this malignancy.

References

MYELOID SARCOMA OF THE URETHRAL PROSTATE: AN UNUSUAL PRESENTATION OF ACUTE MYELOID LEUKEMIA

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Introduction. Myeloid sarcoma (MS) includes all forms of immature myeloid cell infiltrates occurring in an extramedullary site or in bone, in association with acute or chronic myeloid leukemia, myelodysplastic syndromes or myeloproliferative disorders. It may precede or be concomitant with the haematological disorder and may also be the initial manifestation of its relapse. The incidence of MS varies between 3.1% and 9.1% in patients with acute myeloid leukaemia (AML). Urinary tract involvement by MS is uncommon, and prostatic localization of this process is very rare, presenting with non-specific symptoms. We report a case of a MS of the urethral prostate revealing AML.

Method. A 67-year-old man presented an acute urinary retention. Physical and ultrasound examination revealed an enlarged prostatic gland. Peripheral blood smear and prostate-specific antigen (PSA) test at admission were within normal limits. The patient underwent a transurethral resection of the pseudopolypoid mass of the urethral prostate.

Results. Microscopic examination of the prostate tissue chips showed a dense neoplastic proliferation replacing the parenchyma, with only a few residual atrophic or ectasic glands. The tumoral infiltration was diffuse and composed of large cells with slightly irregular nuclei, a fine chromatin and small nucleoli. Eosinophilic granulocytes at different stages of maturation were observed. Immunohistochemically, neoplastic cells were positive for CD45 (LCA), CD34, CD15, CD117, MPO and negative for Tdt, CD30, CD3, CD5, CD4, CD7, CD8; CD20, CD79a, CD10, PAX-5, PSA, chromogranin A, synaptophysin, CKAEE1/AE3 and desmin. The diagnosis of MS of the prostate was performed. Haematological findings in the post-operative follow-up showed a leukocytosis with a severe anemia and thrombocytopenia. In bone marrow biopsys (BOM) we observed a prevalence of myeloid series with a few dyserythropoiesis, dysmorphic megakaryocytes with hyperchromic nuclei and blasts number with CD34 < 5%. The diagnosis of myelodysplastic syndrome was established in accordance with WHO classification. Chemotherapy was started, but the patient died of sepsis one months after the initial diagnosis.

Conclusion. Urinary tract involvement by myeloid sarcoma (MS) is uncommon, and prostatic localization of this disease is very rare with only 17 reported cases in the literature, among which less than 10 were well documented and reviewed. MS of the prostate presents with non-specific urinary or systemic symptoms, and should be therefore considered among the differential diagnosis of prostatic lesions and neoplasia for therapeutic purposes. MS treatment consists of combining surgical excision and chemotherapy. Radiation therapy had been efficient in controlling two cases of localized MS of the prostate with complete remission, and might be beneficial for this condition. Allogenic haematopoiestic stem cell transplantation is also a promising therapy for MS of all sites.

References
**APOTOPSIS Deregulation in Ocular Adnexal Lymphomas**

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**Introduction.** Non-Hodgkin lymphoma is the most common malignancy arising in ocular adnexa, comprising 10-20% of all primary neoplasms in this location. Extra-nodal marginal zone lymphoma of MALT type is the most common form, but follicular lymphoma, mantle zone lymphoma and aggressive types like diffuse large B cell lymphoma are frequently seen. The clinical manifestations are heterogeneous and its management requires a multidisciplinary approach involving ophthalmologists, hematologists and radiotherapists. Treatment is associated with local and vision-threatening toxicities. Despite this clinical importance, molecular features of ocular adnexal lymphomas remain poorly characterized. Nevertheless some chromosomal translocations associated to the development of extra-nodal marginal zone lymphoma are currently known: t(14;18) (q32;q21), t(11;18) (q21;q21), t(1;14) (p22;q23), t(3;14) (p14;q32). Aberrant over-expressed proteins derived from translocations -BCL10, MALT1 and API2-MALT1- determine hyperactivation of NFkB pathway.

**Material and methods.** We performed immunohistochemical and molecular characterization of 83 cases of primary ocular adnexal lymphomas diagnosed between 2000 and 2013, regarding molecules involved in apoptosis and cell cycle regulation. Tissue microarray sections were investigated for immunohistochemical expression of A20, p-NFkB p65 (RelA) and c-Myc and for 6q23.3 deletion, including A20 gene, using fluorescence in situ hybridization. Patients medical records were reviewed retrospectively for information on clinical and prognostic characteristics, including treatment response.

**Results.** Expression of p-NFkB p65 (RelA) was found in 39 out of 67 cases (58.2%), while expression of A20 in 15 out of 65 (23%); expression of c-Myc was not found in any of the cases. Typical 6q23.3 deletion was found in 10 out of 72 cases (13.9%), atypical 6q23.3 deletion in 16 out of 72 cases (22.2%); a gain was found in 9 out of 72 cases (12.5%). No deletion was found in 37 out of 72 cases (51.4%). A statistical association was found between 6q23.3 deletion and A20 overexpression (p 0.020), particularly in MALT lymphoma. But no association was found of both A20 overexpression and 6q23.3 deletion with p-NFkB p65, probably because its deregulation could be determined by other genetic alterations. Finally a trend of association has been found between short PFS and A20 overexpression.

**Conclusion.** Deregulation of A20 could be considered a further pathogenetic mechanism of MALT lymphoma aggressiveness and it could be studied through immunohistochemistry, strictly relating 6q23.3 deletion.

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BONE MARROW HEMATOPOIETIC ADAPTATION AS A SENSOR OF EARLY, PRE-INVASIVE, EPITHELIAL MALIGNANCIES

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The main hypothesis of this study is that signs of the cross-talk between elements of the tumor microenvironment and the bone marrow (BM) can be identified in the very early phases of cancer development being finalized to the instruction of a tumor-promoting hematopoiesis. By integrating in situ BM histopathological and immunophenotypical analyses with flow cytometry and gene profiling of hematopoietic populations in a spontaneous mouse model of breast carcinogenesis (MMTV/NeuT) we investigated the occurrence of early cancer-adapted hematopoiesis and correlated in silico with the gene transcripts found modulated in the BM transcriptional profile could be linked with the incipient malignancy by the reprogramming of miRNA circulating in the peripheral blood. To test this hypothesis, plasmatic miRNAs were profiled at the late and early cancer development stages and correlated in silico with the gene transcripts found modulated within the BM.

In conclusion, our data lay a first demonstration that BM hematopoietic adaption to cancer is not confined to the engendering of a general immunosuppressive state associated with advanced cancers, rather it represents an early process co-evolving with malignant clone expansion. These results, besides casting light on the identification of potential new biomarkers for early cancer detection, offer a whole new prospect on investigating the significance of early cancer-adapted hematopoiesis.

OSTEOPONTIN CONTROLS DLBCL LYMPHOMAGENESIS ASSOCIATED WITH DEREGLUTED IMMUNE RESPONSES

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Conditions of persistent antigenic stimulation such as autoimmunity disorders have been associated with increased risk of lymphoid malignancies, particularly Non-Hodgkin’s Lymphomas, yet, the mechanisms driving the evolution from deregulated autoimmunity responses towards lymphomagenesis remains elusive. We have recently demonstrated that defective extracellular matrix control of myeloid cell activation owing to SPARC deficiency, favors the transition of systemic lupus erythematosus (SLE)-like autoimmunity (Faslpr/lpr) towards an indolent CD5+ B-cell lymphoma. Another matricellular protein, osteopontin (OPN) has been associated with SLE pathogenesis, being the first cytokine up-regulated in the serum of SLE patients and expressed at foci of autoimmune tissue damage. Moreover, genetic variants of the Spp1/Opn locus lead to higher OPN production and are associated with autoimmune diathesis. As far as lymphoid malignancies are concerned, OPN has been involved in lymphoblastic leukemia dormancy in the bone marrow but no studies have investigated its impact on lymphomagenesis associated with deregulated immune stimulation in secondary lymphoid organs (SLO). To test the role of OPN to autoimmunity-driven lymphomagenesis, Fasopb mutation has been transferred into the OPN-deficient background. Double-transgenic mice showed severe and accelerated SLO remodeling and spontaneous evolution towards an aggressive large-cell lymphoma with diffuse architecture (DLBCL) and immunoblastic morphology, upon ageing (6-8 months of age). We found an accumulation of an atypical CD19+ B-cell population with IgM-IgD-CD138-, variable B220 expression, IRF4+ BCL2+ and BCL6- phenotype, which was suggestive of a diffuse large B-cell lymphoma of non-GC type. Proof of malignancy came from the transfer of splenocytes from 8 month-old opn-/-Faslpr/lpr mice into immuno-compromised nude mice where a robust expansion of an atypical CD19+ IgM fraction was observed, recapitulating the parental phenotype. These results are suggestive of an unexpected, protective role of the matricellular protein OPN in DLBCL lymphomagenesis associated with deregulated immune responses.
THE PIVOTAL ROLE OF MOLECULAR TECHNIQUES IN IDENTIFYING MALIGNANT NEOPLASMS IN LOW-RISK INDETERMINATE DIAGNOSES (TIR3A) OF THE ITALIAN REPORTING SYSTEM


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Aim. The 2014 Italian six-tiered Reporting System for Thyroid Cytology has introduced the separation of the “indeterminate” category (TIR 3) into two subclasses (TIR 3A and TIR 3B) with expected different risks of malignancy and different clinical managements. Aim of the present work is to define the clinical impact of the new cytological classification in the first 2 years of application and the role of molecular techniques in identifying the malignant cases which, in spite of the clinical recommendation for a strict clinical follow-up, need to be addressed to surgery.

Methods and materials. From April 2014 to July 2016, 4,897 thyroid nodules were evaluated by US-guided FNAB at the Foundation “Agostino Gemelli” School of Medicine of Rome. The cases processed by liquid-based cytology (LBC) with the method ThinPrep 5000 (Hologic, Italy) diagnosed as LRIL-TIR3A were 249 (5.1%). ICC for HBME1 and Galectin-3 expression was performed in all cases on additional slides. Mutational analysis for BRAF V600E was carried out in 14 selected cases (5.6%). Forty patients (16.1%) underwent surgery.

The malignancy rates of thyroid nodules has been calculated on the nodules which underwent surgery. The sensitivity, specificity, PPV, NPV, accuracy for HBME1 and Galectin 3, considered as single tests or in combination, were evaluated. The κ Cohen concordance between the two molecular markers has also been considered and Fisher’s exact test has been performed and all p values are 2-sided, with p < 0.05 considered statistically significant.

Results. At the final pathologic diagnoses, 6 nodules (15%) resulted hyperplastic, 28 (70%) were follicular adenomas, and 6 (15%) were malignant neoplasms: 1 FVPTC (2.5%), 2 classical PTCs (5%), 1 papillary microcarcinoma (miPTC; 2.5%), 1 FTC (2.5%) and a FTC with a minimal poorly differentiated component. The FVPTC was a non-invasive subtype, recently reclassified as Non-Invasive Follicular Tumor with Papillary-Like Nuclear Features (NIFTP).

Nodules with a LRIL-TIR3A diagnosis on FNAB were more likely to be a non-neoplastic hyperplasia, adenomatous follicular nodule or follicular adenoma (34 nodules out 40; P for trend < 0.001) than a malignancy. The malignancy rate (ROM) was 15% in TIR3A nodules. Because case 1 was a non-invasive EFVPTC, the malignancy rate decreased to 12.5% when we reclassified non-invasive EFVPTCs to NIFTP. The combined expression of HBME-1 and Galectin 3 in TIR3A nodules increased significantly the sensitivity of malignancy detection on FNAB samples (1; Fisher’s exact test P value = 0.0013).

HBME-1 immunocytochemical test showed high levels of sensitivity (0.83), specificity (0.9) and accuracy (0.89), with a PPV of 0.63 and a NPV of 0.97 (Fisher’s exact test P value = 0.0007). Galectin 3 immunocytochemical test showed higher levels of sensitivity (1), lower levels of specificity (0.74) and accuracy (0.78), with a PPV of 0.43 and a NPV of 1 (Fisher’s exact test P value = 0.0013). Concordance analysis between the two molecular markers showed a good agreement (κ = 0.624), with an overall concordance of 0.838. Finally, only 1 out 14 cases (7.14%) was positive for the BRAF V600E mutation and it was a multifocal PTC which also resulted positive for the two antibodies.

Conclusion. After two years of application of the 2014 Italian six-tiered reporting system we can summarize the following preliminary findings:

- the ROM (risk of malignancy) for TIR3A is compliant with the standards (within 15%);
- the recently suggested new classification of non-invasive EFVPTCs to NIFTP decreases the malignancy rates of thyroid nodules with indeterminate preoperative results;
- the immunocytochemical screening, based on the use of the two antibodies in combination (HBME-1 and Galectin 3) is useful in distinguishing nodular lesions that should be addressed to surgery from those which might be clinically followed-up;
- BRAF V600E mutation analysis performed in the cytological preoperative setting has little utility in identifying those PTC which need to be addressed to surgery.

References

CYTOLOGICAL AND HISTOLOGICAL FEATURES OF BENIGN MULTICYSTIC MESOTHELIOMA IN A LIVER TRANSPLANTED HCV-RELATED CIRRHOSIS

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Objectives. We report the first case of a benign cystic mesothelioma of the testicular tunica vaginalis in a liver transplanted with radiological, cytological and histological correlation.

Methods. A 50 year-old male liver transplanted for HCV-related cirrhosis was referred for a voluminous scrotal mass. Physical examination, US, CT and cytology sampling were performed before surgical excision and pathological examination.
Results. US and CT study showed scrotal hernia, hydrocele and a multilobular cystic expansive extra-testicular mass that dislocated both testis. Cytological smears revealed mesothelial reactive cells. Gross specimen consisted of a multiloculated cystic structure emanated from the right didymus. Microscopically, cysts were lined by flat or cuboidal mesothelial cells lacking atypical features, with cytokeratin5/6 (+), calretinin (+), WT1 (+) immunophenotype.

Conclusions: We reported the first case of a liver-transplanted patient with scrotal benign cystic mesothelioma, describing cytological and histological correlations and considering possible differential diagnoses.

Figure 1. CT study showed scrotal hernia, hydrocele and a multilobular cystic mass.

Figure 2. Cytological smears revealed mesothelial reactive cells (40X).

Figure 3. Gross specimen.

Figure 4. Cuboidal mesothelial cells lining the cysts. Intervening septae showed fibrosis (20X).

Figure 5. Immunohistochemical analysis documented expression of mesothelial cells for calretinin (20X).

Figure 6. Immunohistochemical analysis documented expression of mesothelial cells for CK5/6. (20X).

Prognostic and Predictive Value of Tumor-Infiltrating Lymphocytes in HER2-Positive Breast Cancer

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Goal. Tumor-infiltrating lymphocytes (TILs) are emerging as biomarkers of anti-tumor immune response in a wide range of malignancies including epithelial ovarian carcinoma, colorec-
HISTOLOGICAL FEATURES OF A NEWLY EMERGED CLUSTER OF CUTANEOUS LEISHMANIASIS IN NORTH-EASTERN ITALY

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Goals. The incidence of Cutaneous leishmaniasis (CL) is increasing in Mediterranean Europe, thus pathologists need to be aware of this entity when evaluating skin biopsies. The aim of the present study was to describe the histopathological features of CL in detail.

Methods and materials. We performed a retrospective, observational study on CL cases diagnosed in the Bologna area between January 2013 and December 2015. Biopsy specimens were handled following routine laboratory procedures.

Results. During 2013-2015, 30 cases of CL were identified in the Bologna province, but only 26 of them were available for a second histopathological evaluation. Histological characteristics included intracytoplasmic amastigotes in 17 of 26 cases (65%), whereas in the remaining cases the diagnosis was reached by PCR. The most common epidermal changes were hyperkeratosis (100%), parakeratosis (65%), exocytosis (65%) and basal cell vacuolization (73%). In all cases the dermis displayed a mononuclear infiltration of lymphocytes, histiocytes and plasma cells, admixed with occasional eosinophils. Most cases (65%) presented well-formed, non necrotizing granulomas, whereas half of the cases displayed non-specific granulomatous tissue reaction characterized by dermal aggregates of epithelioid and foamy macrophages.

Conclusions. CL may present with a broad spectrum of histological features that are often non-specific. Therefore histological findings may be mistaken for nonspecific chronic inflammation and/or granulomatous reactions when amastigotes are not readily identified. Knowledge of this diagnostic issue should prompt microbiological consultation in cases where a clinical suspicion arises.

CLINICAL AND PATHOLOGICAL CHARACTERIZATION OF PRIMARY NEUROENDOCRINE NEOPLASMS OF THE GALBLADER AND EXTRAHEPATIC BILE DUCT.

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Goal. Neuroendocrine neoplasms of the gallbladder and extrahepatic biliary tract are rare, representing less than 0.5% of all neuroendocrine neoplasms, and are mostly described as case reports or small series. The goal of this multicentric study was to analyze an unselected series of neuroendocrine neoplasms of the gallbladder and extrahepatic biliary tract to depict the most relevant clinical, pathological and immunophenotypical characteristics.
Methods and materials. Nineteen cases of neuroendocrine neoplasms primary of the gallbladder and extrahepatic biliary tract were collected from four Institutions (Turin, Orbassano, Varese and Pavia). Detailed morphological revision was performed assessing major pathological characteristics and immunohistochemical typing, including Ki-67, hormone production, somatostatin receptor type 2A, site-specific transcription factors and mTOR-pathway molecules. Clinical information included type of therapy administered and survival.

Results. The gallbladder location was the most frequent (14/19, 74% of cases). Histotype distribution was 3 neuroendocrine tumors (NET, 2 G1 and 1 G2), 9 neuroendocrine carcinomas (NEC, 6 of the large-cell type), and 7 mixed adeno-neuroendocrine carcinomas (MANEC), without any significant difference as compared to their location. Male to female ratio was 1:2; 5:4 and 4:3 for NET, NEC and MANEC, respectively. Mean age was significantly lower in NETs (39 yrs) as compared to NEC and MANEC (70 yrs) (p=0.001). Mean tumor size was 8, 46 and 26 mm for NET, NEC and MANEC, respectively, although not reaching statistical significance. When available, the presence of associated gallstones was recorded in 1 case of NET, none of the NEC and 4 MANEC. Associated intestinal metaplasia was absent in NET and present in 3 cases of NEC and MANEC, each. High grade glandular dysplasia (BilIN3) was present in the MANEC group only (3/7 cases). No case was associated with hormonal hypersecretion, although heterogeneous immunohistochemical positivity for peptide hormones was observed (2 cases positive for somatostatin, all NEC; 3 cases positive for gastrin, 2 NET and 1 NEC; 8 cases positive for serotonin, including 2 NET, 5 NEC and 1 MANEC, of those with more than 20% of positive tumor cells). Somatostatin receptor type 2A and phospho-mTOR were positive at a variable extent in 13/19 (68%) and 12/19 (63%) cases, respectively. TTF-1 and PAX-8 were positive in one case, each, whereas CDX-2 was positive in 5 cases (1 NET, 1 NEC and 3 MANEC). P53 was over-expressed in 11 cases, all NEC or MANEC. As to concern overall survival, 3/5 MANEC patients and 5/6 NEC patients died of the disease with a median survival of 47 month for MANEC and 26 months for NEC (with no statistical difference at univariate survival analysis according to Kaplan Meier curves, p=0.64), whereas all 3 NET cases were alive and well at a median follow up of 43 months (range 5-108).

Conclusions. Primary neuroendocrine neoplasms of the gallbladder and extrahepatic bile duct are more frequently high grade neoplasms (either NEC or MANEC), associated in about one third with intestinal metaplasia, and show an EC-like phenotype (serotonin and/or CDX-2 positivity) in about 50% of cases. The high rate of expression of both somatostatin receptor type 2A and phospho-mTOR opens to a possible therapeutic strategy with biological/targeted therapies in these aggressive neoplasms.

CELL SURFACE PEPTIDASES CD13 AND CD10 IN RENAL CELL NEOPLASMS

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Goal. The current study attempts to evaluate and compare the CD13 and CD10 immunohistochemical staining in a large series of renal cell neoplasms. The cell surface peptidase CD10 has a diagnostic value among renal cell neoplasms. The aminopeptidase N/CD13 has been reported in some renal cell neoplasms, however little is known about its immunoeexpression according to different histotypes of renal cell neoplasms. Methods and materials: we investigated 174 renal cell neoplasms, including 79 clear cell, 31 papillary, 24 chromophobe renal cell carcinomas (RCC), 21 renal oncocytomas, 11 MIT family translocation renal cell carcinomas: of which 4 t(6;11)/TFEB, 7 with Xp11 translocations, 2 of which are rare cystic variants and 1 has the translocation t(X; 17), and 8 clear cell papillary renal cell carcinomas by CD13 (38C12, Novocastra) and CD10 (56C6, Novocastra) immunohistochemical expression. GATA-3 was added as controls. Results: a membranous staining pattern for CD13 was observed in 63/79 (80%) RCC, in 25/31 (81%) papillary RCC, in 3/8 (37, 5%) clear cell papillary RCC, in MiT family RCC: t(6;11)/TFEB 1/4 (25%); for Xp11 translocation 2/7 (28,5%)/carcinoma; and in 1/7 (14%) t(X; 17) RCC. No staining was observed in all chromophobe RCC (0/24) and oncocytomas (0/21). CD10 was observed in 76/79 (95%) clear cell, 15/31 (48%) papillary RCC; CD10 was also detected in 10/24 (42%) of chromophobe RCC, in 4/21 (19%) oncocytomas, and stained weakly in 1/8 (12,5%) of clear cell papillary RCC (mainly the cystic part). MiT translocation RCC: t(6;11)/TFEB 1/4 (25%); for Xp11 translocation 2/7 (28,5%)/cell carcinoma; and in 1/7 (14%) t(X; 17) RCC. GATA-3 was positive in 3/8 (37,5%) clear-cell papillary RCCs and negative in all remaining RCCs, except a single chromophobe RCC and a single oncocytoma. Conclusion: we concluded that: 1) CD13 is always absent in chromophobe RCC and oncocytomas, whereas CD10 can be immunoexpressed in both chromophobe RCC and oncocytoma; 2) CD13 is a relatively sensitive and specific marker for clear cell and papillary RCC; instead CD10 is equally sensitive but less specific 3) CD13 should be included in a panel of antibodies to distinguish “proximal renal tumors” from “distal renal tumors”. 4) When present, GATA-3 is specific for clear cell papillary RCC.

DISTINCTIVE PATHOLOGICAL AND CLINICAL FEATURES OF HIGH PROLIFERATING LUNG CARCINOIDs.

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Goal. Lung carcinoids are sub-classified according to the 2015 WHO scheme into typical and atypical forms based on mitotic rate and the presence of necrosis. This sub-classification is the best predictor of clinical behavior. Although proposed recently by several groups, there is no official grading for these tumors at variance with neuroendocrine neoplasms of the gastrointestinal tract and pancreas. A potential role of Ki-67 determination to improve the prognostic stratification of lung carcinoids has been proposed (setting the cut-off close
to 4%) but is still controversial. Moreover, the existence of a distinct group of lung carcinoids with high proliferation but well differentiated morphology – similarly to the concept of “G3 NETs” in the gastro-entero-pancreatic area – is unexplored. Therefore, the goal of the present study is to characterize lung carcinoids with a high proliferation rate (with a cut-off arbitrarily set at 10%) to test if they are associated to peculiar clinical and/or pathological characteristics.

Methods and materials. Two-hundred-thirty-nine lung carcinoids with major clinical and pathological information and leftover paraffin embedded material available were collected from 1990 to 2014. Ki-67 was assessed by using MIB1 monoclonal antibody and evaluated either manually counting the percentage of positive tumor cells among 2000 counted nuclei (all cases) or using an automated digital image analyzer (108 randomly selected cases; Aperio Technologies).

Results. Ki-67 manual and automated counts were strongly correlated (Spearman’s R: 0.78; p<0.0001). Atypical tumor histotype was significantly associated with shorter time to progression and overall survival (p<0.0001). Ki-67 >4% was also associated with shorter overall survival (p=0.003). For statistical purposes, cases were then subdivided according to the Ki-67 index into three groups, having <4% (147 cases), 4-9% (60 cases) and ≥10% (range 10-43; 32 cases). The latter group was significantly associated with the atypical carcinoid histotype (24 atypical vs 8 typical carcinoids; p=0.0001), the presence of vascular invasion (p=0.03) and aggressive disease (alive with disease or died of disease status; p<0.0001), whereas the former two groups were not, if compared each other. Moreover, lung carcinoids with Ki-67 index ≥10% showed a significant shorter survival at both time-to-progression and overall survival analysis (p=0.005 and p<0.0001, respectively), whereas cases with Ki-67 <4% and 4-9% failed to reach statistical significance, if compared each other. Finally, a cut off of 10% for Ki-67 retained statistical significance at univariate overall survival analysis in the atypical carcinoid group analyzed separately.

Conclusions. Atypical vs typical carcinoid histotype is the most relevant predictor of poor prognosis in lung carcinoids. However, a specific subgroup of high proliferating tumors (≥10%) exists, encompassing both typical and atypical carcinoids, with peculiar clinical and pathological characteristics and a specific disease-related prognosis.

THE ROLE OF IMMUNOHISTOCHEMISTRY IN THE IDENTIFICATION OF BRAFV600E MUTATED COLORECTAL CANCER

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Goal. BRAF proto-oncogene activating mutations are found in about 8% of human neoplasms and over 90% of BRAF mutated tumours display the V600E mutation. BRAF mutation is detected in about 10% of colorectal cancers (CRCs), more frequently in tumours with microsatellite instability (MSI). BRAF status evaluation is a crucial step in the diagnostic algorithm for the identification of hereditary (Lynch syndrome) CRCs. Moreover, BRAF mutation is a strong prognostic and predictive factor especially in patients with metastatic disease, and is considered a fundamental parameter in any proposed molecular classification of CRC. In clinical practice BRAFV600E mutation is commonly detected by DNA-based molecular methods. While having very good sensitivity and specificity, molecular analysis is burdened by high costs and needs for skilled professionals, factors limiting its widespread availability. Immunohistochemistry (IHC) using monoclonal antibody VE1 directed against BRAFV600E mutated protein has been recently proposed as a less expensive and widely employable method, with shorter turn-around time. Several studies proved its accuracy in a variety of tumours, with excellent concordance rates between IHC and molecular methods. Aim of our work was to compare direct DNA sequencing with IHC in the detection of BRAFV600E mutation in colorectal carcinomas.

Methods and materials. The study was performed on 103 CRC specimens surgically removed in the years 2015 and 2016. DNA was extracted from either frozen or formalin-fixed paraffin-embedded cancer samples, followed by PCR amplification of BRAF exons 15 and Sanger direct sequencing using ABI PRISM 310 Genetic Analyzer. Presence of the mutation was always confirmed in two (forward and reverse) sequences, run separately. MSI status was also determined using a fluorescence based PCR method and mononucleotide markers. Immunohistochemical assay with the VE1 monoclonal antibody was performed on 4μm sections cut from paraffin embedded tumour samples, using the Ventana BenchMark ULTRA automated immunostainer. Expression of the Mismatch Repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 was also studied by IHC using specific monoclonal antibodies.

Results. BRAF mutation was detected by exon 15 direct sequencing in 28 cases (27.2%), while the other 75 were classified as non-mutated (72.8%). Twenty-seven out of the 28 mutated tumours carried the BRAFV600E point mutation and only one bore a different mutation (BRAFK601E). All the 27 carcinomas with BRAFV600E mutation were classified as BRAF positive by IHC and all the 75 BRAF wild type tumours were negative. The tumour with the rare BRAFK601E mutation was BRAF negative by IHC. Therefore, IHC with VE1 antibody showed a 100% sensitivity and a 100% specificity for the identification of CRCs harbouring BRAFV600E mutation. As expected, BRAF mutation was detected more often in MSI than in microsatellite stable carcinomas (61.1% vs 9%, p<0.001). Among MMR deficient CRCs BRAF mutation was only found in tumours displaying loss of MLH1 protein expression. Seventy-three per cent (22/30) of MLH1 negative tumours demonstrated BRAF mutation while all the MSI tumours with different MMR immunohistochemical patterns were BRAF wild type (p<0.001). In the group of MSI MLH1 negative carcinomas BRAF mutation frequency was higher among patients ageing 70 years or more (20/25, 80%) than among patients younger than 70 years (2/5, 40%; p<0.001).

Discussion and conclusions. IHC assay with the VE1 monoclonal antibody, specific for the BRAFV600E mutated protein, has been proposed as a complementary or alternative technique to define BRAF mutational status in CRC as well as in several other tumour types. In agreement with the available literature, our series of CRCs demonstrated an excellent concordance between BRAF mutation molecular analysis and IHC, the single discordant case being represented by a tumour with a different BRAF mutation not recognized by the VE1 antibody. As more than 90% of mutations in CRC are BRAFV600E, IHC represents a reliable method to determine BRAF status in this tumour type. CRCs classified as positive by IHC showed moderate and less frequently intense staining of the cytoplasm in all cancer cells while the negative ones
showed no reactivity. A few tumours displayed focal and weak staining deemed aspecific and were always BRAF wild type at molecular analysis. Superficial mucosal epithelial cells stained variably and muscularis propria cells were usually moderately positive. Therefore, in our experience evaluation of stained sections was quite simple and the results unequivocal. In conclusion, our study confirms the value of IHC for detection of BRAF\(^{V600E}\) mutation in colorectal cancer, with advantages in terms of feasibility of the method and time and cost saving.

EVALUATION OF PD-1, PD-L1, PD-L2, FOXP3 AND CCR7 EXPRESSION AS PROGNOSTIC FACTORS IN PATIENTS WITH PRIMARY TESTICULAR LYMPHOMA

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Introduction. Primary testicular lymphomas (PTLs) are extranodal large B-cell lymphomas with poor response to current therapies. This might reflect their sites of origin considered to be an immune privileged sanctuary. The presence of the blood-testis barrier hinders the passage of antibodies and local production of anti-inflammatory cytokines, and may induce the development of an immune escape phenotype in the lymphoma cells. Interestingly, a recent paper have described frequent 9p24.1/PD-L1/PD-L2 copy number alterations in PTL which may represent structural bases of immune evasion. However, a complete assessment of the immune escape mechanisms eventually acting in this type of disease is missed. The aim of the present study is to determine the clinico-pathological impact of immune invasion in PTL by evaluating the expression of the programmed cell death 1 (PD-1)/PD-L1/PD-L2 copy number alterations in PTL which may represent structural bases of immune evasion. The pilot statistical evaluation seems to suggest an adverse prognostic impact of PD-L1 on patient’s outcome and an inverse correlation with PD-1 and FOXP3 expression. Nonetheless, statistical analysis is working.

Material and methods. Formalin-fixed paraffin embedded (FFPE) tumor samples and clinical data from 52 patients with PTLs was obtained from the Section of Pathology and the Oncology Unit of Siena and Firenze University. The pathology of all cases has been reviewed and diagnosed according to the updated WHO classification of tumours of hematopoietic and lymphoid tissue. All the cases were classified as germinal center or activated B-cells lymphoma.

Immunohistochemistry staining was performed by an automated staining system (Ventana BenchMark ULTRA, Roche diagnostic, Monza-Italy) with appropriate positive and negative controls. The following antibodies were tested: PD-1, PD-L1, PD-L2, FOXP3, CCR7 and MYC. To study neoplastic population, double staining for PD-1 and PAX-5, PD-L1/PD-L2 and PAX-5 was evaluated. To analyze tumor cell microenvironment, double staining for PD-1 and CD68, PD-L1/PD-L2 and CD68 was carried out. Tumor infiltrating small lymphocytes (TILs) have been also estimated by means of LCA, CD3, FOXP3/CD4 and CCR7/CD8 double stainings.

Statistical analysis was performed using a statistical software package (SYSTAT-7). We assessed the correlation of PD-1, PD-L1\(^1\), PD-L2\(^1\), PD-L1\(^TILS\), PD-L2\(^TILS\), FOXP3, CCR7, with the main clinical features of PTLs. Germinal center/activated B-cell phenotype as well as MYC protein pattern expression was compared with PD-1, PD-L1, PD-L2, PD-L1\(^TILS\), PD-L1\(^TILS\), PD-L2\(^TILS\), FOXP3, CCR7 staining, patients’ prognosis.

Results. The Activated B-cell phenotype was observed in 50% of cases, thus confirming previous data from literature, and partially accounting for the historically poor outcome of PTLs. MYC protein expression was detected in 45% of cases, in a percentage of neoplastic cells ranging from 50% to 70%. Forty percent of cases were double expressor (MYC and BCL-2 positive). We detected PD-L1 expression only in neoplastic cells, whereas PD-1, FOXP3 and CCR7 only in the tumor microenvironment (reactive lymphocytes). The TILs were represented mainly by CD3-positive, CD8-positive T-cells. Interestingly, CD8-positive T-cells also co-expressed CCR7. Moreover, cases with lower CD4-positive/FOXP3-positive T-cells number tended to have higher PD-1 expressing cells. However, immunohistochemical evaluation needs to be completed on all the cases. Although preliminary, an important finding suggested by our study is that the PD-1/PD-L1 pathway may preferentially mediate its immunosuppressive effects during direct presentation of tumor antigens. In fact, we observed preferentially a depletion of T-reg immunity than of T- cytotoxic and central memory response.

Conclusions. Recent years have brought new insights into the network of interaction between lymphoma cells and host immune defense demonstrating that impaired host immunity plays a role in the pathogenesis and progression. Although therapies targeting immune escape are highly effective, their current response rate leave much to be desired. In fact, predictors of response to immune checkpoint inhibitors could be related to tumor associated factors (oncogenic pathways activation, mutational burden, protein expression of PD-1 pathway, FOXP3, CCR7, etc.) or host factors (e.g. viral infection, stage, etc.). On the contrary, a complete assessment in PTL as well as the standardization of immunohistochemical evaluation, are lacking. Understanding what factors determine if a patient will respond is a crucial step in selecting the more appropriate therapeutic approach. Therefore it is important to address what these marker are and their relative importance. If our preliminary results will be confirmed, the present study will open new avenues for identifying predictive biomarkers that may guide therapeutic choices and eventually improve the prognosis of these diseases.

CHEMOTHERAPY RESPONSE SCORE IN TUBO-OVARIAN HIGH GRADE SEROUS CARCINOMA: VALIDATION OF A THREE TIER SCORING SYSTEM TO QUANTIFY HISTOPATHOLOGIC RESPONSE

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Goal. To validate the three-tier chemotherapy response score (CRS) recently proposed by Böhm et al. for measuring the histopathologic response to neoadjuvant chemotherapy in interval debulking surgery specimens of tubo-ovarian high-grade serous carcinoma (HGSOC), and the interobserver reproducibility to use this CRS system.

Patients and methods. The CRS system was applied to a series
of 121 patients (collected between 2001 and 2015) treated with neoadjuvant chemotherapy and interval debulking surgery for stage IIIC and IV tubo-ovarian HGSC. Adnexal and omental sections were independently scored by three trained pathologists. Tumors were also reviewed and classified as recently described2,3 in two morphological categories: classic predominant HGSC histology (“classic”) and solid, pseudoendometrioid, and transitional predominant HGSC histology (“SET”).

**Statistical analysis.** Fleiss’ kappa statistics and Kendall’s coefficient of agreement were calculated for levels of agreement between pathologists. Survival analysis was conducted by using Cox proportional hazards regression and the log-rank test to assess the prognostic value of the CRS, adjusting for age, stage, and debulking status. Survival functions were estimated by using the Kaplan-Meier method. For contingency tables, odds ratios were calculated, and Fisher’s exact test was applied.

**Results** Interobserver reproducibility for CRS system and morphologic pattern (“classic” versus “SET”): The interobserver reproducibility of the three-tier CRS system showed 60% of absolute agreement and Kappa Fleiss score of 0.68 indicating high agreement. Kendall’s coefficient of concordance was high (0.88), indicating a narrow range of disagreement on individual cases (ie, a frequent single score difference). The interobserver reproducibility of morphologic pattern showed 50% of absolute agreement and Kappa Fleiss score of 0.42 indicating a moderate agreement. Kendall’s coefficient of concordance was 0.53, indicating a moderate concordance.

**CRS system morphological pattern and prognostic significance:** The three-tier CRS system applied to omental sections showed prognostic significance for progression free survival (CRS 1 and 2 v 3: median, 12 v 19 months). When adjusted for age, stage, and debulking status, the score predicted progression-free survival (adjusted hazard ratio, 0.27; 95% CI, 0.11 to 0.7, p value = 0.007). The three-tier CRS system applied to omental sections also showed a benefit in overall survival for CRS 3 group (CRS1 and 2 28 v 130). No significant association was found between the two morphological categories (“classic” v “SET”) and progression free survival or overall survival.

**Conclusion.** The three-tier CRS system is easy to use in practice, cost-free, and highly reproducible and shows independent prognostic significance for high-grade serous carcinoma after neoadjuvant chemotherapy. In particular, CRS system allow to identify a group of patients with higher risk to early recurrence (i.e. CRS 1 and CRS 2 v CRS 3), providing information in addition to debulking status. Based on our experience, incorporation in the pathological reporting is recommended for its potential impact on patient care and research.

**References**
Corner Giovani “Gli Under 35yIN...FORMANO”

Venerdì, 25 novembre 2016
AREA POSTER – 13.30-14:30

THE ONCOTYPE DX BREAST CANCER ASSAY FOR NO HIGH GRADE DUCTAL CARCINOMA IN SITU: NAVIGATING THE COMPLEX WATERS OF DCIS MANAGEMENT

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Goal. Ductal Carcinoma in Situ (DCIS) is non-invasive breast cancer. Because it is limited to being inside the duct of the breast, it is classified as being Stage 0. DCIS progresses as invasive breast cancer in 20-50% of cases. Despite DCIS is heterogeneous it is usually treated by surgically removing the cancer, lumpectomy in most cases. Routine radiation therapy after DCIS surgery is common, but newer DCIS treatment guidelines say that radiation therapy after surgery doesn’t have to be given routinely to all women, but only to those with high risk of local recurrence. The difficulty lies in identifying which women will benefit from radiation therapy.

The Oncotype DX Breast Cancer Assay for DCIS has been developed and validated for risk recurrence in ductal carcinoma in situ. It is a genomic test that measures the expression of 12 genes of an individual tumor to generate results as a score. The DCIS Score quantifies the 10-years risk of an invasive or a DCIS local recurrence by clarifying the complexities of individual tumor biology. Depending on the recurrence score number, the DCIS has a low (less than 39), intermediate (between 39 to 54), or high (55 or higher) risk of recurrence. The aim of our study was to evaluate the impact of the DCIS Score on recommendations for adjuvant radiation therapy for no high grade DCIS.

Methods and materials. The Oncotype DX was performed on 17 no high grade DCIS diagnosed in our department between 2012 to 2015. 10 were low grade DCIS (DCIS-G1) and 7 were intermediate gradeDCIS (DCIS-G2). For each DCIS sample the test was performed sending 15 consecutive 5-μm-thick unstained sections of formalin-fixed, paraffin-embedded archival material to the central laboratory of Genomic Health in USA.

Results. Of 10 DCIS-G1 patients included in the study age at the time of diagnosis was from 44 to 79 years-old (average age: 55,9and median age: 54). Tumor size was from 5 to 38 mm (average size: 13,4 mm and median size: 10 mm). Based on clinical and pathological factors only 2/10 patients were treated by lumpectomy with negative margin (>0,5 cm) and 8/10 patients were treated by breast conservatory surgery (lumpectomy) with negative margin (≥0,5 cm), adjuvant radiation therapy and adjuvant hormonal therapy. DCIS Score for each patient of this category was low (range: 0-28; average: 12,3; median: 10).

Of 7 DCIS-G2 cases included in our study age at the time of diagnosis was from 50 to 73 years-old (average age: 60,7 and median age: 64). Tumor size was from 7 to 20 mm (average size: 12,9 mm and median size: 13 mm). Based on clinical and pathological factors only 1/7 patient was treated by lumpectomy with negative margin (≥0,5 cm) and the other 6/7 were performed to breast conservatory surgery (lumpectomy) with negative margin (≥0,5 cm), adjuvant radiation therapy and adjuvant hormonal therapy. DCIS Score was low in 2 cases, intermediate in 3 patients and high in the other 2 (range: 18-72; average: 46,4; median: 49).

Conclusion. The correlation between low tumor grade and DCIS Score is evident: in all 10 cases of DCIS-G1 there was no indication to adjuvant radiation therapy, regardless of the tumor size. The test changed treatment recommendations in 80% of cases. For the intermediate grade category (DCIS-G2) results showed greater variability. It is necessary to test a large number of cases with a longer follow-up. The Oncotype DX increases clarity and confidence in patient’s personalised treatment plan.

“NON-INVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES” (NIFTP): WHAT DO THEY LOOK LIKE IN FNAB?

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Papillary thyroid carcinoma (PTC) is the most frequent carcinoma in the thyroid and among PTC, the follicular variant is the most common. It includes encapsulated forms (EFVPTC) which account for 10-20% of all thyroid cancers and are known to have an indolent behavior, with a very low risk of recurrence or other adverse events. For this reason an international multidisciplinary group of authors recommended to reclassify tumors with a follicular growth pattern, complete encapsulation or clear demarcation from adjacent thyroid tissue, no invasion and papillary cancer-type nuclei as “Non-invasive follicular thyroid neoplasm with papillary-like nuclear features” or “NIFTP”, thus eliminating the term “carcinoma”. This proposal will have a profound impact on patient management.

Goal. In this study, we provide a detailed analysis of a large series of NIFTP from three Italian Institutions to characterize their cytological features and correlate them with histological outcomes.

Materials and methods. Weretrospectively collected cases with a histological diagnosis of NIFTPand an adequate fine needle aspiration biopsy (FNAB) sample performed in the same thyroid nodule. For comparison, benign follicular lesions and invasive EFVPTCs were also included. All FNAB were
classified according to Bethesda classification system; a nuclear score based on the presence of specific nuclear features (nuclear size, nuclear membrane irregularities and chromatin clearing) was assigned to cases and controls, both in cytological and histological samples.

**Results.** FNAB cytology from NIFTP nodules yielded the diagnosis of “suspicious for follicular neoplasm” (Bethesda category IV) in 56% of cases, “suspicious for malignancy” (category V) in 27%, “atypia of undetermined significance/follicular lesion of undetermined significance” (category III) in 15%, and “malignant” (category VI) in 2%. We found good correlation (k=0.62) of nuclear features between histological and cytological specimens. NIFTP nuclear features were significantly different from those of benign nodules (χ², p <0.0001), but not from those of invasive EFVPTC (χ², p 0.15). Nuclear scores 2 or 3 (recognizing two or three of the above features) induced a higher suspicion of PTC and in FNAB material should now be considered as marker of NIFTP in a well demarcated follicular patterned nodule.

**Conclusion.** Our data indicate that nuclear features found in cytology samples are reproducibly identified in corresponding histology samples both in cases of NIFTP and controls. Among specific nuclear features, membrane irregularities had the best correlation, while nuclear size was the least well-performing microscopic feature. Nuclear features of NIFTP are significantly different from those of benign follicular tumors and hyperplastic nodules, but not from those of EFVPTC with invasion. Our results point out that most of NIFTP nodules yield an indeterminate cytological diagnosis in FNAB cytology and therefore cannot be reliably diagnosed preoperatively, but should be listed in the differential diagnosis of all indeterminate categories of thyroid cytology. Recent cytology papers on FVPTC/NIFTP confirm that this diagnosis will significantly impact on the evaluation of malignancy risk associated with each Bethesda diagnostic subgroup, definitely reducing the risk in intermediate categories (IV and V).

**VASCULAR MODIFICATIONS IN HEPATOCELLULAR CARCINOMAS: A MULTIDISCIPLINARY STUDY.**

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**Goal.** The relationship between tumour vasculature and architecture in hepatocellular carcinoma (HCC) is well known[1], albeit very few morphological and molecular studies analyzing the typology and the phenotype of tumoral neoangiogenesis in human HCC are available in the literature [2]. The specific aims of our work are: (i) to identify the different morpho-phenotypical patterns of the vascular modifications in human HCCs (immunohistochemistry/IHC, RT-PCR and miRNA analyses); (ii) to correlate the patterns of vascular modifications with tumor aggressiveness and serum miRNAs; (iii) to correlate the pattern of vascular modifications with contrast-enhanced magnetic resonance imaging (MRI).

**Materials and methods.** Ninety-six HCCs (46 from non-cirrhotic livers and 50 from cirrhotic livers) were selected from 80 patients, 68 (85.0%) men and 12 (15.0%) women. Clinical and follow-up data, as well as contrast-enhanced MRI – when available – were collected. IHC for CD34 and Nestin was automatically performed. RT-PCR was carried out for Nestin, IGF1R and TGF-1β. miRNA analysis was conducted by means of microRNA arrays (microfluidic cards) on tissue and serum.

**Results.** Combining tumor morphology and IHC endothelial expression for CD34 and Nestin, we observed four main pattern of vascular modifications [3]: (A) Microtubular/acinar architecture, CD34+/Nestin–, sinusoids, occasional arteries; (B) Microtubular/acinar architecture, CD34+/Nestin–, sinusoids, occasional arteries; (C) CD34+/Nestin– endothelial cells lining macrotrabeculae; (D) Primarily solid architecture, several arteries, eventually sinusoids. These four patterns showed peculiar expressions of Nestin, IGF1R and TGF-1β mRNA, and in particular a “boost” of expression was observed between pattern A HCC (basal levels) and the other patterns. Pattern A HCCs are more common in cirrhotic livers (p=0.024, chi-squared test) and are likely to represent an earlier HCC stage [3] than other HCCs. The four HCC vascular patterns showed also different miRNA profiles: 4 miRNAs were deregulated in pattern A HCCs, 16 in pattern B, 56 in pattern C and 36 in pattern D HCCs. Interestingly, miR125b-2# and miR1228#, deregulated in patterns B and D HCCs respectively, were found deregulated also in serum of patients with advanced HCC. Finally, in 25 cases a comparison with MRI before and after administration of gadoxetic acid was done: of note, pattern B HCCs were always hyperintense in T1 weighted images (wi), features of the so-called radiological “glycogen nodules” without hyperintensity in T2 wi in which appeared isointense. Moreover, HCCs with pattern D were always isointense both in T1 and T2 wi, albeit they were always detected by using contrast-enhanced images. This could mean that the more aggressive pattern D HCCs do not follow the regenerative-dysplastic-neoplastic progression [4], but they are likely to start as aggressive cancers.

**Conclusion.** Tumor architecture and IHC identifies four different vascular patterns, each reflecting a different stepin tumor progression (different RT-PCR and miRNA profilas well as peculiar MRI features). These results are likely to be applicable in the routine practice, from MRI diagnostics to the identification of serum miRNAs predictive of tumor progression and aggressiveness.

**References**


**COMPARATIVE ANALYSIS OF PD-L1 EXPRESSION IN MATCHED SAMPLES OF PRIMARY TUMOR AND METASTASES FROM COLORECTAL CANCER PATIENTS.**


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**Goal.** Immune checkpoint blockade targeting the programmed death-1 pathway has shown efficacy in several types of cancers, including colorectal cancer associated with mismatch-repair deficiency. In some tumor types, programmed death-ligand 1 (PD-L1) expression detected by immunohistochemistry has shown utility as a predictive marker for response to anti-PD-1 therapies. This utility, however, remains to be
determined in colorectal carcinoma, which shows a generally low prevalence of expression in tumor cells, with a predilection for cases associated with mismatch-repair-deficiency. In addition, no data are available in colorectal cancer on the eventual modulation of PD-L1 expression during the course of tumor progression and/or as the result of chemotherapy administration. The goal of this study was therefore to compare PD-L1 expression in tumor and tumor-associated immune cells from primary and metastatic tumor samples, either after chemotherapy or therapy naïve.

**Methods and materials.** A series of 43 colorectal cancer patients with matched primary tumor sample and synchronous or metachronous metastases was analyzed by means of immunohistochemistry using a monoclonal anti-PD-L1 antibody (clone CAL10, Biocare). PD-L1 expression in tumor cells (TC) was scored as positive if a moderate/intense positivity was present in at least 1% of cells, as proposed in the recent literature of colorectal cancer, whereas PD-L1 positivity in tumor-associated immune cells (IC) was semi-quantitatively scored into 4 grades based on the percentage of the tumor area colonized by PD-L1 positive lymphocytes (score 3: ≥10%, score 2: 5%-9%, score 1: 1-4%, and score 0: <1%), as proposed in the lung cancer model. PD-L1 expression in tumor and tumor-associated immune cells was also correlated with major parameters both in primary tumor and metastatic tumor samples.

**Results.** PD-L1 expression in tumor and tumor-associated immune cells was increased in metastatic samples overall (TC: 4/43, 9%; IC: 11/43, 26%) as compared to primary tumors (TC: 3/43, 7%; IC: 9/43, 21%), but highly heterogeneous with discordant results in 3/5 cases positive in tumor cells either in the primary or the metastases (one positive in primary only and 2 positive in metastases only) and in 12/16 cases positive in tumor-associated inflammatory cells in tumor cells either in the primary or the metastases (5 positive in primary only and 7 positive in metastases only) Interestingly, the increase of TC and IC PD-L1-positive cells was not influenced by chemotherapy administration, although the percentage of discrepant cases as to concern tumor-associated inflammatory cells in primary vs metastatic lesions was higher in chemotherapy treated patients (10/24, 42%) vs untreated patients (2/19, 11%) (p=0.04). PD-L1 expression in tumor cells of the primary tumor was associated with right colon location (3/3 positive cases; p=0.05) but not with other parameters, including sex, age, presence of mucinous features, tumor grade and stage, vascular invasion of the extent of tumor-associated lymphocytic infiltrate. By contrast, the extent of positive immune cells in metastatic samples was not associated with clinical or pathological parameters in the primary tumor but was more prevalent in the lung location (7/11 positive cases; p=0.05).

**Conclusions.** PD-L1 expression in tumor and tumor-associated immune cells is slightly increased in metastatic tissues of colorectal cancer as a suggestive mechanism of tumor progression and is possibly influenced by chemotherapy administration prior to metastatic development. However, the wide heterogeneity of PD-L1 expression among different tumor samples from the same patient should be kept in mind for the use of PD-L1 immunohistochemistry as a predictive biomarker of response to immune checkpoint inhibitors.

**SOCS3 IMMUNOHISTOCHEMICAL EXPRESSION SEEMS TO SUPPORT THE 2014 INTERNATIONAL SOCIETY OF UROLOGICAL PATHOLOGY (ISUP) GLEASON GRADING SYSTEM.**

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**Goal.** In the 2014 International Society of Urological Pathology (ISUP) Consensus Conference redefines Gleason grading system. [1] In a recent report for the first time we demonstrated that a hypermethylation of SOCS3 with a significant reduction of its mRNA and protein expression identifies a subgroup of prostate cancer with a more aggressive behavior. [2] In this study we evaluate the immunohistochemical protein expression of the suppressor of cytokine signaling (SOCS) proteins 3 (SOCS3) in different Gleason grade prostatic adenocarcinoma: classical Gleason grade 3, classical Gleason grade 3 upgraded to Gleason grade 4 according to the ISUP modified grading system and classical and modified Gleason grade 4.

**Methods and materials.** Two different pathologists (PP and GP) on the basis of strictly classical (1977) and modified (2014 ISUP) criteria, assigned Gleason pattern in 148 different areas of prostatic adenocarcinoma. [1] The Gleason grade were stratified into 3 different categories on the basis of histologic pattern: (1) 26 prostatic adenocarcinoma with classical and modified Gleason pattern 3, (2) 57 prostatic adenocarcinoma with classical Gleason pattern 3 upgraded to Gleason pattern 4 by 2014 ISUP Conference and (3) 65 prostatic adenocarcinoma with classical and modified Gleason pattern 4. In particular we collected 15 neoplastic areas with cribriform pattern and 5 cases with glomeruloid features, assigning a Gleason grade 4. Immunohistochemical analysis for SOCS3 was performed and the SOCS3 staining intensity were evaluated in three different ways on the basis of the intensity of cytoplasmatic staining: positive (strong positivity in all neoplastic glands and in all the cells in the same neoplastic gland); negative (negativity in all neoplastic glands and in all the cells in the same neoplastic gland) and weakly positive (weak intensity staining in all neoplastic glands and in all the cells or with isolated negative cells in the same neoplastic gland). Colonic mucosa were used as positive control according to manufacturer’s instruction. 

**Results.** Considering the immunohistochemical expression of SOCS3 we found that the SOCS3 pattern staining negative (-) or weakly positive (+/-) increase progressively in concomitance with the rise of Gleason grade and SOCS3 positivity (+) correlate with classical or modified Gleason grade 3. Moreover comparing the prostatic adenocarcinoma with classical and modified Gleason grade 3 and the prostatic cancer with classical Gleason grade 3, upgraded to Gleason grade 4, we found that the SOCS3 negative staining and SOCS3 with weak intensity staining correlate with the upgraded group suggesting a difference between two groups not only for histological aspects but also for a molecular background. Interestingly in cases with prostatic adenocarcinoma with glomeruloid features a weak staining for SOCS3 in glomerularion structures was observed.

**Conclusions.** In conclusion our data seem to support, from a molecular point of view, the modified criteria by 2005 International Society of Urological Pathology (ISUP) Consensus Conference and by 2014 International Society of Urological Pathology (ISUP) as well as the hypothesis that the diagnosis of Gleason cribriform pattern 3 virtually does not exist and
cribriform glands—regardless of their size—are nearly always considered pattern 4. The data concerning the SOCS3 immunohistochemical expression in glomeruloid pattern also for the limited number of cases, due to relative rarity of glomeruloid features, suggest additional studies to validate our observation.

References


STRA6 EXPRESSION PATTERN IN HUMAN PLACENTA IN PREGNANCIES AFFECTED BY GESTATIONAL DIABETES MELLITUS (GDM)

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Background and objective. The vitamin A (retinol) metabolite, all-trans retinoic acid (RA), is a signaling molecule that plays key roles in the development of the body plan and induces the differentiation of many types of cells. Even if the role of some key transport and metabolizing proteins have been investigated, the mechanisms regulating embryonic acquisition and utilization of retinoids from the maternal circulation via placenta remain largely unknown. STRA6, the membrane receptor for the RBP4-retinol complex, has been described as strongly expressed at the level of blood-organ barriers like the emochorial placenta. Furthermore, recent research results indicate that STRA6 seems to be not only a vitamin A transporter but also is a cell-surface signaling receptor activated by the RBP-retinol complex involved in activation of JAK2/STAT5 cascade responsible for insulin resistance. The aim of this study was to compare the pattern of STRA6 localization at the placental site in human placenta, both during physiology and in case of gestational diabetes mellitus (GDM).

Materials and methods. In this study we analyzed data from a single-center retrospective study considering placental tissue from seven singleton pregnancies affected by GDM and from seven controls of normal developed pregnancies. The pattern of STRA6 distribution was examined by IHC on placental samples collected at the time of delivery and by qRT-PCR. We also analyzed the mRNA expression by qRT-PCR of the proteins LRP1, LRP2 and MTTP, involved in the intracellular intake of retinoids via lipoproteins, and of RXRγ, a vitamin A transporter but also is a cell-surface signaling receptor. The vitamin A (retinol) metabolism involves the enzymes LRP1 and LRP2, seems to play a role in the pathogenesis of GDM. Unexpectedly, STRA6 expression was found to be reduced during GDM. Further studies need to be performed in order to better understand the underlying pathogenic mechanisms.

RESULTS.

The mean gestation age was 32.43 weeks (±7.28) in GDM group and 33.75 weeks (±4.06) in control group. We found a decreased expression of STRA6 in GDM placentas than in controls in ICH and qRT-PCR analysis. Furthermore, LRP1 mRNA expression was found to be increased in cases compared to controls and LRP2 was shown to be significantly and positively correlated with STRA6 mRNA expression.

Conclusions. Placental STRA6, as well as the enzymes LRP1 and LRP2, seems to play a role in the pathogenesis of GDM. Unexpectedly, STRA6 expression was found to be reduced during GDM. Further studies need to be performed in order to better understand the underlying pathogenic mechanisms.

CORRELATION BETWEEN GESTATIONAL DIABETES MELLITUS AND ETHNICITY IN PLACENTAS. THE ITALIAN EXPERIENCE AND ITS UNEXPECTED RESULTS.

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Objectives. We studied the association between placentas histomorphometry in gestational diabetes mellitus (GDM) and race/ethnicity.

Methods. We collected a total amount of 412 placentas, from singleton full-term live births, from January 2015 till June 2016 (18 months): 292 where from Pordenone Hospital and 120 where from Monselice Hospital. Of the 412 placentas specimens, 146 had a clinical or sub-clinical diagnosis of GDM (110 from Pordenone and 36 from Monselice).

Results. Placentas of non-Caucasian women without GDM where (27%) whereas placentas with GDM where (34%). In contrast with literature, our data showed an higher incidence of GDM among Caucasian than non-Caucasian women.

Conclusions. Studies so far published indicated that GDM in placentas of non-Caucasian ethnicity has ahiger incidence, yet results of our investigation shows an opposite trend. Further investigations are in progress to better define the reason of these conflicting data.

TUMOR INFILTRATING LYMPHOCYTE (TIL) AND CD8+ CELLS IN TNBC: NEW PROGNOSTIC MARKERS

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Goal. Triple negative breast cancer (TNBC) stands out for a remarkable morphological and biological heterogeneity associated with a high propensity for systemic metastases and poor survival in comparison with the different subtype of breast cancer. The efforts to adopt specific target therapies are complicated by the difficulty to identify representative and reproducible markers. Recently, there is increasing evidence of the role of tumor infiltrating lymphocytes (TILs) in this subtype of breast cancer in terms of improving disease-free (DFS) and overall survival (OS) rates. Moreover, the presence of TILs in the tumor microenvironment can also predict responses not only to adjuvant but also to adjuvant chemotherapy treatments. Following this strong evidence, recent efforts have been made to develop a standardized methodology for evaluating TILs.

In our study we assessed the expression of TIL in TNBC patients, as a quantitative histological parameter, and the specific CD8+ lymphocytes subset. Then, we investigated the correlation between these histological markers with clinico-pathological features and DFS and OS for the evaluation of their prognostic value.

Methods and materials. A total of 105 TNBC patients diagnosed by the Section of Anatomic Pathology and Biomolecu-
lar Diagnostic of the University of Ferrara between 2000 and 2011, and followed up by the O.U. of Oncology, were included in this study. The main criteria of selection were the ER and PgR immunoreactivity <1% and the absence of Her2 overexpression or amplification.

TILs were assessed in the hematoxylin and eosin-stained sections, following the methodology proposed by the International TIL Working Group: the boundaries of the invasive tumor were identified, and only the mononuclear cells (lymphocytes and plasma cells) in the stromal compartment within those borders were quantified, reporting as a percentage value. The immunohistochemistry expression of CD8+ lymphocytes marker was also assessed.

Results. High TIL expression (>41%) was positively related with smaller tumor size (74% ≤2cm) and negative lymph node status (60% pN0). At the univariable survival analysis, the group of patients with high TIL expression had a better DFS and OS in comparison with patients with a lower TIL expression (<10%): DFS=11.3 years and OS=11.4 years vs DFS=6.2 years and OS=6.2 years (p < 0.0001). Multivariable analysis confirmed that TILs represent an independent prognostic factor for both DFS and OS.

The total number of CD8 cells in lymphocytic infiltration was inversely correlated with node status and distant events: patients with CD8+ expression >50% were pN0>pN+ (77% vs 23%) and M0>M1 (82% vs 18%). Moreover, rich CD8+ infiltrate lymphocytes predicted superior OS and DFS (p=0.001).

Conclusion. Our data support the presence of TILs in the tumor microenvironment as a positive prognostic factor, and the favourable role of CD8+ lymphocytes in breast tumor immunity.

The recent evidence suggests also a predictive function of TILs in TNBC patients, underlining the possibility to use them as a marker to stratify the risk in this group of patients and to identify those who could benefit from target therapy, including new immunomodulatory drugs.

Materials and methods. Formalin-fixed paraffin-embedded sections of 169 GCs, well characterized for Epstein-Barr infection (EBV+, 33 cases), microsatellite instability (MSI, 59 cases) and CD8 intraepithelial lymphocytes (IELs) were analysed by immunohistochemistry for the expression of PD-L1 (clone 405.9A11, Cell Signaling Technology and/or clone SP142, Spring Bioscience) and PD-1 (clone NAT105, Ventana Medical System). PD-L1 immunostaining was observed in membrane and cytoplasm of both neoplastic and immune cells. A case was considered PD-L1 only if 5% or more tumour cells displayed membranous staining. Statistical analysis was performed using Fisher’s exact test, Chi Square test and Wilcoxon rank-sum test; Kaplan-Meier estimator test (log-rank test) was used for survival analysis evaluation.

Results. Overall, PD-L1+ GCs were 31/169 (18%), in detail 15/33 (46%) EBV+, 14/59 (24%) MSI and only 2/77 (3%) MSS/EBV+ GCs (p=0.001). PD-L1 expression was higher in EBV+ (mean value of PD-L1+ cells in positive cases: 33%, range 5-90%), than MSI (mean value: 18%, range 5-40%) and MSS/EBV- (mean value: 18%, range 15-20%) GCs. Histologically, PD-L1+ GCs were prevalent in poorly differentiated (22/87, 25% of G3, vs 8/69, 12% of G1-G2, p=0.05) intestinal type (26/118, 22% of intestinal, vs 1/21, 5% of diffuse and 4/30, 13% of indeterminate type) GCs. According to histotype-based prognostic classification, the majority of PD-L1+ cases were high lymphoid response (HLR, 28/77, 36%) GCs. Among MSS/EBV+ GCs the only 2 PD-L1+ cases were a HLR and a cohesive carcinoma with a minor tumour component rich in lymphocytes, respectively.

Finally, PD-L1+ cases were significantly associated with the presence of high levels of CD8+ IELs (29/107 (27%) GCs with CD8+ IELs > 9.5 cells for HPF vs only 2/48 (4%) GCs with low CD8+ IELs, p<0.001), low tumour stages (22/86, 26% of I/II, vs 9/81, 11% of III/IV, p=0.05) and better survival (p=0.01). In addition, the subgroup of patients with PD-L1+ GCs and high levels of CD8+ IELs showed a better prognosis than all the other subgroups.

PD-L1+ IELs were significantly higher in EBV+ than MSI and MSS/EBV+ cases (17.5 cells/HPF vs 7 and 3.1 cells/HPF, respectively; p=0.01). No correlation was found between PD-L1 expression and survival.

Conclusions. PD-L1/PD-1 pathway seems to be selectively activated in HLR GCs, in particular in EBV+ and MSI carcinomas. In these subsets of GCs, PD-L1/PD-1 pathway could be considered as possible target for therapy.
are further examined through Human Papillomavirus (HPV) test (Hybrid Capture-2®/HC2) to discriminate healthy ones from those potentially at risk of developing cancer. HC2 includes a cocktail of probes designed to detect 13 HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), classified as carcinogenic. Some studies have shown cross-reactivity with other HPV strains. We tried to assess the accuracy of HC2 test through HPV genotyping in cases close to the cut-off of positivity (1 pg/ml of HPV DNA) in order to estimate the frequency of false positive or negative results and their impacts on clinical management.

Methods and materials. Among the 173 cases that underwent HC2 test between January and April 2016, 34 showed results close to the cut-off (12 negative and 22 positive) and were selected for the study. HPV genotyping was performed on DNA samples using DNA extraction QIAamp DNA FFPE Tissue. DNA was amplified using the kit HPV sign and positive PCR were typed with the kit HPV sign by “PyroMark Q96 ID system”.

Results. 9 of the 12 negative cases tested (75%) were confirmed, while 3 (25%) showed the presence of HPV (types 18, 67, 90). In 20 (91%) of the positive cases tested the presence of HPV was confirmed (types 16, 18, 33, 56, 31, 35, 42, 66, 73, 90), while in 2 samples (9%) no virus was identified. The Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were respectively 0.91 [95% C.I. 0.71-0.99], 0.75 [0.43-0.95], 0.87 [0.66-0.97] and 0.82 [0.48-0.98].

Conclusions. Accuracy of HC2 test is high also in the borderline range, as evidenced by the low rate of false positive and false negative results (0.09% and 0.25% respectively). The study showed 18.2% of cross-reactivity with viral types not included in the probe cocktails (types 42, 66, 73 and 90).

References


Routine immunohistochemistry (IHC) with D5F3 mAb is an efficient and cheap tool for identification of ALK-positive lung adenocarcinomas: a prospective study of 234 cases

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Goal. To validate a diagnostic algorithm based on routine immunohistochemical staining with D5F3 mAb for detection of patients with ALK-rearranged adenocarcinoma of the lung.

Materials and methods. In the period June 2015-May 2016, paraffin sections of 234 consecutive cases of lung adenocarcinoma (mean age = 67.5 ± 10.35, ranging from 17 to 87; male = 57.2%; 7.3% were ≤ 50 years of age) were tested at the Sant’Andrea Hospital of Rome for expression of ALK protein using D5F3 mAb (Cell Signalling, USA) and a Dako automated immunostainer. Cases were classified as IHC-positive (2+, diffuse and intense cytoplasmatic staining), uncertain (1+, weak diffuse cytoplasmatic staining) and negative. Confermative fluorescence in situ hybridization (FISH) (Vysis LSI ALK Dual Colour, break-apart rearrangement probe kit; Abbott Molecular) was used in all IHC-positive cases (1+/2+/3+) and in IHC-negative cases with signet/mucinous histology and/or age ≤50 years. Investigated cases included: 1. All surgical specimens (76.5% of all investigated cases). 2. All small biopsies in which there was a sufficient amount of tumour tissue to allow also investigation of EGFR mutations. 3. All small biopsies of patients ≥ 50 years of age and/or with signet/mucinous tumours independently of the amount of tumour tissue. The results of the IHC screening were compared with those obtained in 319 cases (mean age = 66.8 ± 11.42, ranging from 18 to 90; male = 60%; 8.1% were ≤ 50 years of age) tested with FISH only in the period 2014-2015. X2 test was used for statistical analysis.

Results. ALK-IHC+ cases (2+/3+) were 190 of 234 (81.1%); 16 cases were confirmed by FISH (IHC+/FISH+). The three IHC+/FISH-cases showed ALK gene amplification not associated with translocation (1 case) and ALK translocation in < 5% of neoplastic cells (2 cases). All cases with weak immunoreactivity for ALK protein (membrane, granular or weakly diffuse cytoplasmic pattern) were FISH-negative. All IHC-negative cases with clinico-pathological features suggestive of ALK translocation (histology and/or young age) were confirmed to be ALK-negative also by FISH. It is of interest that the percentage of ALK+ cases detected using IHC (8.1%) was 1.8-fold higher than that obtained in the previous two years using FISH as detection system (4.4%; 14 of 319 cases; X2 test, p = 0.06266).

The cost analysis of IHC screening + FISH confirmation versus FISH screening only, revealed that IHC screening had the following advantages: 1. It was cheaper. In fact, the cost for detection of an IHC-ALK+ patient (552€) was significantly lower (3.3-fold) than that for detection of a FISH-ALK+ patient (1822€); this value could be even lowered by eliminating FISH confirmation in 2+/3+ IHC-positive cases. 2. The low cost of routine IHC allows definition of the ALK status in all adenocarcinoma patients, independently of the request of the Oncologist. Conclusion. 1. IHC for ALK protein allows detection of most cases (81.1%) of ALK-positive adenocarcinoma. 2. The percentage of IHC-positive cases is much higher than that obtained with FISH analysis (4.4%), probably because light-microscopy facilitates recognition of small clusters of IHC-positive tumour cells. 3. IHC allows identification of crizotinib-sensitive tumours (high cytoplasmic expression of ALK protein) even when FISH is negative. 4. The time-to-diagnosis is shortened because the ALK status is established at the time of histological diagnosis. 5. Detection of ALK+ cases is much less expensive using IHC as compared to FISH. 6. Routine immunohistochemistry with the use of D5F3 mAbs is an extremely efficient tool for identification of ALK+ cases.
HIGH PREVALENCE OF ALK+/ROS1+ CASES IN PULMONARY ADENOCARCINOMA OF ADOLESCENTS AND YOUNG ADULTS

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Goal. To investigate prevalence of ALK/ROS1-translocated and EGFR-mutated adenocarcinomas in patients ≤50 years of age.

Materials and methods. This is a retrospective study conducted on a series of pulmonary adenocarcinomas collected at Sant’Andrea Hospital of Rome in the period 2012-2015. Paraffin sections of tumors were tested for EGFR mutations (n=789 cases; median age = 68.0±11.7; M:F = 1.39), ALK fusions (n=637 cases; median age = 67±12; M:F = 1.47), and ROS1 fusions (376 cases; median age = 67±13; M:F = 1.36) in the period January 2012- August 2015. Immunostaining for ALK protein (D5F3) and ROS-1 protein (D4D6) (Cell Signalling, USA) was introduced as a diagnostic tool since June 2015. EGFR mutations (T790M, Exon 19 Deletions, L858R, L861Q, S768I, G719X, Exon 20 Insertions and Exon 21 Insertion) were tested using mutant-specific Real-Time PCR (EGFR Mutation Analysis Kit For Real-Time PCR, EGFR-RT52, ENTROGEN, CA, USA) able to identify 33 somatic mutations of exons 18, 19, 20, 21 of the EGFR gene. Fluorescence in situ Hybridization was performed on paraffin-embedded tissue sections according to protocols of Tissue Digestion Kit (KBI-60007, Kreatech, Resnova, Italy) using a break-apart probe specific to the ALK locus and to the ROS-1 locus (Vysis LSI ALK Dual Color, break-apart rearrangement probe and 6q22 ROS1 break-apart RUO kit; Abbott Molecular, Abbott Park). Positive cases were defined as those with >15% positive tumor cells. X2 test was used to assess the association between gene alterations and age of the patients.

Results. 55 of 637 cases (8.6%) of pulmonary adenocarcinoma had mutually exclusive ALK (47/637 cases, 7.4%) or ROS1 (8/376 cases, 2.1%) fusions. When patients were stratified for age, it was found that six of six cases (100%) diagnosed in patients <30 years of age were translocated for ALK (4 cases) and ROS1 (2 cases). With the increase of age, there was a gradual decrease in the percentage of positive cases. In fact, ALK+/ROS1+ cases were 5 of 17 cases (29%) in the 31-40 years age-group, 6 of 46 cases (13%) in the 41-50 years age-group, and 38 of 568 cases (7.0%) in patients older than 50 years. Prevalence of ALK+/ROS1+ cases in patients <30 years of age was significantly (p<0.001) different as compared with the other age groups. The six patients <30 years of age (5F/1M), included two pediatric patients (≤18 years old). All presented with stage IV disease, were never or light smoker, and had no family history of pulmonary tumors. Four of the six patients were treated with crizotinib and had an objective response. At variance with ALK+/ROS1+ adenocarcinomas, prevalence of EGFR-mutated cases was poorly influenced by age. In fact, the mean age of 126 EGFR-mutated cases (67.0 years ±11.3) was similar to that of 663 EGFR-WT cases (68.0 years ±11.7).

Conclusion. Our findings provide evidence that ALK or ROS1 translocations are crucial events in tumorigenesis of pulmonary adenocarcinoma of very young patients, including pediatric patients.
IMMORTALIZED HUMAN ADIPOSE-DERIVED STROMAL CELLS: EVIDENCE OF CELL PROJECTION WITH ESEM

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¹ DETO, Bari; ² Consorzio Carso

Background. Human adipose-derived stromal cells (hASCs) have ability to secrete several angiogenic factors. To prolong their limited cultore life span, our previous studies demonstrated the capacity to immortalize them through infection with hTERT/SV40 (hASCs-Ts) and hTERT/HPV E6/E7 (hASCs-TE).

In order to evaluate cell morphology and growth aspect we performed an ultrastructural study with environmental scanning electron microscopy (ESEM).

Methods. hASCs were transduced with the human telomerase reverse transcriptase (hTERT) gene alone or in combination with either SV-40 or HPV E6/E7 and cultured on microscope slides for three days. Then cells were fixed with 2.5% glutaraldehyde solution for three hours. After dehydration with scalar alchols, the samples were dried with hexamethyldisilazane (HMDS) and submitted to Au ionization with ionic sputter.

Results. Non immortalized cells (hTERT alone) showed large amount of cellular prolongations up to 200 μ (microtubules and nanotubules), tiny (70-100 μ), sometimes branched, often communicating with other target cells. Isolated small vesicles (100 μ) were present on the slide surface (probably exosomes). On their body cell surface several blebs (up to 2 µ) were observed. HPV E6/E7 immortalized cells were scanty, with few and short cellular prolongations and rare blebs.

On the other side, SV-40 immortalized cells were numerous and showed few cellular prolongations and a large amount of blebs.

ROSAI-DORFMAN DISEASE OF THE PAROTID GLAND. A CASE REPORT IN ELDERLY WOMAN

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¹ DETO, Bari; ² Laboratorio P. Pignatelli, Lecce; ³ Clinica Città di Lecce, Lecce

Introduction. Rosai- Dorfman disease is a rare benign histiocytic proliferative disorder of unknown origin and a distinct clinicopathologic entity also known as Sinus Histiocytosis with Massive Lymphoadenopathy (SHML). The disease can involve extranodal tissue and rarely can present a salivary gland enlargement without significant lymphadenopathy.

Case report: a 76 year old women with parotid swelling for several months. FNAC was suggestive for lymphoma and negative for CD1a, factor XIIIa and CD34. The SHML cells are strong S-100 protein and CD 68 positive, and negative for CD1a, factor XIIIa and CD34.

Materials and methods. The histopathologic features include the presence of glandular atrophy and a heavy lymphoid infiltrate alternating with pale-appearing areas composed of histiocytes characterized by round to oval, vesicular to hyperchromatic nuclei, with abundant amphophilic to eosinophilic, granular, foamy to clear cytoplasm. The nuclei do not demonstrate nuclear lobation, indentation or longitudinaly grooving granular, foamy to clear cytoplasm. The nuclei do not demonstrate nuclear lobation, indentation or longitudinaly grooving.

The SHML cells are strong S-100 protein and CD 68 positive and negative for CD1a, factor XIIIa and CD34. On the base of morphological pattern we did the diagnosis of Rosai- Dorfman disease of parotid gland.

Conclusion. Differential diagnosis include Sjogren’s Disease and Lymphoma. Sjogren’s Disease is characterized by too large follicles, polyclonal cell and destruction of the glandular component instead lymphoma shows extensive monomorphism, scarce presence of CD68 + cells and monoclonality.
MOLECULAR PROFILE OF AMELOBLASTOMA: A RETROSPECTIVE STUDY TO ADDRESS NEW CLINICAL TREATMENT

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Introduction. Ameloblastoma is a benign but locally infiltrative odontogenic neoplasm. Although ameloblastomas rarely metastasize, recurrences together with radical surgery often result in facial deformity and significant morbidity. Development of not invasive therapies has been precluded by poor knowledge of molecular background of ameloblastoma pathogenesis. There was mounting evidence suggesting that activation of the mitogen-activated protein kinase (MAPK) pathway plays a prominent role. In this study we investigate the BRAF V600 status of 21 ameloblastoma patients observed in our institute to offer a rationale to test drugs targeting mutant BRAF as novel therapies for ameloblastoma.

Methods. Between 1998 and 2015 on the basis of the postsurgical histologic diagnosis, 21 case of ameloblastoma in patient aged 9 to 88 years (12 male and 9 female) were selected from archive tissues to verify mutational status of BRAF gene. All the cases were reviewed by pathologist and reclassified according to WHO 2005 indication. For seven patients, FFPE tissue was available from primary tumors and recurrences. Genomic DNA was extracted from 32 manually macro-dissected FFPE tissue samples with at least 10% of tumor cells using a QIAamp DNA FFPE Tissue Kit (QIAGEN, Germany) and dosed by Nanodrop spectrophotometer.

Table I. SALVI POSTER PATOLOGIA PLEURO POLMONARE (crizotinib therapy: FISH analysis of MET/ALK/ROS1 gene

<table>
<thead>
<tr>
<th>Patient code</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Hyalosy</th>
<th>Location</th>
<th>Recurrence</th>
<th>BRAF V600 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>70</td>
<td>S/M * follicular</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>63</td>
<td>S/M follicular/plexiform</td>
<td>mandible</td>
<td></td>
<td>wt</td>
</tr>
<tr>
<td>3A</td>
<td>M</td>
<td>31</td>
<td>S/M follicular/plexiform</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>3B</td>
<td>M</td>
<td>32</td>
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<td>V600E</td>
</tr>
<tr>
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<td>M</td>
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<td>S/M</td>
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<td>M</td>
<td>72</td>
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<td></td>
<td>V600E</td>
</tr>
<tr>
<td>6A</td>
<td>M</td>
<td>9</td>
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<td>mandible</td>
<td></td>
<td>wt</td>
</tr>
<tr>
<td>6B</td>
<td>M</td>
<td>9</td>
<td>Unicystic (var. basal)</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>6C</td>
<td>M</td>
<td>9</td>
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<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>7A</td>
<td>F</td>
<td>17</td>
<td>S/M (var. luminal)</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>7B</td>
<td>F</td>
<td>34</td>
<td>S/M</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>8</td>
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<td>38</td>
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</tr>
<tr>
<td>9</td>
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<td>38</td>
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<td></td>
<td>V600E</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>69</td>
<td>peripheral</td>
<td>mandible (mucosal)</td>
<td>16 months</td>
<td>V600E</td>
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<tr>
<td>11</td>
<td>M</td>
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<td></td>
<td>V600E</td>
</tr>
<tr>
<td>12</td>
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<td>V600E</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
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</tr>
<tr>
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<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>14B</td>
<td>M</td>
<td>26</td>
<td>S/M follicular</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>15A</td>
<td>M</td>
<td>43</td>
<td>S/M follicular</td>
<td>mandible (gingival mucosa)</td>
<td>4 months</td>
<td>V600E</td>
</tr>
<tr>
<td>15C</td>
<td>M</td>
<td>53</td>
<td>metastasizing</td>
<td>mandible</td>
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<td>15D</td>
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<td>57</td>
<td>long</td>
<td>mandible</td>
<td></td>
<td>14 years V600E</td>
</tr>
<tr>
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<td>F</td>
<td>88</td>
<td>S/M</td>
<td>mandible</td>
<td></td>
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<tr>
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<td>56</td>
<td>S/M plexiform clear cells</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>17B</td>
<td>F</td>
<td>57</td>
<td>S/M plexiform clear cells</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>17C</td>
<td>F</td>
<td>58</td>
<td>S/M plexiform clear cells</td>
<td>mandible</td>
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<td>V600E</td>
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<tr>
<td>17D</td>
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<td>73</td>
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<td>V600E</td>
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<tr>
<td>18</td>
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<td>73</td>
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<td>mandible</td>
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<td>V600E</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
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<td>mandible</td>
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<td>V600E</td>
</tr>
<tr>
<td>20A</td>
<td>F</td>
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</tr>
<tr>
<td>20B</td>
<td>F</td>
<td>23</td>
<td>Unicystic (var. luminal)</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
<tr>
<td>21B</td>
<td>F</td>
<td>27</td>
<td>Unicystic (var. luminal)</td>
<td>mandible</td>
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<td>V600E</td>
</tr>
<tr>
<td>21A</td>
<td>F</td>
<td>29</td>
<td>Unicystic (var. luminal)</td>
<td>mandible</td>
<td></td>
<td>V600E</td>
</tr>
</tbody>
</table>

* S/M: solid multicystic
Mutation status for codon V600 BRAF gene was assessed by real-time PCR using fluorescent ARMS-Scorpion allelic specific probe (therascreen® BRAF RGQ PCR kit, QIAGEN) and confirmed by pyrosequencing (therascreen® BRAF Pyro® Kit QIAGEN). The samples resulted non mutated in BRAF V600 were addressed to mutational analysis of KRAS and NRAS genes by pyrosequencing of codons 12,13 and 61 (therascreen® RAS Pyro® Kit, QIAGEN).

Results. BRAF V600E mutation was initially identified in a pulmonary metastasis of ameloblastoma in 57 year old male while being evaluated for clinical trials. The same mutation was seen in his primitive tumor and previous metastasis resected 14 and 4 years before. Archive FFPE slides from 21 patients (median age 43yr, range 9-88yr), referred as ameloblastoma were reviewed and reclassified according WHO 2005 in: ameloblastoma solid/multicystic (9 cases), unicystic (9 cases), peripheral (2) and desmoplastic (1). Detailed histological stratification is reported in table 1. 18 out of 21 primary tumors were localized in the mandible, two in the maxilla and one in the palate. 32 out of 33 specimens (20 primary tumors, 10 locally recurrences and 2 extra cranious metastasis) not subjected to decalcification from 20 patients were addressed to molecular analysis. The V600E BRAF mutation was detected in all unicystic ameloblastomas and in 50% of solid/multicystic ameloblastomas. Overall frequency of BRAF V600E mutation was 75% of samples analyzed. All Primary tumors and recurrences showed the same molecular profile suggesting that the mutation in BRAF gene is acquired early and it is stable in the neoplastic development of the lesion. 6 primary tumors not mutated for BRAF codon V600 were evaluated also for KRAS and NRAS mutational status in codon 12, 13 and 61 by pyrosequencing. No mutations were detected in the regions analyzed. (See table1)

Conclusion. This retrospective study on 21 case of ameloblastoma treated in our Institution has confirmed the high frequency of BRAF V600E mutation in this odontogenic neoplasm in according to recent scientific literature. The molecular profile of primary tumor is maintained in recurrent on site tumor and even in metastasis arisen after 10 year from initial surgery. The presence of BRAF activating mutations suggests the involvement of RAS-RAF-MAPK pathway in the pathogenesis of ameloblastomas. These results support the rationale for the use of BRAF inhibitors in the treatment of ameloblastoma to improve outcome and minimize functional and cosmetic morbidity.

References

A RARE CASE OF PAPILLARY SQUAMOUS CELL CARCINOMA OF THE TONSIL

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Introduction. Papillary squamous neoplasms of the upper respiratory tract are rare variants of squamous cell carcinomas. They are characterized by an exophytic, papillary growth and have a favorable prognosis. The tumor has been described in the upper aerodigestive tract. In this context, most common sites of involvement are the larynx and hypopharynx, and rarely the oral cavity and oropharynx.

Background. The limited studies and the small number of published cases of papillary squamous cell carcinoma have induced us to make a complete analysis of this tumor by analyzing the clinical, radiological, virological and therapeutic aspects that are not always present in the literature.

Case report. A case of papillary squamous cell carcinoma of the palatine tonsil is reported. The lesion (T2N0M0) was located into the left palatal tonsil that hung towards the oral cavity. Both HPV 16 DNA and E6/E7 mRNA were detected. The clinicopathological profile of the neoplasm is presented.

Discussion and conclusions. A comprehensive review of recent literature was made by analyzing the epidemiological, etiopathogenetic, clinical and therapeutic aspects of this neoplasm.

IDENTIFICATION OF LARYNGEAL LEIOMYOSARCOMA GENETIC ALTERATIONS USING NEXT GENERATION SEQUENCING

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Background. Laryngeal leiomyosarcoma is an extremely rare mesenchymal malignant tumor originating from smooth muscle cells. First described in 1939 by Jackson and Jackson (Jackson C, et al.1939), fewer than 50 cases are described in literature. Laryngeal mesenchymal tumour per se is a rarity (<1%), within which leiomyosarcoma is one of the most uncommon types, accounting for less than 0.1% (Thompson LDR et al., 2005). Few reports are reported describing the use of Next Generation Sequencing for the identification of genetic alteration in soft tissue sarcomas. Due to the rarity of these tumors DNA sequencing has been a challenge mostly for Leiomyosarcoma for which the genetic changes still remain to be discovered. In this study we performed NGS analysis of 409 cancer-related genes in order to identify novel mutation associated to these rare tumors.

Methods. A 67-yr-old man was operated for a mass of the glottis region of the larynx, with symptoms of obstruction. Histological examination showed a mesenchymal tumor composed of spindle cell, with pleomorphism and mytotic activity, covered by a hyperplastic squamous epithelium. Immunohistochemical (IHC) analysis showed positivity of tumor cells for Vimentin and Smooth Muscle Actin, negativity for Cytokeratin. DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor and normal tissues were subsequently used for library preparation. A total of 409 full coding sequence of cancer-associated genes were evaluated using the Ion AmpliSeq™ Comprehensive Cancer Panel on the Ion Torrent Proton platform. The sequence data were mapped to the human genome (hg19) by the Ion Reporter Software to perform variant calling and mapping. The resulting nucleotide variants, including single-nucleotide substitution, small insertions and deletions, were subsequently annotated as pathogenic or
possibly pathogenic. In addition bioinformatic analysis was performed in order to detect copy number variation in the panel genes.

**Results.** The diagnosis based on morphological and immunohistochemical analysis revealed that the tumor was a laryngeal leiomyosarcoma. The molecular analysis allows the detection of genetic alteration in genes that are currently targetable, like MYC, FGFR1 and AURKA, and in genes that are still non-targetable like PTEN, TP53 and NOTCH1.

**Conclusions.** The identification of molecular abnormalities by comprehensive genomic analyses could help the development of target therapies and improve the outcomes for patients affected by this rare and aggressive mesenchymal tumors.

**PATOLOGIA ENDOCRINA**

**HYALINIZING TRABECULAR TUMOR MISDIAGNOSED AS PAPILLARY THYROID CARCINOMA ON FNAC: A DECEPTIVE AND PROBLEMATIC CASE**

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**Introduction.** Thyroid hyalinizing trabecular tumors (HTT) are classified in the last WHO as rare tumors of follicular origin with a trabecular pattern of growth and marked intratrabecular hyalinization. This definition replaces the term hyalinizing trabecular adenoma proposed by Carney et al in 1987 that firstly described this pathological entity. In the past there were many controversial debates about this tumor because some considered it a precise entity, some as a variant of papillary carcinoma and others as a nonspecific histological pattern of many variety of thyroid lesions. It is characterized by circumscription or encapsulation, trabecular growth pattern, polygonal and elongated cells, nuclear cytoplasmic inclusions and grooves, hyaline material, dilated sinusoids, laminated calcispherites, and cytoplasmic yellow bodies. However, it can be mistaken for thyroid carcinoma on fine needle aspiration cytology (FNAC). In fact, cytological diagnosis becomes challenging because HTT cytomorphology overlap with both papillary and medullary carcinoma. Due to the complexity of the cytological diagnosis, patients sometimes undergo a surgical overtreatment because the major diagnoses on FNAC are carcinoma, especially papillary carcinoma for the grooves and medullary carcinoma for the hyaline material that mimicks amyloid, or suspicious for carcinoma. Despite its rarity, it would be desirable to establish more precise cytological criteria that help to conduct a right surgical treatment. Herein we describe a case of thyroid hyalinizing trabecular tumor previously diagnosed as a papillary carcinoma by FNAC. For this reason it would seem reasonable to pay serious attention to the cyt morphological aspects of the thyroid FNAC smear, because in this setting, sometimes it is very difficult and challenging to differentiate HTT from papillary thyroid carcinoma, as in our case.

**Materials and methods.** The specimen was fixed in 10% formalin solution, paraffin embedded, sectioned at 5 μm and histologically examined using standard Hematoxylin–Eosin (HE) staining method. Afterwards, the following IHC reactions were performed: Thyreoglobulin, TTF1, CK7, pS100, CK19, p53, 34BetaE12, Ki67. A REAL-TIME PCR (RT-PCR) analysis was performed on formalin-fixed and paraffin-embedded tissues (FFPE) from the surgical specimen of the thyroid neoplasm to evaluate a possible mutation at the exon 15 of the B-RAF gene.

**Results.** A 57-year-old woman, without relevant previous medical history, in May 2015, underwent total thyroidectomy after a FNAC diagnosis of papillary carcinoma performed in another institution. The macroscopical examination showed two solid whitish-grey nodule located at the upper of the left thyroid and lobe at the istmus, the first measuring 11 mm and the second measuring 2 mm. Microscopical examination revealed an encapsulated tumor surrounded by a thin capsule and characterized by trabecular structures separated by minimal fibrous stroma. The tumor cells were polygonal and oval with an acidophilic cytoplasm. The nuclei were round with inconspicuous small nucleoli, with prominent grooves and less frequent pseudoinclusions. There were also few Zellballen, intermingled in a vascular network, similar to those noted in paragangliomas. Atypical cells, mitotic figures, necrosis and capsular invasion were not evident.

Immunohistochemical studies revealed that the neoplastic cells were strong positive for TTF-1, CK 7, CK19; the Ki 67 proliferation rate was very low and there wasn’t membranous labelling.

There was no evidence of molecular mutation of BRAF gene. On the bases of the morphological and immunohistochemical results the diagnosis of HTT was performed and an accurate follow-up was required. Today the patient is healthy without signs of recurrence.

**Conclusions.** HTT of the thyroid is a neoplasm with distinctive morphologic features described by Carney et al in 1987 and classified by the last WHO as “hyalinizing trabecular tumor” due to its exceedingly low to nonexisting malignant potential. Its cytomorphological pattern of presentation on FNAC smear leads cytopathologists to confuse it with papillary thyroid carcinoma because of the grooves and cytoplasmic inclusions (“holes”) and with medullary thyroid carcinoma because of the homogeneous acidophilic hyaline material that mimicks amyloid. HTT can be a solitary nodule or can display a pattern of multiple nodules that are well circumscribed and encapsulated. The major histological features of the tumor cells in the classic trabecular pattern of HTT are: the hyaline cytoplasm with inclusions, the presence of matrix and the presence of nuclei with prominent grooves. Immunohistochemistry shows positivity for thyroglobulin and TTF-1. A particular stain is the cytoplasmic expression of MIB-1 (Ki-67) in HTT that is useful to distinguish it from PTC although HTT is misdiagnosed almost uniformly in FNAC specimens. Some authors have proposed a cytological distinction between HTT and papillary carcinoma of the thyroid, but its cytological diagnosis remains challenging. It has been observed that a lack of papillary architecture, the presence of a unique architecture features like trabecular pattern, in combination with a bloody background, radially oriented cohesive cells, nuclei with very frequent cytoplasmic inclusions and grooves, and the presence of hyaline material, suggest a diagnosis of HTT. The differential diagnosis with MTC can also pose diagnostic difficulties because hyaline acellular material in the aspirates can be misdiagnosed with amyloid. These tumors can be distinguished from MTC by Congo red negativity, positive thyroglobulin immunoreactivity, and negative calcitonin immunoreactivity.

In our case the patient presented at our hospital after a FNAC diagnosis of PTC formulated at other institution. For this
MALIGNANT SOLITARY FIBROUS TUMOR OF THE PLEURA: ANALYSIS OF TWO CASES

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Introduction. Solitary fibrous tumor of the pleura is a rare neoplasm arising from subepithelial mesenchymal fibroblast cells. It accounts for less than 5% of primary pleural tumors and occurs in middle age, without gender differences. It is usually benign but 10-20% of cases may be more aggressive and can develop metastases or recurrences despite primary complete resection. One third of the cases are asymptomatic while most show atypical symptoms like chest pain, breathing difficulties and a cough. We describe two female patients who underwent resection of the pleural tumor.

Material and methods. CASE 1: a 47-year-old female came to our hospital in February 2016 for left pleural effusion, dyspnea and cough lasting some weeks. She denied other diseases in her medical history. CT scan showed a large left-side mass of the pleural surface composed of heterogeneous, well circumscribed soft tissue with low density areas, as well as bone and cerebral lesions, suspicious for metastases. The patient underwent surgical resection of the tumor with lung-sparing because of the size of the mass. The macroscopic examination revealed a 21 centimeter nodular mass, uniformly grey with some necrotic and hemorrhage areas. The tumor was composed of short spindle cells, with perivascular hyalinization. Mitotic activity was >4/10 HPF. The cells showed immunophenotypical expression for CD34, Bcl-2, vimentin and CD99 and were negative for CK pool, CK5/6, CK7, calretinin, WT-1, EMA, desmin, actin, S100, TTF1, and HMB45. The final diagnosis was a malignant solitary fibrous tumor, based on the histological features and the presence of metastasis.

CASE 2: a 58-year-old female was admitted to our hospital in February 2016, previous admission March 2015. CT scan was performed and showed a voluminous nodular mass measuring about 13 centimeters in the left peripheral lung. A first percutaneous CT-guided biopsy was conducted but was not diagnostic, because of the absence of neoplastic cells in the sample. In the second biopsy the samples were composed of fascicles of fusiform cells with interstitial collagen. The cells expressed CD34 and vimentin and were negative for actin, S100, CK pool and CD31. The immunophenotype was coherent with a solitary fibrous tumor. The diagnosis was confirmed by the positivity for CD99 and Bcl-2. The patient underwent left thoracotomy. During the resection an extemporary examination was required. The provisional diagnosis described a neoplastic proliferation of spindle cells organized in clusters next to dense collagen. The morphological features did not exclude a neuroendocrine origin of the neoplasm. The mass, some mediastinal lymph nodes, and a fragment of rib were removed. The nodes and the rib were negative for neoplasm. The analysis of the full mass showed hypercellularity, moderate atypia and an increased mitotic activity (>4/10 HPF; KI67>40%). The tumor was judged malignant.

Results. The two patients described were diagnosed in the fifth and sixth decade of life, in agreement with data in literature. The tumors were voluminous and symptomatic. Surgical resection was necessary to avoid respiratory failure, even if only the first case was clinically malignant and metastatic. The second was diagnosed as malignant after histological examination.

Conclusions. There are no definitive criteria for malignancy in solitary fibrous tumors. The most common are hypercellularity, nuclear atypia, mitotic activity>4/10 HPF, necrosis and hemorrhage. No pathognomonic clinicopathological features have been identified. In a recent study the fusion variant of NAB2-STAT6 that drives STAT6 nuclear expression was revealed in a majority of intrathoracic solitary fibrous tumors, but was unrelated to the prognosis. More efforts are needed to find any correlations between molecular changes and prognosis.

FISH ANALYSIS OF MET GENE IN MALIGNANT MESOTHELIOMA

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Introduction. Malignant mesothelioma (MM) is a particularly aggressive tumour associated with exposure to asbestos and characterized by a high mortality. To date, there is no curative
therapy for MM and survival tends to be 11 to 12 months after diagnosis. A multidisciplinary approach, involving the use of surgery, chemotherapy and radiotherapy, aims at alleviating the symptoms and lengthening survival (Robinson BW, et al.). The innovative therapies targeting the tyrosine kinase receptors (TKR) that have significantly progressed in many cancers might be used also for MM.

MMNG HOS Transforming gene (MET) is a proto-oncogene located in the 7q31 that encodes the high-affinity receptor for hepatocyte growth factor (HGF). The HGF/MET pathway has been recently proposed as a new cancer treatment, especially for lung and stomach cancers in addition to MM (Kawakami H, et al.) that showed MET gene amplification associated to receptor over-expression.

Moreover, MET gene amplification occurs in about 20% of non-small cell lung cancer (NSCLC) patients with acquired resistance to anti-epidermal growth factor receptor (EGFR) TKI treatment as gefitinib or erlotinib. Therefore, amplification of MET is considered as a primary mechanism of resistance to EGFR-TKI (Ayoola A, et al.). In this study, we evaluated, by FISH, the status of MET gene in MM, thus providing the incidence of MET amplification and high polysomy in MM.

**Patients and methods.** The protocol of this study was approved by the Liguria Region Ethics Committee (P.R. 207REG2014) and the written informed consent was obtained from all the patients (Varesano S, et al.). We analysed 60 MM (male: 66.7%) including: 36 epithelioid, 12 sarcomatoid, 8 biphasic, 2 desmoplastic and 2 papillary subtypes. Thirty cases of MM were from a tissue microarray (MS801, US Biomax Inc, Rockville, MD, USA), 12 cases of formalin-fixed paraffin-embedded tissues from the Division of Histopathology Cytopathology, IRCCS AOU San Martino-ISt (Genova) and 18 tissues from the Division of Histopathology Cytopathology, ASL n°5 “Spezzino (La Spezia). MET gene amplification was investigated by FISH using MET/CEP7 probe cocktail (Vysis MET Spectrum Red FISH Probe Kit reagent/Vysis CEP 7 (D7Z1) SpectrumGreen Probe, both reagents from Abbott Molecular, Des Plaines, IL USA). IHC staining of MET was performed by Anti-c-Met Antibody IHC-plus™ LS-B2812 (LSBio, Seattle, WA).

**Results.** Using the University Colorado Cancer Center scored-system (Varella-Garcia M, et al.) we found 55 FISH-negative cases (Figure 1, panels A, B, C). Moreover, four epithelioid MM showed high polysomy of MET gene (range of 4-10 spots in 60-80% of MM cells) associated with low stain-
ing of MET protein (Figure 1, panels D, E, F). Finally, one epithelioid MM had MET gene amplification (about 8 MET signals on > 70% of cells; MET/CEP7 ratio = 4.0) associated with moderate expression of MET protein in cytoplasm and membrane of MM cells (Figure 1, panels G, H, I).

Conclusions. We report that the amplification and high polysomy of MET gene, with consequent protein expression, is present in a small percentage of MM. These observations may be important for MM treatment as the aberration of MET gene might identify a subset of tumours that better respond to HGF/ MET pathway targeted therapies. 

At present, several agents targeting the HGF/MET pathway are rapidly progressing through various clinical trials and in various cancers. They can be used alone or in combination with other TKI or chemotherapeutic agents. Some of these agents are selective small-molecule inhibitors of MET, such as PHA-665752 and tivantinib, others are antibodies against MET cell-surface receptor, such as onartuzumab or against HGF, such as rivalotumumab; moreover, novel drugs, including perifosine, crizotinib and caboziotinib, show a spectrum of action involving different genes (Kawakami H, et al.).

In MM, MET targeted therapies have already been proposed but, at this time, they are used only for in vitro research showing promising results. For example, treatment of MM cell lines with PHA-665752 shows inhibition of cell proliferation, migration and invasiveness (Mukoora T, et al.) or treatment with tivantinib synergistically enhances the antiproliferative and proapoptotic activity of pemetrexed.

A further possible consequence of MET amplification on the treatment of MM with EGFR targeted therapy is that MET gene amplification in NSCLC is an element of resistance to EGFR inhibitors. (Ayoola A, et al.).

Up to now, routinely molecular biology examinations are not available in MM, but we believe that genetic anomalies, already targets of therapies in other cancers, should be studied also in MM because they may open the door to new treatments for this cancer in the future.

References


CRIZOTINIB THERAPY: FISH ANALYSIS OF MET/ALK/ROS1 GENE STATUS IN MALIGNANT MESOTHELIOMA

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Introduction. Malignant mesothelioma (MM) is a particularly aggressive tumour with poor prognosis and limited possibility of treatment so that the survival tends to be one year after diagnosis (Robinson BW, et al.). New therapeutic hopes may arise from the receptor tyrosine kinases (RTKs)-based targeted therapies significantly progressed in many cancers. RTKs are expressed on the cell membrane, regulate many important functions in normal cells and display a crucial role in oncogenesis. Many molecules, including anti-RTK antibodies and small-molecule inhibitors of RTK kinase activity (TKIs), are able to block RTKs and interfere with their signal transduction. (Gschwind A, et al.).

Early promising results have been reported for Crizotinib (PF02341066) that is a TKI with an affinity for MMNG HOS Transforming proto-oncogene (MET), Anaplastic lymphoma kinase proto-oncogene (ALK) and ROS proto-oncogene 1 (ROS1) kinase domain. Crizotinib has been initially designed to target MET proto-oncoprotein, subsequently approved by the U.S. Food and Drug Administration for the treatment of advanced ALK-rearranged NSCLC and recently extended also to the potential treatment of ROSI-rearranged NSCLC. (Rothschild SI, et al.).

MET amplification, ALK and ROSI translocation constitutively activate the fusion kinases and drive cellular proliferation and transformation.

MET proto-oncogene is located in the 7q31 locus of chromosome 7 and it is found amplified in many cancers including approximately 4% of non-small cell lung cancer (NSCLC). ALK proto-oncogene is mapped on chromosome 2p23 and its variants showing different patterns of chromosomal translocation are present in many cancers and in about 5% of NSCLC. Finally, ROSI proto-oncogene is mapped on chromosome 6q22 and its variants of rearrangement occur in many types of cancer including approximately 1% of NSCLC. (Rothschild SI, et al.).

While MET FISH-positivity was reported in about 9% of MM (2% amplification and 7% high polysomy gene status) (Varensano S, et al. Ref. 4) and ALK gene rearrangement was described in only one case of biphasic paediatric MM (Loharantweethong K, et al.), to our knowledge, no data have been reported on the status of ROSI proto-oncogene in MM. In this study, we evaluated, by FISH, MET, ALK and ROSI status in order to verify the number of MM patients who may benefit from treatment with crizotinib.

Patients and methods. We analysed a total of 79 MM (47 males, median age 49 years) including 42 (53%) epithelioid, 28 (35.4%) sarcomatoid, and 9 (11.4%) biphasic subtypes, from different stages and organ locations. The formalin-fixed and paraffin-embedded tissues were derived as follows: 30 cases of MM from the tissue microarray MS801 (US Biomax Inc, Rockville, MD, USA) and 49 from the tissue microarray MS1001 (US Biomax). MET gene amplification was investigated by FISH, according to the University Colorado Cancer Center-scored system (Varella-Garcia M, et al.), using MET/CEP7 probe cocktail (Vysis MET Spectrum Red FISH Probe Kit reagent/Vysis CEP 7 (D7Z1) (Abbott Molecular, Des Plaines, IL USA). ROSI gene translocation was investigated by FISH using Spectrum Green Probe, Vysis 6q22 ROSI Break Apart FISH Probe (Abbott).

ALK gene translocation was investigated by Vysis LSI ALK Dual-Color Break Apart Rearrangement Probe.). All FISH reagents from Abbott Molecular, Des Plaines, IL USA. (Abbott).

Results. All 79 (100%) cases of MM were negative for ALK or ROSI gene translocation. By using the University Colorado Cancer Center-scored system (Varella-Garcia M, et al.) we did not find any MET...
amplification (according to MET to CEP7 ratio ≥2 or at least 15 copies of MET signals in ≥10% of the tumour cells). In contrast, 5/79 (6.3%) MM (3 epithelioid, 1 sarcomatoid, 1 biphasic) showed high MET polysomy (according to mean ≥4 copies/cells in ≥40% of tumour cells) in a range of 4-10 spots of MET gene in about 60-80% of tumour cells. Finally, all the other 74 FISH-negative cases (93.7%) were disomic for MET gene (Table 1).

Conclusions. Crizotinib is a TKI of ALK/MET/ROS1 proto-oncogene alteration. In the present study, we confirmed our previous report in which analysis by FISH, in a small number of MM patients, showed that MET may be present in a high polysomy status (Varesano S, et al. Ref. 4). Furthermore, our data reinforce the notion that the rearrangement of the ALK gene in MM is a very rare event (Varesano S, et al. Ref. 7); indeed no ALK gene rearrangement was detected in our set of MM so that the patients reported by Loharamtaweethong K, et al (Ref. 5), remain the only cases of MM harbouring ALK gene rearrangement in literature. Moreover, we analyzed ROS1 gene, which is the third possible target for crizotinib TKI and we did not detect any ROS1 translocation. To our knowledge, this is the first study which analyzes ROS1 rearrangement in MM.

In conclusion, our results indicate that, for treatment of MM patients with crizotinib, MET is the only candidate target gene and consequently this gene is the only one that should be evaluated by FISH. However, since both the ALK and ROS1 translocations may be rare events in cancers, we cannot exclude with certainty that they can happen in a small number of cases. However, our data are to be considered preliminary and should be re-evaluated in a larger series of MM patients.

References

Table 1. SALVI POSTER PATOLOGIA PLEURO POLMONARE (Crizotinib therapy: FISH analysis of MET/ALK/ROS1 gene

<table>
<thead>
<tr>
<th>Gene status</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK translocation</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>ROS1 translocation</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>MET amplification</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>MET high polysomy</td>
<td>5 (6.3)</td>
</tr>
<tr>
<td>MET diploidy</td>
<td>74 (92.7)</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
</tr>
</tbody>
</table>

Background. Lung cancer is the leading cause of cancer-related mortality and targeted therapies have revolutionized the treatment of lung cancer in the past decade. Epidermal growth factor receptor (EGFR) activating mutations and anaplastic lymphoma kinase (ALK) gene rearrangements represent the two genetic drivers of non-small cell lung cancer (NSCLC) with an impact on current clinical practice. Discovery of actionable mutations in EGFR and translocations in ALK have identified subsets of patients with excellent tumor response to targeted agents with manageable side effects. EGFR tyrosine kinase inhibitors (TKIs) and crizotinib are the standard of care for the treatment of EGFR mutant and ALK gene rearranged advanced NSCLC patients. The frequency of EGFR mutations and ALK rearrangements varies according to not only ethnicity but also gender, smoking status and the histological type of NSCLC. Somatic mutations in the EGFR gene are present in approximately 20% (Caucasians) to 50% (East Asians) and rearrangements in ALK gene are found in about 2%-8% of NSCLC patients.

ALK gene rearrangements have been reported in 2% to 13% of patients with NSCLC. In the present study, we investigated the distribution of EGFR mutations and the distribution of ALK rearrangements in NSCLC patients. Additionally, we compared the clinicopathological data of NSCLC patients with the mutational status of EGFR and ALK.

Materials and methods. A total of 80 unselected histological cases (stage IV) examined between January 2013 and December 2015 entered the present study. Forty four cases out of 80 referred to a diagnosis of primary lung adenocarcinoma (ADC) and the other 36 cases referred to ADC metastases. The assessment of EGFR mutations in exons 18-21 was carried out by DNA sequencing in 64 cases. ALK rearrangements were tested in 80 patients by fluorescent in situ hybridization (FISH) using the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe® (Abbott Molecular, Abbott Park, IL). Additionally, comparisons between clinicopathological data of NSCLC patients with the mutational status of EGFR and ALK were performed with the Pearson χ² test for categorical variables.

Results. Eighty patients were enrolled into this study with a median age of 62 years old (range, 33-82). Among them, 50 (62.5%) were females and 23 (29%) out of 79 cases with available data about smoking history were non-smokers. EGFR mutations were detected in 15 (23%) out of 64 evaluated cases. ALK rearrangements were identified in 12 (15%) out of 80 evaluated cases (Figure 1). One patient (0.01%) out of 80 showed the coexistence of EGFR mutation in exon 19 and the presence of ALK rearrangement. The ALK FISH-positive ADCs showed the two major described patterns as follows: the break-apart pattern (‘classic’ pattern) (BA) was observed in 5 (6%) out of 80 of cases and the isolated red signal pattern (‘atypical’ pattern) (IRS) pattern in 7 (8%) out of 80 tumors. Ten cases out of 12 received Crizotinib treatment and the five ALK FISH-positive cases with the BA pattern showed a better response than the ones with the IRS pattern.
Clinicopathological data of the enrolled patients and their association with EGFR and ALK status are shown in Table 2. Statistical analysis evidenced that the incidence of ALK rearrangements was much higher in EGFR wild-type patients than in those with EGFR mutations (P=0.08). Additionally we found the frequency of ALK rearrangements to be significantly higher in non-smokers patients (P=0.006), whereas no association was demonstrated regarding age, sex and disease site (lung/metastases). No statistical associations were found between EGFR mutations and clinicopathological data.

Conclusions. We may hypothesize that different ALK variants confer differential sensitivity to ALK inhibitors. ALK rearrangements are associated with non-smoker status and EGFR wild type status.

Future studies on larger patient groups would provide more accurate data to exhibit the relationship between EGFR mutations and ALK rearrangements and the clinicopathological status.

References

Table I. Characteristics of 12 ALK rearranged ADC. PD: progression disease, PR: partial remission, CR: complete remission.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Tumor site</th>
<th>Tumor sample</th>
<th>Histology</th>
<th>main pattern</th>
<th>ALK pattern</th>
<th>Treatment with Crizotinib</th>
<th>Response</th>
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<tr>
<td>1</td>
<td>61</td>
<td>M</td>
<td>Lung</td>
<td>Surgical</td>
<td>Solid</td>
<td>iRS</td>
<td>Yes</td>
<td>pD</td>
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<tr>
<td>2</td>
<td>73</td>
<td>M</td>
<td>Lung</td>
<td>Surgical</td>
<td>Solid</td>
<td>iRS</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>F</td>
<td>Lung</td>
<td>Surgical</td>
<td>Acinar</td>
<td>BA</td>
<td>Yes</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>M</td>
<td>Lung</td>
<td>Surgical</td>
<td>Solid</td>
<td>iRS</td>
<td>Yes</td>
<td>pD</td>
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<tr>
<td>5</td>
<td>50</td>
<td>F</td>
<td>Lung</td>
<td>Surgical</td>
<td>Acinar</td>
<td>iRS</td>
<td>Yes</td>
<td>pD</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>M</td>
<td>Lung</td>
<td>Surgical</td>
<td>Solid</td>
<td>iRS</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>73</td>
<td>F</td>
<td>Lung</td>
<td>Surgical</td>
<td>Acinar</td>
<td>BA</td>
<td>Yes</td>
<td>PR (ongoing)</td>
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<tr>
<td>8</td>
<td>81</td>
<td>M</td>
<td>Lung</td>
<td>Surgical</td>
<td>Solid</td>
<td>iRS</td>
<td>Yes</td>
<td>PD</td>
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<td>9</td>
<td>42</td>
<td>F</td>
<td>Pleura</td>
<td>Biopsy</td>
<td>Solid</td>
<td>BA</td>
<td>Yes</td>
<td>PR (ongoing)</td>
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<td>10</td>
<td>43</td>
<td>F</td>
<td>Lung</td>
<td>Surgical</td>
<td>Acinar</td>
<td>BA</td>
<td>Yes</td>
<td>CR</td>
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<tr>
<td>11</td>
<td>76</td>
<td>F</td>
<td>Pleura</td>
<td>Biopsy</td>
<td>Acinar</td>
<td>iRS</td>
<td>Yes</td>
<td>PD</td>
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<tr>
<td>12</td>
<td>58</td>
<td>F</td>
<td>Lung/Lymph node</td>
<td>Surgical</td>
<td>Solid</td>
<td>BA</td>
<td>Yes</td>
<td>PR (ongoing)</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Clinicopathological features of the enrolled 80 patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EGFR wild type cases (n=49)</th>
<th>EGFR mutated cases (n=15)</th>
<th>ALK wild type cases (n=68)</th>
<th>ALK rearranged cases (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking history</td>
<td>Non-smokers 12 (24.49%) 6 (42.86%) 15 (22.39%) 8 (66.67%)</td>
<td>Smokers 37 (75.51%) 8 (57.14%) 52 (77.61%) 4 (33.33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female 34 (69.39%) 8 (53.33%) 45 (66.18%) 5 (41.67%)</td>
<td>Male 15 (30.61%) 7 (46.67%) 23 (33.82%) 7 (58.33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td>Primary 29 (59.18%) 9 (60.00%) 36 (52.94%) 8 (66.67%)</td>
<td>Metastases 20 (40.82%) 6 (40.00%) 32 (47.06%) 4 (33.33%)</td>
<td></td>
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</table>

Figure 1. Lung ADC showing ALK rearrangement.
Background. The discovery of anaplastic lymphoma kinase (ALK) rearrangement in NSCLC in 2007 and the approval of crizotinib for the treatment of advanced ALK rearranged NSCLC in 2011 represents a landmark in the development of targeted oncology therapy. The reported incidence of EML4-ALK fusions in NSCLC is low (5%–7%) but can be higher (13%–18%) if the population is selected on specific clinicopathologic characteristics. Patients with EML4-ALK fusions are more commonly younger and non-smokers. Moreover, EML4-ALK+ carcinoma is characterized by adenocarcinoma (ADC) with a distinct histologic appearance and less frequently is squamous cell carcinoma (1%) (SCC). Additionally, most EML4-ALK fusion carcinomas are wild type for EGFR and KRAS. Accurate identification of EML4-ALK+ NSCLC is essential for the selection of appropriate therapy. These tumors do not respond to EGFR antagonists but respond to specific ALK kinase inhibitors like Crizotinib. It is an approved molecule for the treatment of advanced ALK rearranged NSCLC. Different technologies are available to assess ALK gene rearrangements. Fluorescence in situ hybridization (FISH) is the accepted standard because it has been used as a reference method in clinical trials; however, it is an expensive, time consuming, and labor intensive assay. An alternative diagnostic method based on the detection of ALK fusion protein expression is IHC analysis. Several studies showed that IHC is sensitive and specific for determination of ALK protein expression and is an accessible cost effective and rapid alternative to the ALK FISH assay. Recent regulatory changes have allowed the diagnostic use of IHC analysis for the identification of patients with NSCLC. The U.S. Food and Drug Administration has approved the VENTANA ALK (D5F3) Assay (Ventana Medical Systems, Tucson, AZ) as companion diagnostics, and the Italian Medicines Agency has recognized this IHC analysis as a diagnostic test indicating an algorithm for patient selected who are eligible for treatment with ALK inhibitors such as Crizotinib. Crizotinib may rapidly induce tumor regression in certain patients even if not all patients respond. In our study, we studied the concordance between these two methods evaluating the ALK status using FISH and an automated standardized IHC assay in a series of 95 cases of advanced NSCLC derived from our clinical workflow, giving emphasis to discordant cases on potential biological considerations such as the percentage and the rearrangement pattern of ALK and of rearranged cells on FISH and their significance to outcome with crizotinib.

Patients and methods. Consecutive 95 NSCLC specimens, wild-type EGFR, were tested for ALK rearrangements. Preliminary data on the outcome of crizotinib-treated patients were recorded. Tumor samples were fixed in formalin, embedded in paraffin and histologically classified on the basis of hematoxylin and eosin and IHC staining according to the WHO classification of lung tumors. We tested our NSCLC cases for ALK rearrangement by two IHC assays (Ventana ALK D5F3 antibody and D5F3 antibody Cell Signaling with the Optiview DAB IHC detection kit and Optiview Amplification kit) and by fluorescence in situ hybridization (FISH). Tumor samples were considered ALK FISH positive (ALK rearranged) if more than 15% of the tumor cells showed split red and green signals (signals separated by one or more signal diameters) and/or single red 3’ signals (deleted green signal) in addition to fused and/or broken apart signals and gene amplifications or polysomy. Otherwise, the samples were considered FISH negative. Clinical characteristic and response to crizotinib were reviewed.

Results. ALK testing on FISH analysis gave positive results in 13 (13.6%) of the 95 cases investigated. We observed that ALK IHC correlated well with ALK FISH in 88 cases (92.6%). They comprise 82 (86.3%) FISH/IHC negative cases and 6 (6.3%) FISH/IHC positive cases. The last group had concordant ALK protein overexpression and ALK rearrangement by FISH. However, some discordant cases were identified. Seven cases (7.3%) had discordant results between the ALK FISH and expression protein ALK IHC assay. They were ALK FISH+/IHC negative. None case showed ALK FISH negative/IHC positive. The discordant group consisted of four female and three male patients with mean age of 71.8 (range, 66-77 years). All, less one case, were negative by IHC assessment with the Ventana system CE IVD (clone D5F3) and two cases showed SCC while the others were ADC histotype. One case was equivocal result (score 1+) by Ventana ALK (clone D5F3) CST system and it had a solid adenocarcinoma histotype. Of this group the mean number of FISH positive rearranged nuclei was 40.2% (range 20-54%). All cases showed coexistent split signals pattern positivity with mean percentage 19.1% (range 10-32%), 5' deletions pattern positivity with mean percentage 21.7% (range 12-34%) and one case also had gene amplifications pattern positivity with percentage of 64%. Moreover, the polysomy was observed in all cases with mean percentage of 45.7% (range of 16-72%). No other driver alterations were seen in these cases detected by NGS. Five patients with discordant ALK FISH and IHC results received crizotinib and four of them had requisite radiologic follow-up for analysis of response. Four patients had disease progression and one of them died. One case had stable disease. Two patients started to receive adjuvant chemotherapy.

Discussion. Regarding the clinical-pathologic characteristics of patients with ALK translocations, our findings are in contrast with previously reported ones which associated ALK translocations with nonsmokers and young age patients. The patients in our series were elderly. The discrepancies observed between the IHC and FISH data revealed unexpected biological events, rather than technical issues, which potentially can have a strong impact on the therapeutic strategy with crizotinib. We found in most of discordant cases a coexistent complex pattern of rearrangements (deleted, inverted and amplified/polysomic patterns) of ALK positive cells in FISH analysis that could reflect a negative IHC analysis and important clinical implications. Currently, in our analysis the ALK FISH+/IHC negative discordant group showed a lower percentage of nuclei positive for ALK rearrangement 19.1% in split signals and 21.7% in 5’ deletions compared with 64% of gene amplification in one case and with those of polysomy (45.7%) observed in all cases. Nevertheless, these complex rearranged cases was not detectable by IHC, probably resulting from a lack of protein expression derived from this trans-
In June 2015, a 61-years-old male, ex light-smoker (1 pack/year), ECOG PS 0, was referred to the medical oncology service after the diagnosis of stage IV lung adenocarcinoma (T2aN3M1a). The bronchial biopsy showed a pulmonary invasive poorly differentiated adenocarcinoma, p63 immunohistochemistry (IHC) negative, TTF1 IHC positive and mutation analysis of EGFR revealed an exon 19 mutation. The patient received Afatinib as first-line treatment with disease stabilization.

Eight months later, disease progression occurred while increasing sieric neuronal specific enolase (NSE) so a liquid biopsy, looking for T790M mutation, it was carried out and it was negative on two repeated samples. At this time, a rebiopsy under bronchoscopy performed at the same primary disease site demonstrated a SCLC transformation, p63 and TTF1 IHC negative, synaptophysin and chromogranin IHC positive. The biomolecular re-assessment didn’t show the primary Exon 19 EGFR mutation neither the T790M mutation. Surprisingly, a different EGFR activating mutation (Exon 21, p.L858R) concurrently with a Exon 18 EGFR mutation were identified by RT-PCR and NGS. Furthermore, the tumor cells showed strong expression of anaplastic lymphoma kinase (ALK) protein at IHC (clone D5F3, CE IVD Ventana). ALK rearrangement was then also confirmed by FISH analysis. (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular). It detected ALK rearrangement in 26% of the analyzed tumor cell nuclei (100 tumor tumor cell nuclei analyzed with a cut-off of 15%) with split ALK signal (Exon 21, p.L858R) and polysomy remains an interesting challenge that needs to be fully understood and interpreted.

Thus, a chemotherapy with Cisplatin plus Etoposide was started, on the basis of the changed histology but, after two cycles, the patient experienced disease progression with pleural effusion and clinical worsening. Interestingly, analysis of pleural effusions showed a TTF1 positive, synaptophysin and chromogranin IHC negative poorly differentiated adenocarcinoma. Determination of ALK protein by IHC was negative and ALK rearrangement wasn’t performed. Determination of assessment of EGFR mutations showed identical Exon 21 EGFR mutation detected in the rebiopsy.

**Discussion.** The mechanisms underlying these multiple biomolecular and pathological findings remain to be proven, but it is speculate that intratumour heterogeneity describes this phenomenon that tumour lesions within one individual may harbour different morphological and genetic characteristics. It is highly likely that intratumour heterogeneity plays a major role in the development of acquired resistance to targeted therapies. For transformation to SCLC during EGFR-TKI treatment, it has been hypothesised that a small population of neoplastic cells with a SCLC phenotype is already present at start of EGFR-TKI treatment. During this treatment, the sensitive NSCLC tumour cells are efficiently eliminated, providing the small clone of resistant SCLC tumour cells the opportunity to proliferate. Here, we observed that the initial EGFR mutation wasn’t detectable in the SCLC tumour cells. This 'selection-hypothesis' may also apply to this case of transformation to a combined SCLC-adenocarcinoma as the different EGFR mutations was detected in the original biopsy as well as in the rebiopsy which harboring different EGFR mutations and concomitant ALK rearrangement. Multiple molecular mechanisms are emerging as causes of acquired...
resistance to treatment with TKI, posing new challenges for targeted therapy of NSCLC. Together with previous reports, our case clearly underlines the need for re-biopsy in patients who develop acquired resistance to TKI such as afatinib. Moreover, our case indicates that the occurrence of multiple driver mutations in TKI resistant tumor cells appear to be a problem strictly related to one another and capable of undermining effective treatment. When separate or multiple drivers occur drug combinations or broader based treatments such as cytotoxic chemotherapies may be required. Fully understanding the basis and frequency of the different mechanisms of resistance to TKI that are emerging will help us to continue to exploit personalized medicine approaches.

References

TEMI DI INTERESSE GENERALE

USE OF FMEA ANALYSIS IN ANATOMIC PATHOLOGY TO REDUCE RISK OF ERRORS IN EXTRA-VAGINAL CYTOLOGY

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Figure 1.

Priority matrix

R 14 Wrong patient identification (name, surname, date of birth)
R 15 Wrong specimen identification (anatomic site)
R 16 Wrong specimen identification (laterality)
R 17 Wrong specimen identification (type of specimen)
R 65 Wrong labelling of slide
R 87 Diagnostic discrepancy (major and minor)

Background. The aim of clinical risk management is to improve the quality of care provided by health care organizations and to assure patients safety. Failure mode and effect analysis (FMEA) is a tool employed for clinical risk reduction (1-2-3-4). We applied FMEA to reduce risk of errors in extra-vaginal cytology.

Method. FMEA steps: (I) process study, we recorded phases and activities; (II) hazard analysis, we listed activity-related failure modes and their effects, described control measures, assigned severity, occurrence and detection scores for each failure mode and calculated the risk priority numbers (RPNs) by multiplying the 3 scores. Total RPNs is calculated by adding single failure mode RPN; (III) planning, we performed a RPNs prioritization on a priority matrix taking into account the 3 scores, and we analyzed failure modes causes, made recommendations a planned new control measures; (IV) monitoring after failure mode elimination or reduction, we compared the resulting RPN with the previous one.

Results. Our failure modes with the highest RPN came from specimen identification, slides labelling and diagnostic misinterpretation in classifying extravaginal cytological cases as inconclusive/uncertain. Then we identified three new activities of improvement plans for standardize accessioning and labelling procedure and diagnostic criteria through sharing and discussing cases at microscope recording the discussion in a specific register. After six months total RPN value decreased from 1700 to 1612 (5%) while RPN of the three specific risks decreased from 304 to 210 (29%).

Conclusions. This report is one the few published so far on the of FMEA in pathology, although the method has been recommended by the Joint Commission on Accreditation of healthcare organisation. Employing FMEA, we work on a few critical activities, and we reduced patients clinical risk
References

PATOLOGIA DELL’OSSO E DEI TESSUTI MOLLI

PRIMARY THYROID BIPHASIC SYNOVIAL SARCOMA: REPORT OF A CHALLENGING CASE

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Introduction. Synovial Sarcoma (SS) is the fourth most common soft tissue sarcoma accounting for 5-10% of these neoplasms and is characterized by translocation t(X; 18) (p11.2;q11.2) between the genes SYT and SSX1/SSX2/SSX4. It usually arises in children and young adults in the extremities, especially around large joints. The diagnosis of SS can be quite straightforward especially in prototypical cases arising at usual locations. However at unusual locations or at atypical age groups achieving a diagnosis of SS may be challenging because of wide spectrum of differential diagnoses. In this setting primary thyroid SS is extremely rare with only a few cases reported in the English Literature even if the head and neck region represents one of most common site of SS origin. However immunohistochemical and molecular analysis for t(X; 18) can be performed and therefore can help supporting the morphological hypothesis. This report describes a case of primary thyroid SS in an adult woman. We would like to discuss potential diagnostic pitfalls due to the overlapping of features between SS and other primary and secondary thyroid malignancies.

Materials and methods. The Fine Needle Aspiration Cytology (FNAC) was performed using a 22 Gauge needle. Direct smears were stained with May-Grunwald-Giemsa and with Papanicolaou stain and a cell block was obtained. The surgical specimens were fixed in 10% buffered formaldehyde and then routinely processed. Sections were stained with H&E. Immunohistochemical studies were performed using the following panel of antisera: cytokeratin 7, Bcl2, calponin, EMA, CD99, CD56, vimentin, Ki67, WT1, NSE, synaptophysin, calretinin, D2-40, CD5, p53, osteoprotein, CD10, p63, PGR, ER, cytocheratin 20, α-inhibin, LCA/CD45, Ca125, SMA, CD117, thyroglobulin (Ventana), TLE1 (Santacruz) and PAX8 (Proteintech). Fluorescence in situ hybridization (FISH) and REAL-TIME PCR (RT-PCR) analyses were performed on formalin-fixed and paraffin-embedded tissues (FFPE) from the surgical specimen of the thyroid neoplasm. A commercially available break-apart dual-color specific probe at 18q11.2 (Vysis-Abbott) for SS18 (SYT) gene rearrangement was applied according to manufacturer’s instructions. Dual Color FISH images were digitally generated using a computer-based imaging system (Cytovision). As for RT-PCR, Total RNA was isolated from FFPE samples using the TRIzol reagents. Two µg of total RNA was reverse-transcribed into cDNA using oligo(dT) primers and reverse transcriptase (Superscript), according to the manufacturer’s recommendations. The integrity of cDNA was tested by the amplification of the ubiquitous gene β-actine. The detection of the putative SYT-SSX1 and SYT-SSX2 fusion gene was carried out with the primers SYT 1100 5′AGGATATAGACCACACAGGC3′, SSX1 5′GGT GTT GTT TCC CAT CG3′ and SSX2 5′GGC ACA GCT TTT CCC CAT CA3′. PCR conditions were the following: 40 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and elongation at 72°C for 30 sec. Each reaction included cDNA from normal mesenchymal tissue as negative control. The amplification products of all the PCR reactions were analysed on 2% agarose gel. All the sequence reactions were carried out using an automated sequencing system (3500 DX Genetic Analyzer, Applied Biosystem) following standard protocols.

Case report. A 42-ys-old female patient was referred to our Hospital for a left cervical swelling. She performed an ultrasonography evaluation (US) of the thyroid revealing, in the left lobe, an isoechoic nodule. All laboratory tests, including thyroid functional markers, were within usual ranges. The patient underwent an US-guided FNAC and a cytological diagnosis of neoplastic lesion, indefinite whether primary or metastatic, was formulated. The patient underwent a total body Computed Tomography (CT) scan, revealing no other neoplastic condition. A complete thyroidectomy was performed. During the surgical procedure the capsule of the left nodule was ruptured and grayish tissue fragments were discharged and collected for histological examination. On histological evaluation, a dual cellular population was appreciated: several glandular structures composed by columnar elements with clear cytoplasm were embedded in a highly cellular stroma. Stromal cells were spindle-shaped, with scant cytoplasm and ovoid nuclei. Vessels had thin walls and a hemangiopericytoma-like appearance. Areas of necrosis and a high mitotic count were also noted. The tumor was encapsulated and separated from thyroid parenchyma by a thick fibrous capsule. Immunohistochemical analysis showed a complex pattern: CD99, and EMA were expressed by spindle and epithelial cells; Bcl2, calponin, vimentin, CD56 were positive on the spindle component while the epithelial one was intensely reactive for cytokeratin 7.

On that ground, a biphasic SS was suspected and therefore additional immunohistochemical and molecular analyses were performed on consultation at the national referral center. The results confirmed the histological diagnosis since positivity for TLE1 and negativity for PAX8 were detected as well as the presence of both rearrangement of the gene SYT (18q11.2) at FISH break apart analysis and the fusion transcript SYT-SSX1 at RT-PCR analysis. Integration of clinical-radiological, histological, immunohistochemical and molecular data led us to a final diagnosis of biphasic SS, grade 3 according to FNCLCC grading system, primitive of the thyroid gland. The patient is alive and free of disease and has actually no other localization since six months.

Conclusions. SS accounts for 5-10% of all soft tissue sarcomas. It usually arises in children and young adults in the extremities, especially around large joints. These latter proto-
typical clinical aspects were missing in our case thus aggravating the patient’s diagnostic evaluation. Histologically two categories of SS can be identified: biphasic and monophasic, based on the presence or absence of both epithelial and spindle cell components. Moreover areas of poor differentiation can be encountered. Cases of SS localization in the thyroid, both as a primary malignancy and as a metastasis from an already documented SS, are very rare with only a few described events. In our case, the patient complained only a cervical swelling due to a thyroid nodule without signs of thyroid dysfunction.

In biphasic SS, as the one we are describing, a dual cellular population can be appreciated with both spindle cells and epithelial elements. Spindle component is characterized by small to medium cells with scant cytoplasm, ovoid nuclei with finely granular chromatin and unapparent nucleoli. The epithelial elements are usually arranged in acinar or glandular structures composed by larger cells with abundant, clear cytoplasm. Other findings we underlined in our case and which are considered usual features of SS are the presence of high mitotic count and of small, thin-walled, branching vessel with a hemangiopericytoma-like appearance. The hypothesis of SS should be confirmed throughout ancillary techniques like immunohistochemistry and molecular biology analyses: in our case the translocation t(X;18) (p11.2;q11.2) was demonstrated on histological material.

As for differential diagnoses, we had at first to rule out primary thyroid epithelial malignancies, like medullary carcinoma and undifferentiated (anaplastic) carcinoma (UTC). The first entity can be very challenging to distinguish because of its higher incidence and of its morphological variety, comprising a spindle cell component. UTC can be characterized by spindle elements but, unlike our case, they display marked nuclear pleomorphism. In our case the expression of cytokeratin by the epithelial cells only, the monomorphous morphology and the absence of marked pleomorphism of the spindle cell component helped us to exclude these entities.

Other neoplasms that should be considered in differential diagnosis are Spindle Epithelioid Tumor with Thymus Like Differentiation (SETTLE) and ectopic thymomas. Like SS, SETTLE is characterized by a dual cellular population (spindle and epithelial components) and with cohesive sheets of spindle cells. However it lacks the high mitotic count, which is typical of SS, and both cellular populations express cytokeratin and vimentin. As for thymomas, the main challenge is to distinguish type A thymoma which usually display spindle epithelial cells with ovoid nuclei and with finely granular chromatin. Unlike SS, mitoses are infrequent. Type B thymomas can be easily ruled out since they are characterized by abundant lymphoid component and by scattered polygonal, neoplastic, epithelial cells. At last, other mesenchymal malignancies, both primary and metastatic, should be taken into consideration, especially sarcomas capable of epithelioid differentiation (i.e. clear cell sarcoma and epithelioid sarcoma, MPNST) and those with hemangiopericytoma-like vessels as extrapleural solitary fibrous tumor.

The present case displays two other additional important features: the first one we would like to mention is that the tumor, although intrathyroidal in location, did not infiltrate the thyroid parenchyma but was separated from it by a thick fibrous capsule. We might speculate that primary thyroid SS may originate from pluripotential mesenchymal cell of the thyroid capsule or of thyroid stromal tissue. The second finding we would like to focus on is the fact that the tumor capsule was ruptured during thyroidectomy: this event has been already described and seems to be related to an increased risk for local and metastatic relapses.

We describe an additional, rare and unusual case of primary thyroid biphasic SS developed in an adult woman and finally we would like to stress the importance of ancillary techniques, especially molecular biology, to confirm the morphological suspicions in deceiving cases.

UROPATHOLOGIA

PATIENTS AFFECTED BY ADVANCED BLADDER CARCINOMA WITH STRONG MDM2 GENE AMPLIFICATION

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Introduction. Despite carcinoma of the bladder is one of the most frequent neoplasm in the adults, the therapeutic management of this tumor is challenging. Based on the recent interests on MDM2 inhibitors as a new cancer therapeutic strategy1 and the reported amplification of chromosomal region 12q13-q15 in bladder cancer2, we investigated the occurrence of MDM2 gene alteration in a series of advanced carcinoma of the bladder.

Methods. We analyzed 40 primary carcinomas of the bladder (24M, 16F; median 68 years). Three tissue microarrays (TMA) were built with 3 cores for each tumor and 3 cores for respective normal tissue, and one TMA was built with lymph-node metastasis from 13 cases. Fluorescence in situ hybridization was performed using MDM2 locus specific probe and centromeric alpha-satellite (CEP) specific for chromosome 12 as control probe. Gene copy number was scored for both probes. Amplification was defined as presence of MDM2 gene locus signals >6 and ratio MDM2/CEP12 >2 in at least 10% of neoplastic nuclei. Polisomy was considered as presence of either MDM2 gene locus signals or CEP12 signals ≥3.

Results. Amplification for MDM2 was found in 6 tumors (15%) (granular pattern, at least >20 fluorescent spots), polisomy was observed in 4 tumors (average of MDM2 signals 4). No lymph-node metastasis showed MDM2 gene amplification, including one case with MDM2 amplification in the primary bladder cancer. Among tumors having amplification, patients (four male and two female; median 73 years) were older than those without amplification, although the difference was not statistically significant (p=0.05).

Conclusion. These data suggest a role of MDM2 as a 12q13-q15 amplification target only in a subset (15%) of patients with advanced bladder carcinoma, thus clinical trials using MDM2 inhibitors in patients affected by advanced bladder carcinoma with MDM2 amplification may be accelerated in this subset.

References
INTRODUCTION. Penile metastasis is an extremely rare entity. Only few cases have been reported in literature. The primary tumor most commonly arises from the genitourinary tract, especially bladder and prostate, with the bladder being the most frequent primary location. The most common symptoms are penile swelling, induration, ulceration, and sometimes also associated priapism. Secondary penile tumors are usually associated with disseminated disease and indicate a poor prognosis. We describe a case of penile metastasis from urinary bladder urothelial carcinoma.

METHODS. A 67-year-old man with a history of radical cystoprostatectomy for high grade urothelial papillary bladder carcinoma in May 2010 (pT4a, pN2, pMx). He had received adjuvant radio and chemotherapy post-operatively. In June 2016 at clinical and instrumental examination showed painful priapism with hard nodules along the penile urethra. The patient underwent total penectomy based on the clinical diagnosis of penile metastasis from urinary bladder carcinoma.

RESULTS. A histopathological evaluation of the resected specimen revealed an infiltration by poor differentiated carcinoma with a nests of atypical cells permeating the glans penis, vascular spaces of the corpus spongiosum and corpus cavernosum consistent with his primary urinary bladder urothelial carcinoma. Immunohistochemistry of tumor cells demonstrated positive staining for CK7, p63 and CK34B12 (useful marker of urothelial carcinoma) and negative staining for CK5/6.

CONCLUSION. Tumors metastasizing to the penis are rare. This event is believed to be due to abundant vascularity of the penis and its proximity to pelvic organs and the metastasis mechanism is thought to be secondary to venous retrograde flow because of the intense pelvic, lumbar and penile vein communication, lymphatic and arterial dissemination, as well as contiguity. 35 percent of all metastasis to the penis originates to urinary bladder carcinoma. Penile metastasis tends to show a poor prognosis because the metastasis to the penis, in most cases, tends to be part of widely disseminated disease. The majority of patients die within one year.

REFERENCES

INTRODUCTION. Mixed epithelial stromal tumours (MEST) of the male genital organs have been named, in the previous literature, with several different terms including epithelial stromal tumors, cystic epithelial stromal tumors, phyllodes tumors, cystomioma, mesenchymoma, muellerian adenocarcinoma-like tumor and cystoadenomas. This circumstance has led to a varied and confusing nomenclature to describe the spectrum of epithelial and stromal tumors of seminal vesicles and vas deferens.

Male pelvic floor cystic lesions are rare clinical entity usually benign that, from an anatomical standpoint, are divided into intraprostatic (medial, paramedial and lateral) and extraprostatic (seminal vesicles, vas deferens and Cowper duct). From an etiopathogenetic perspective, they include: Wolffian duct remnants, Mullerian duct remnants, cysts of the seminal vesicles, prostate and ejaculatory duct-vas deferens cysts. Cystoadenoma of the lower male genitourinary tract are challenging diagnostic abnormalities because they are uncommon. Their origin is uncertain and they are often difficult to diagnose, therefore only a few reports have been published. Vas deferens cystoadenomas are located along the course of the vas deferens, superior to the prostate. Accurate diagnosis depends mainly on the anatomic location of the cyst. Transrectal ultrasonography (US) is excellent to detect and characterize the nature and the exact anatomic origin of these cysts. Magnetic resonance imaging (MRI) is the most useful tool to determine the spatial relationship between a cystic lesion and the vas deferens. On the bases of the neoplasm classification, the recent literature categorizes cystoadenoma as a MEST. Herein, we present a case of a cystoadenoma of the vas deferens as an incidental finding, in a patient who underwent a radical prostatectomy for a prostatic adenocarcinoma in order to evaluate clinicopathological features of this malignant tumor. We would also like to provide a brief review of the relevant published literature, considering the possible histogenesis of cystoadenoma of vas deferens. Moreover an essential differential diagnosis will be reported.

MATERIALS AND METHODS. The specimen consisting of radical prostatectomy, described according to standard guidelines, was fixed in 10% formalin solution for 24 hours and then was totally sampled. Samples were routinely processed and paraffin embedded. Blocks were sectioned at 3 mm and slides were stained using standard Hematoxylin–Eosin. A panel of immunohistochemical stains was performed including CK7, PSAP, and SMA to better define the features of both epithelial and stromal cellular components of the cystic lesion.

RESULTS. A 56-year-old male was referred to the Urology Unit of our Hospital due to a recent diagnosis of prostatic adenocarcinoma, performed at another center on biopsy specimen. Our patient did not complain any specific symptom like hematuria, hematospermia or painful and ejaculation disorder. Radiological exams and imaging studies of the pelvic region were negative for lesion or malformations related to ejaculatory ducts. Moreover he was not affected by Zinner Syndrome. A radical prostatectomy was carried out in order to evaluate the pathological features of prostatic adenocarcinoma, and
the surgical specimen was sent to the Pathology Unit for histological diagnosis. Gross pathological examination revealed a prostate of normal volume with rubbery consistency. On the left vas deferens, a cystic, well-circumscribed, oval mass, measuring 1.3 cm in greatest diameter was detected. The lesion was clearly separated from the prostate and was characterized by a smooth external surface while, inside, it was filled with serous fluid.

Histopathological prostatic sections showed the presence of prostatic adenocarcinoma, involving only the left lobe of the prostate without extraprostatic dissemination and with negativity of pelvic lymph nodes for metastatic disease. Moreover, microscopically sections from the cystic lesion of the left vas deferens demonstrated dilated cysts, containing homogeneous eosinophilic material with histocytes and without spermatozoa. A fibromuscular wall, lined by a single layer of epithelial cells with uniform dark, centrally located nuclei and distinct cell membranes was also observed. A patchy mild chronic inflammatory infiltrate was present in the wall. Mitoses, atypia and tumour necrosis were not present in both epithelial and stromal component. On immunohistochemistry, the basal cell layer of the cysts demonstrated uniform staining for CK7 and negativity for PSAP, that excluded a prostatic origin. The scattered stromal cells stained for SMA. These microscopic findings were consistent with a histological diagnosis of cystadenoma of the vas deferens. The patient’s postoperative recovery was satisfactory and he is healthy.

Conclusions. MEST including cystoadenoma of vas deferens and seminal vesicles are unexpected diseases, with an incidence lower than 0.005% and with a complex clinico-therapeutic management. Their origin is uncertain with a spectrum of different theories comprising genital infections, obstruction of ejaculatory ducts and congenital abnormalities. They are often difficult to diagnose and treatment is still controversial, being laparoscopic resection of the cystic lesions the main therapeutic best options in this pathological setting. Various types of cystic lesions can affect the lower male genitourinary tract and may lead to serious complications. Despite their rarity, it is essential to be familiar with these entities in terms of associations with other syndromes and with pathological classification. In consideration of this latter problem, the recent literature categorizes cystadenoma as a MEST. It should demonstrate a biphasic composition and the presence of neoplastic epithelial cells and stromal elements without atypia, although in some cases, classified in the pubmed literature as cystadenoma, the cystic component predominates. In this setting single to few layers of bland cuboidal to low columnar epithelial cells line cystic walls and are intermingled with variable amounts of stroma that resembles usual seminal vesicle fibromuscular stroma. The term cystadenoma should be therefore restricted only to those rare benign tumors that demonstrate hypocellular stroma without atypia as in our case. Important considerations in the differential diagnosis include simple cystic enlargement, muellerian duct cysts, diverticula of the ejaculatory ducts or ampulla of the vas, prostatic cysts and malignant tumor of the seminal vesicles.

In particular, cystic lesions of the vas deferens are very uncommon and unexpected, causing still diagnostic problems regarding their precise anatomical identification. The spatial relationship between a cystic lesion and the vas deferens is most likely to be determined by MRI. In fact, physical examination may be negative and only transrectal ultrasonography, CT scan and MRI may reveal them. Moreover, it must be kept in mind that these lesions are usually asymptomatic and only occasionally come to medical attention due to intermittent haematospermia, perineal pain, emptying phase symptoms.

At the best of our knowledge, a review of the recent literature highlights about 20 reports of seminal vesicle cystoadenomas and only one similar case occurring in the vas deferens. Our case is noteworthy to mention because cystoadenoma of the vas deferens is especially exceptional and can further add to a gradually growing body of literature on MEST of the vas deferens. Because of the high association between MEST and developmental abnormalities, this finding should ring a bell and prompt further clinical investigation in order to rule out congenital disease like Zinner Syndrome. Lastly, it is important for the pathologist to identify the nature of this lesion, in order to recognize and better classify this unusual pathological entity in the scenario of a correct nomenclature, following the recent guide lines of last WHO classification of the male genital organs. Moreover, this appropriate approach could help to avoid unnecessary overtreatment like radical surgery, and also to preserve fertility and erectile function.

References

WHO classification of tumours of the urinary system and male genital organs. Lyon, France 2016.


ZINNER SYNDROME: A RARE WOLFFIAN MALFORMATION. SURGICAL, DIAGNOSTIC AND EMBRYOLOGICAL CHALLENGES

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Goal. To Report on a rare, congenital, urologic malformation. Zinner syndrome is a rare male Wolffian congenital malformation of the genitourinary tract which includes seminal vesicle cyst, ejaculatory duct obstruction and ipsilateral renal agenesis or dysplasia. It may be totally asymptomatic, make its first appearance acutely, or be related to reproductive dysfunctions.

Case report. A 37 year-old male who referred to San Martino’s Hospital Urology Department after abdominal ultrasound performed for weight loss. First level diagnostic imaging exams showed a pelvic cystic mass between bladder and rectum. Left kidney was absent. Further workup with magnetic resonance imaging and endoscopy demonstrated the cyst to be of urogenital origin. The big size of the lesion prompted surgery. At pathological gross examination the resected specimen showed a nodular formation measuring
7x2x1cm, originally identified as abnormal kidney remnant, and a tract of ureter 16 cm long with a blind-ending branch in continuity with ball-like cyst, measuring 8,6 cm of maximum diameter and containing proteinaceous fluid. From the cystic lesion departed a second blind-ending tubular structure 5 cm long, considered as being the deferential duct as a first step. To elucidate the nature of the resected tissues and their embryological origins, the surgical specimen was extensively sampled for further histological investigations. Haematoxylin and eosin-stained sections of representative specimen blocks from each area described before were examined to assess morphological diagnosis. The study was completed with immunohistochemistry (IHC) essays, with specific monoclonal antibodies for cytokeratins 7 (CK 7), 20 (CK 20) and 34 (CK-34), Caldesmon, Paired box 8 (PAX8), prostate specific antigen (PSA) and prostate specific acid phosphatase (PSAP) in order to furtherly characterize each site’s features. Pathological examination of the surgical specimen demonstrate the mass to be a left seminal vesicle cyst secondary to a malformation of the ejaculatory duct and embryological related to ipsilateral renal agenesis. Such findings were consistent with the diagnosis of Zinner syndrome. 

Discussion. Maldevelopment of the distal part of mesonephric duct leads to atresia of the ejaculatory duct and abnormal ureteral budding, which may display as renal agenesis or dysplasia. The obstruction at the level of ejaculatory duct leads to gradual accumulation of secretions in ipsilateral seminal vesicle with consequent cyst formation. The syndrome has also wide phenotypic variability depending on the time of insult during the embryogenesis. If the noxa occurs prior to week 7, the entire mesonephric duct and its derivatives will be adversely affected, usually leading to renal agenesis, as in our patient.

This is a peculiar case of completely silent anamnesis and accidental finding of the syndrome; to our knowledge this is one of the biggest cyst successfully excised trough laparoscopic approach. A thorough anatomic pathology examination, both gross and microscopic, is necessary to identify and confirm the syndrome triad consisting on seminal vesicle cyst, ejaculatory duct obstruction and ipsilateral renal agenesis. A panel of few immunostains was useful and sufficient to correctly identify the observed structures.

MALIGNANT SOLITARY FIBROUS TUMOR OF THE PROSTATE: A CASE REPORT WITH LITERATURE REVIEW

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Introduction. Solitary fibrous tumor is uncommon soft tissue neoplasm with intermediate biological behavior, rarely metastasizing. Solitary fibrous tumor is characterized by a broad morphological spectrum and are currently divided into three main variants that have the NAB2-STAT6 gene fusion as their unifying molecular lesion: usual, malignant and dedifferentiated forms. The most common localization is pleural, whereas only 0,6% are of extrapleural localization.

Discussion. The differential diagnosis of spindle cells tumors arising in the prostate is broad and includes lesions of epithelial and mesenchymal origin. Differential diagnosis includes primary prostatic lesions like STUMP and stromal sarcoma and anatomically ubiquitous soft tissue neoplasms. Histopathological examination of the prostatectomy specimen showed patternless architecture with hypocellular and hypercellular areas and haemangioepicytoma-like vessels. In some fields the neoplasm was characterized by mitotic figures and cellular atypia. Immunohistochemically there were positivity to CD34, bcl2, CD99 and STAT6, partial positivity to PgR and negativity to S100, SMA, CD10, CD117, desmin, EMA, ER, B-catenin and keratins. The neoplasm was diagnosed as a malignant solitary fibrous tumor. There were no recurrences with a follow-up of 8 years after surgery.

Conclusion. Solitary fibrous tumors should be considered in cases of prostatic tumors with a spindled morphology. Malignant solitary fibrous tumor is extremely rare in the prostate. A review of literature showed only two other cases. Considering the unpredictable biological behavior and the possibility of recurrence, a long-term clinical and instrumental follow-up is recommended.

References

UNCLASSIFIED SEX CORD/GONADAL STROMAL TESTIS TUMOUR WITH PREDOMINANCE OF SPINDLE CELLS. A CASE REPORT

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Introduction. If germ cell and hematopoietic tumours are excluded, most primary testicular neoplasms are designated as sex cord/gonadal stromal tumours (SCSTs). They account for approximately 5% of all primary testis tumours. Occasional SCSTs cannot be readily categorized into the prevalent granulosa cell and Sertoli cell families and are diagnosed as mixed or unclassified SCSTs. When these neoplasms are identical to their ovarian counterparts, the classification is relatively straightforward. There remains, however, a small subset of spindle cell tumours for which there is disagreement with regard to their exact taxonomy.

Methods. The patient was a 83-year-old man who was referred to Hospital of Novi Ligure for a right and left scrotal enlargement and a palpable, painless firm mass in the left testis. Serum alpha-fetoprotein and beta human chorionic gonadotropin in levels were normal. A magnetic resonance imaging confirmed the presence of a mass with near-complete
replacement of the left testis. The patient underwent total orchiectomy.

**Results.** The left testis measured 9 cm in its largest diameter. It contained a 7x7x5.5 cm gray-yellowish rubbery, solid and cystic, firm mass that replaced nearly all the testis. Microscopic examination of hematoxylin-eosin stained sections showed a highly cellular lesion arranged in vague fascicles with occasional storiform structures and intermixed oedematous areas and hyalinized collagen bundles. The neoplastic spindle cells had eosinophilic cytoplasm, plump spindly hyperchromatic nuclei with small nucleoli. The mitotic count was low (1 per 10 HPF). Areas of tumor necrosis were never seen. The neoplastic cells were negative for epithelial markers (EMA, Cam 5.2 and AE1/AE3 cytokeratins), desmin, CD34, calretinin, CD99, MART1 and PLAP, but showed a diffuse positive reaction with S-100 protein and vimentin and focal expression of inhibin and alpha-smooth muscle actin. Ki-67 nuclear expression in the tumor cells was low (<5%). Based on these features, this tumor was designated as unclassified SCST with predominance of spindle cells.

**Conclusions.** Although unclassified SCST with predominance of spindle cells has unique localization and characteristic histological features, underrecognition and rarity of this entity would pose a diagnostic challenge. Differential diagnosis includes a wide variety of other tumours and tumour-like conditions, such as Sertoli/Leydig cell tumours, smooth muscle tumours, solitary fibrous tumours, follicular dendritic cell sarcomas, spindle cell carcinomas, neurotumours and rete testis tumours. Immunohistochemistry might represent a very useful tool in the differential diagnosis (1). Experience with this extremely rare neoplasm is very limited, and thus, the histological features with prognostic significance as well as optimal management and treatment of these patients have not yet been defined.

**References**

**PELVIC PHLEBOLITHS AND THE RISK FOR UNNECESSARY, WIDER RESECTIONS IN RADICAL PROSTATECTOMY SPECIMENS**

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**Introduction.** Phleboliths are areas of calcification within a vein, representing the end product of thrombosis. They are frequently found within the pelvis, in the veins around bladder, prostate, uterus, and rectum (1). Sometimes it may be difficult to distinguish them from distal ureteral stones on radiographs (2), but pelvic phleboliths are generally considered of no clinical significance (1). For this reason they are actually neglected in radiology and histopathology reporting and, to the best of our knowledge, the presence and significance of phleboliths in radical prostatectomy specimens has never been described in literature.

After a preliminary report dealing with the occasional observation of periprostatic phleboliths in radical prostatectomy specimens submitted to histopathological examination (3), we would like to update the case series. The aim of this study is to determine the main features of periprostatic phleboliths and evaluate their role as a mimic of extraprostatic extension and a potential pitfall for unnecessary, wider resections in prostate cancer.

**Methods.** After routine histopathological examination of radical prostatectomy specimens, the incidental presence of phleboliths was noted in a total of 7 cases, in a time span of 2 years. According to international protocols, the prostate had been serially sectioned and entirely submitted to histology, with at least a section of each sample stained with hematoxylin-eosin and microscopically observed (4). The age of the patients, tumor extension (pT), site of extraprostatic extension, margin status, site of positive margins, and location of phleboliths were recorded. An abdominopelvic computed tomography scanning was available for one patient only; no mention of phleboliths appeared in the report, but retrospective evaluation of the scans allowed to identify multiple bilateral periprostatic calcifications.

**Results.** The age range of the patients was 55-75 years (mean: 64.3; median: 65). Three cases were in pT2c category, three were classified as pT3a, and the last one was in the pT3b category. Two out of seven patients had positive margins (R1), both in the left prostatic apex. Phleboliths, with the classic concentric calcification pattern (figures 1-2), were observed in the periapical region in 1 case, near the prostatic base in 3 cases, outside the left lateral aspects of the gland in 3 cases, and outside the right lateral aspects in 4 cases. In all cases, the phleboliths were surrounded by abundant normal periprostatic tissue and located far from intra- or extraprostatic neoplastic tissue. In each prostate specimen, different stages of periprostatic venous thrombosis were also evident. The main features of patients and specimens are summarized in table 1.

**Conclusions.** Such reports suggest that phleboliths represent a common finding in periprostatic tissues as they may be frequently identified during histopathological examination of radical prostatectomy specimens. The most frequent locations of the phleboliths are outside the posterolateral regions of the prostate, where the neurovascular bundles adhere to the gland. Tactile evaluation is often used during nerve-sparing surgery, relying on cancer tissue firmness identified by palpation (5). Phleboliths may simulate extraprostatic extension of prostate carcinoma and therefore be responsible of unnecessary wider resections to achieve negative margins. Pre-operative recognition of phleboliths could be of help in distinguishing them from extraprostatic cancer and limit the entity of the resection to guarantee safe margins. Further investigation on wider series is needed to understand the real entity of the problem. A retrospective evaluation of prostatectomy specimens is planned, in order to document the frequency of this finding and its role in surgical resection policy.

**References**
THE ACTIVATION OF GPR30 INDUCES APOPTOTIC PATHWAYS IN HUMAN HUMAN CARCINOMA IN SITU AND SEMINOMAS

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Introduction. Estrogens are steroid hormones that play important role in testis development and reproductive functions. They act on cell and tissues not only by binding two classical cognate receptors ERα and ERβ, but also through a member of a seven transmembrane receptor family named GPR30 responsible for the mediation of non classical genomic effects. ERβ subtype is the principal mediator of estrogen action in promoting germ cell survival and development. In our recent work (Chieffi et al 2015) we have demonstrated that GPR30 over-expression is frequently associated to ERβ downregulation in human testicular Seminoma and Carcinoma in situ.

Aim and methods. In the present work we have evaluated immunohistochemical expression of ERβ, GPR30 and Phospho-ERK followed by Tunel assay for apoptosis detection in a Tissue Micro Array (TMA) including 63 Seminomas and 35 Carcinoma in Situ (ITGCNU) in order to evaluate a possible role of GPR30 in proliferation and survival in testicular carcinogenesis.

Results and conclusion. Frequently a significant high cytoplasmic and membranous GPR30 expression was found in Seminomas and ITGCNU (p<0,005). All positive sample showed a contemporary absent ERβ immunostaining in seminomas or weak expression in ITGCNU in according to our previous data. Furthermore ph-ERK over-expression and apoptotic index was directly and inversely correlated with higher GPR30 immuno- staining respectively (p<0,05 and p<0,04). Taken toghether these results confirm a strictly correlation between estrogen signalling pathways and GPR30 and have emphasized a central role of the latter in cell survival and proliferation.In conclusion GPR30 could represent a potential molecular marker for new target therapies in this subset of testicular tumor.

References

Table I - Main features of patients and prostatectomy specimens.
G. Russo, S. Trabucco, T. Lettini, M.A. Cascarano, V.F.V. Carnevale, M. Mastromauro, L. Resta
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Introduction. Germ cell tumor is the most common malignancy in 15–35 year old man. About five percent of the malignant germ cell tumors are extragonadal in origin. We present a case of embryonal carcinoma of the right cryptorchid testicle histologically regressed after a cycle of chemotherapy.

Case report. A 31 year old man undergoes to emergency room with a distention of the abdomen with pain since few days. Furthermore the patient refers weight loss since 6 months.

The chest CT shows pulmonary nodule of 4 mm in the left lower lobe. A neoplastic mass is present in the left anterior mediastinum extending in the supraclavicular and laterocele- rical regions with a maximum of 10x5 cm in diameter. In abdomen an expansive retroperitoneal lesion of 26x25 cm to polycyclic contours is described.

A biopsy of the supraclavicular lymph node is performed. Histological diagnosis is compatible with a metastatic embryonal carcinoma probably of testicular origin.

Ultrasound examination shows the left testis within the scrotal sac in the high position, with inhomogeneous echogenicity. The right testis is not detectable into the scrotal sac. In the right inguinal canal a solid formation of 20x8 cm is present. The patient receives 4 cycles of chemotherapy with cisplatin, etopside, and bleomycin and he is submitted to surgical removal of the abdominal tumor. The neoplastic mass weights 1,250 g and present a homogeneous grey cut surface. The removed retroperitoneal lymph nodes are enlarged.

The histological examination excludes a residual tumor. The mass comprehends a residual atrophic didymus, a large amount of lytic necrosis circumscribed by a xantocytic granuloma. The lymph nodes are largely necrotic and fibrotic. Immunohistochemistry results negative for CK pool, EMA, PLAP, CD 45(LC), S100 protein and CD 34. CD 68 is positive in reactive histiocytes.

The diagnosis is: “post-chemotherapy regressed cell germ tumor”.

Comment. The effectiveness of chemotherapy in testicular carcinomas is today very frequent also in patients with a large tumor. In our case the tumor is documented only with pre-therapeutic biopsy of node metastasis. The cryptorchidism is a condition which favors the occurrence of a hidden giant tumor.
CLEAR CELL CYSTADENOMA OF THE TESTIS: IS THERE A NEED FOR FURTHER INVESTIGATION IN UNILATERAL CASES?

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Introduction. Neoplasms of the epididymis are rare. Benign epididymal neoplasms include adenomatoid tumor, leio-myoma, serous (non-papillary) cystadenoma, cavernous hemangioma, and melanotic neuroectodermal tumor. Papillary cystadenoma is the second commonest benign neoplasm of the epididymis following adenomatoid tumor. It is an uncommon epithelial tumour which is thought to develop within the efferent ductules of the head of the organ; the clear cell variant is even rarer. To date, only 59 cases have been reported in the literature since the original report by Sherrick in 1956. However, only 20 of them are histologically well documented. It is important to recognize epididymal papillary cystadenoma not only as a lesion occurring in some male patients with von Hippel-Lindau (VHL) disease and one that may mimic metastatic renal cell carcinoma but also as a potential diagnostic indicator in patients who have not yet had other physical manifestations of VHL disease.

Material and methods. A 67-year-old male presented for a left testicular mass. There was no history of any urogenital complaints or abnormalities. His past medical and surgical history was unremarkable. On physical examination no evident superficial or deep lesion other than the painless testicular enlargement was found. Spermatic cord and adnexae were unremarkable on palpation. Urinalysis and laboratory tests for tumor markers, including α-fetoprotein and β-human chorionic gonadotropin, were negative. Sonography of the left testis revealed a hypoechoic mass with increased vascularity near the mediastinum testis measuring 20×15 mm. The right testis was unremarkable. The left hypoechoic mass was thought to be an intra-testicular mass. Accordingly, the patient underwent scrotal exploration. The lesion was completely excised with the epididymis and the didymus because it was difficult to separate the lesion from the former structures. The patient’s recovery was uneventful. The surgical specimen consisted of left testis and spermal cord. The sample underwent standard procedures for sampling and processing.

Results. Grossly, the tumor was encapsulated and well-circumscribed with an expansive growth pattern and measured 2×1.5 cm in size. Cut surface showed multiple grey white cysts filled with yellowish serous, sometimes haemorrhagic, fluid. The cavities of the tumor were separated by thin fibrous septa. Histologically, the proliferation was composed predominantly by epididymal tissue with ectasia of the afferent ducts and numerous cysts filled with prominent intracystic papillary structures. The papillary formations contained fibrovascular cores and were lined by single-layered cuboidal and columnar cells; cilia were occasionally present. The cells were arranged in tubules and nests, and presented prominent cell borders. Cytoplasmic clearing and vacuolization were conspicuous. Complex papillary processes completely filling the cystic spaces were seen in a few ducts; other cysts contained colloid-like eosinophilic material. Nuclei were small and round and showed no atypia. Mitoses were not identified. The supporting stroma was collagenous. Hemorrhagic area, tumor necrosis, scarring, psammoma bodies were not observed. Invasion of surrounding structures was absent. The tumor was located within the testis and separated from the tunica albuginea by thin fibrous tissue sparsely containing atrophic seminiferous tubules. The mediastinal portion of the rete testis was compressed peripherally by the tumor and no connection between the cysts and channels of the rete was found. In situ germ cell neoplasia was not found. Immunohistochemical profile showed positivity for Pan-cytokeratin (clone MNF-116), Cytokeratin 7, Epithelial Membrane Antigen. The tumour cells were negative for Cytokeratin 20, Alpha fetoprotein, placent al alkaline phosphatase, CD10, PAX-8, Melan-A. Based on morphological and immunohistochemical features, the final diagnosis was clear cell papillary cystadenoma confined to the epididymis with no testicular involvement.

Conclusions. Clear cell papillary cystadenoma, a benign epithelial tumor occurring in the head of the epididymis, is a rare lesion that has been found significantly more frequently (60%) in patients with VHL disease. Unilateral presentation may rarely be found in the general population. Since the histologic resemblance to clear cell renal cell carcinoma (RCC) can be striking and that RCC often occurs in VHL patients, the most important lesion in the differential diagnosis of clear cell papillary cystadenoma is metastatic clear cell RCC. Features common to the 2 lesions include cystic, tubular, and nested architecture, prominent vascular stroma and clear cells. A CK7-positive, CD10-negative profile has been shown to differentiate the two entities. However, discordant results were reported, and CD10 and RCC marker positivity must be interpreted in the context of the overall morphologic features. Other differential diagnoses include: serous adenocarcinoma of the paratestis (nuclear atypia and invasive growth), para-testicular papillary mesothelioma (absence of clear cells and presence of calretinin positivity), serous borderline tumor of the paratestis (absence of clear cells, presence of a stratified lining, and occasional presence of psammoma bodies), serous (non-papillary) cystadenoma (lack of papillary architecture). Treatment of clear cell papillary cystadenoma consists of testicle-sparing surgical excision although some patients do undergo orchiectomy. It has been suggested that patients be followed after excision. There is 1 report of recurrence (possibly due to incomplete initial excision) and 2 reports of transformation to cystadenocarcinoma. The possibility of VHL disease should be considered in all patients with bilateral clear cell papillary cystadenoma, since they may be at risk of developing other VHL-associated tumors and radiologic or genetic testing for VHL disease is warranted. In fact, two thirds of patients with bilateral tumor had stigmata or were found to be VHL positive. The association of unilateral clear cell papillary cystadenoma with VHL disease is weaker (20.3%) and unilateral clear cell papillary cystadenoma has never been reported as the initial presentation of VHL disease. Therefore, a dilemma arises concerning the necessity of investigating patients with unilateral clear cell papillary cystadenoma. We reviewed the only 8 cases for which a long period (18 months-15 years) follow-up was available. No one developed VHL disease, therefore we could assert that in advanced-age patients with unilateral lesion no genetic testing is required in the tumor follow-up that should be limited to whole body CT-scan.
Gene-specific methylation profiles in prostate cancer. A preliminary study of a series of hormonally treated and untreated patients

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Background. DNA promoter hypermethylation is a frequent epigenetic event in prostate cancer (PCa) and many genes have been found to be aberrantly methylated in PCa. Recently, DNA methylation of several genes has been implied in progression to hormone refractory PCa, suggesting that the analysis of the methylation profile of a panel of selected genes may be useful to identify patients which will not benefit from hormonal therapy.

Design. Using the candidate-gene approach, we performed promoter methylation analysis of a panel of genes involved in hormonal (AR, ESR1, ESR2) and tumor progression (RASSF1, APC, CD44, CDH1, BCL2) pathways. A series of 48 PCa cases were retrospectively collected, 25 patients had surgery alone (non-treated) while 23 received androgen deprivation therapy for 3 months before surgery (treated). Clinical-pathologic data, including age, histology, Gleason score, stage and margin status, were recorded. Biomolecular analysis was performed by pyrosequencing and methylation levels were assessed by calculating the average of methylation for each gene. Kruskal-Wallis test was used for statistical purposes.

Results. No significant differences emerged in methylation levels for the AR, ESR1, ESR2, RASSF1, CDH1, APC, ZEB1 and BCL2 genes in treated and non-treated patients. Mean CD44 methylation levels were higher in non-treated tumors (p=0.0005). Unsupervised hierarchical cluster analysis revealed two groups of clustered genes. One group, characterized by low methylation rates (<18%), included AR, ESR1, ESR2, BCL2, APC and ZEB1 genes. The second with high methylation rates (>18%) comprised RASSF1, CDH1 and CD44 genes. Kruskal-Wallis test was used to identify possible associations between methylation levels and clinicopathologic parameters in non-treated and treated PCa cases. A correlation between APC methylation and nodal involvement (p=0.04) was noted in non-treated patients. ESR1 methylation was associated with nodal involvement (p=0.04) and surgical margin status (p=0.03) in PCa patients undergoing total androgen ablation. Gene-specific methylation profiles were not associated with the other prognostic parameters analyzed.

Conclusions. Our results showed that the methylation profiles of the genes investigated did not significantly vary in relation to hormonal therapy. Nonetheless, the frequency of CD44 hypermethylation was higher in non-treated than in treated PCa cases.

References
Conclusions. In this work, for the first time two regions from myxoma tumor specimen were investigated on the proteome phenotype. Histology-directed on tissue approach, derivatization chemistry and high-resolution mass spectrometry coupled with liquid chromatography were used. Moreover, immunohistochemistry was used to confirm the expression of periostin-C. Our results confirm a pivotal role of periostin in the development and proliferation of cellular component of cardiac myxoma.

References


PATOLOGIA FETO-PLACENTARE

A third-trimester placenta with cysts: placental mesenchymal dysplasia

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Introduction. A 26-year-old caucasian woman (gravida 1, para 0) presented to our hospital for preterm prelabour rupture of membranes at 29 weeks and 4 days of gestation. Fetal ultrasound performed two months earlier at another facility reportedly showed no abnormalities. On admission, there was umbilical cord prolapse requiring delivery by cesarean section. She gave birth to a male infant weighing 1147 g (37th centile), with Apgar scores of 8 and 9 at 1 and 5 minutes. No dysmorphic features were appreciated on physical examination. However, there were scattered cysts on the maternal side of the placenta, which was fixed in formalin and sent to Pathology. The baby was transferred to the Neonatal Intensive Care Unit on continuous positive airway pressure.


Results. The fixed placenta weighed 915 g (>95th centile), had a large chorionic plate (20x14 cm) with meandering dilated vessels, and was irregularly thickened (up to 5 cm). On sectioning, the parenchyma was unevenly spongy, with scattered grape-like vesicles (Fig. 1A). Histology revealed enlargement of the main stem villi, characterized by edematous to myxoid stroma, with occasional cistern formation (Fig. 1B, H&E, 2x), and also rich in fibroblasts and collagen (Fig. 1C, H&E, 10x). Groups of intermediate villi showed chorangiomatosis-like changes, and distal villi were either normal or dysplastic, without trophoblast hyperplasia. Some areas showed an increase in perivillous fibrin deposition. There was thickening of chorionic vessels, with intimal fibrin cushions and subocclusive thrombi; many stem villous vessels displayed recanalization with septation (Fig. 1C, inset, H&E, 10x), and there were scattered large foci of terminal avascular or karyorrhreic villi.

Our primary diagnosis was placental mesenchymal dysplasia (PMD), associated with fetal vascular malperfusion. There was mesenchymal cell-confined loss of p57kip2 protein expression on dysplastic villi (Figure 1D; SABC, 20x), a phenomenon previously recognized in a fair number of PMD cases1. p57kip2 is the product of a maternally expressed but paternally imprinted gene: accordingly, the aforementioned differential pattern of expression may parallel androgenetic/biparental mosaicism, which is a consistent finding in PMD2.

During the first weeks of life, the infant experienced several episodes of non-hypersulinemic hypoglycemia. He also suffered from neonatal jaundice, anemia, hypothyroidism, retinopathy, and cystic periventricular leukomalacia, likely related to chronic hypoxia due to severe placental dysfunction. Liver function tests were within normal limits; however, liver ultrasound revealed scattered hypoechoic lesions, the largest of approximately 35 mm. MRI highlighted five nodules with liquid content, affecting multiple hepatic segments, and suggesting mesenchymal hamartomas. The lesions had considerably grown in size (up to 61.1 mm) when the patient was discharged home 81 days after birth (41 weeks and 1 day post-menstrual age). Two months later, a percutaneous puncture was performed with fluid aspiration from the largest cyst; an 18G tru-cut biopsy retrieved small fragments for histology, comprising scant mesenchymal tissue with few small bile ducts, dilated blood vessels, and plasma cells.

The infant is presently 7 months old (corresponding to 4 months and 3 weeks post-menstrual age) and is in good clinical conditions. Liver function tests are within the normal range, and neurological and ultrasound follow-up are ongoing. The last scan, performed 2 months after fluid aspiration, showed a reduction in size of the cysts.

Conclusion. PMD has been recognized since 1991 as a cause of placentomegaly, mainly distinguished from molar pregnancy by its vascular anomalies, in the absence of trophoblast proliferation or triploidy3. Subsequent studies reported associations with female karyotype, intrauterine fetal growth restriction or demise (possibly through fetal vascular malperfusion), visceral and cutaneous hemangiomas, hamartomas, and Beckwith-Wiedemann syndrome (BWS; OMIM 130650)4. The latter represents a condition of constitutional overgrowth and predisposition to cancer; it is linked to genetic and epigenetic alterations (often in a mosaic state) mainly at 11p15 region, which encompasses a cluster of imprinted genes including CDKN1C(p57kip2), H19, and KCNQ1OT1. However, the pathogenesis of PMD is still controversial. In fact, only 20% of infants born with PMD actually have BWS5, and other chromosomes hosting imprinted genes might also be involved6. As of today, long-term follow-up studies of patients born with PMD are not available, but hepatic tumors have been reported in about 17% of cases6, with or without BWS. Most of these are mesenchymal hamartomas of the liver, often congenital. We are also aware of at least one example of androgenetic/biparental mosaicism, both in the placenta and the liver6.

For our case, MS-MPLA analysis is underway in order to detect aberrant methylation or possible deletions/duplications in the 11p15 BWS critical region.
This report underlines the importance of a timely diagnosis of PMD, prompting early detection of concurrent anomalies in the newborn. No ultrasound data was available for this patient’s placenta, pointing out the importance of careful evaluation of this organ in cases with incomplete obstetrical information or suboptimal prenatal care.

References

Introduction. Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) is a rare congenital condition of severe functional intestinal obstruction with, up to date, 230 cases in literature, most of whom (71%) are female gender. This syndrome has an unknown etiology and is associated with microcolon, decreased or absent intestinal peristalsis and abnormally dilated non-obstructed urinary bladder.

Material and methods. We present a case of a male foetus of 14 week gestation in secundigravid primipara without family history of genetic pathology. An Ultra-sonography (US) carried at 12 weeks + 2 days of gestational age (G.A.) showed

**Figure 1.**
megacystis (mm 21x11x14), bilateral pelvicaliectasis and single umbilical artery. The mother decided for Voluntary Interruption of Pregnancy (VIP). After abortion, was requested an autopsy that revealed in addiction: microcoloon (15mm), imperfectated anus and hypoplastic and cystic right kidney. Diagnosis of MMISH was performed.

Conclusion. Megacystis microcolon intestinal hyperperistalsis syndrome (MMISH) is a very rare congenital anomaly, particularly in male gender characterized by several signs difficult to evaluate at the ultrasonography or at the autopsy. This is an example of how the close cooperation, between gynecologists and pathologists can be decisive for a correct diagnosis and a good clinical management especially in case of rare pathologies.

References
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TRAP SEQUENCE: A REPORT OF TWO CASES

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Introduction. Multiple gestation, an event which occurs in 1.5% of all pregnancies, is considered an independent risk factor for congenital malformations. Twins are reported to be affected by congenital anomalies twice as often as singletons (1). Acardia is a rare anomaly (1: 35,000 births) associated with monozygotic twin pregnancies, in which the sick fetus survives only on account of the presence of anomalous placental anastomoses enabling a reversed arterial perfusion at the expense of the other twin, the so-called “pump” twin. Therefore, the acronym TRAP (Twin Reversed Arterial Perfusion) or TRAPS (Twin Reversed Arterial Perfusion Sequence) is commonly used to refer to such a birth defect. The establishment of reversed arterial perfusion is lethal to the perfused twin due to the reduced oxygen tension of the blood supplied and leads to its abnormal development. Nevertheless, it causes the death of the other twin, even when normal, in more than 50% of cases, determining hemodynamic imbalance with subsequent intrauterine heart failure (2). Diagnosis is usually made on ultrasonography; the demonstration of a reversed perfusion, with arterial blood flowing from the placenta towards one malformed fetus is the pathognomonic sign of TRAP. Herein we report two cases of TRAP.

Materials and methods. Two cases with a diagnosis of acardiac malformation/TRA sequence were identified searching our database for perinatal autopsies performed in 2015 in our institute (University of Napoli ‘Federico II’, Department of Advanced Biomedical Sciences, Pathology Section). We compared maternal and fetal information, obstetric history, imaging during pregnancy, fetal X-ray examination and gross autopsy findings relative to both acardic fetuses.

Results. Ultrasonographic diagnosis of acardiac malformation was definitely confirmed by pathology. Our observations support evidence that the spectrum of anomalies and malformations found in the acardic fetus is wide and manifold; the most common and severe involve the cranial vault, facial structures, brain, upper limbs, lungs, pancreas and upper intestine. Conclusion. Twin reversed arterial perfusion (TRAP) sequence is a rare syndrome complicating approximately 1% of monochorionic twin pregnancies (3). Though the pathophysiology of this defect has been comprehensively explained, its aetiology is not fully understood. However, a high frequency of karyotypic abnormalities has been associated to the acardic anomaly; these include monosomies, trisomies, deletions, mosaicism and polyploidy. The pump twin is most often morphologically and genetically normal.

In such a context, perinatal autopsy and pathological examination may prove helpful to disclose those mechanisms and anomalies underlying the development of this dramatic type of fetal malformation.

References

GINECOPATOLOGIA

SCREENING WITH HPV TEST IN ASL2 SAVONESE: DATA ANALYSIS

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Introduction. Assess the performance of the four years of screening (2012-2015) with primary HPV testing on the territory of ASL2-Savonese where it was not active before a screening program organized with pap-test.

Methods. In December 2011, it has started a feasibility study for the screening of organized cervical cancer with the use of hr-HPV testing at five-year intervals for women aged between 30-64 years (69. 213). In case of a positive HPV test is programmed triage with pap-test. If the outcome of cytology is negative indicates the HPV test is repeated at 12 months while in the presence of ASC-US+ result or inadequate is to be sent to colposcopy.

Mode of invitation letter with fixed appointment at the time without accepting to non-respondents.

Results. To December 2015 we were invited 65.676 women and 31.655 (48,2%) had accepted the invitation.

Hr-HPV test was positive for 2.728 women (8,6%) which was examined the pap-test. The Pap test was negative by 1.352 women for whom it was
given the repetition of hr-HPV testing at 12 months. The 3.7% of cytology (n = 100) was considered inadequate and 46.8% (n = 1,276) showed cell changes (ASC-US+); total were referred to colposcopy 1,376 women (43.3% of screened). The accession to colposcopy was equal to 90.3% (1,242/1,376) and 969 women (70.4%) biopsies were practiced (969/1,376). Detection Rate (DR) CIN2+: n. 250/31,655 (7.9%).

Positive Predictive Value (PPV) for CIN2+: n. 250/1,376 (18.2%).

The women participating in the 12 month recall were 806/1,019 with a participation rate of 79.1%. Women do not comply were solicited by telephone. The lesions histologically proven after successful appeal are: CIN2 + n. 25/1,019 (2.4%)

Conclusions. Satisfactory adhesion, since the project started with no previous experience of organized screening programs. The introduction in the dunning program for not meeting could significantly increase membership.

The PPV of Pap-test triage for CIN2+ (18.2%) is higher than the national average (15-16%) with a higher frequency in the bands 40-44 and 45-49 probably due to the absence, until now, organized screening.

The DR (7.9%) is well above the national average (5.7%). The use of HPV testing has allowed the start of screening for cervical cancer in the province of Savona efficiently with the ability to recruit a large number of women without introducing additional resources in the system; Parallel decreased in an important way the Pap test opportunistic at the ASL structures making available resources, especially human, to be committed in the project.

The data, however, are subject to change have not yet completed the 1° ROUND.

\[\text{LSIL with some features suggestive of the presence of a concurrent HSIL: evaluation of positive predictive value (VPP) in cytology triage}\]

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Introduction. The new 2014 Bethesda System for reporting cervical cytology takes into account the use of new terminology to cases with cytological characteristics intermediate between LSIL and HSIL (LSIL who cannot exclude HSIL or LSIL-H).

We started to apply this indication in the cytology triage screening with HPV testing in primary ongoing since December 2011 in our province.

The aim of the study is:

Evaluate the applicability of this terminology in cytology triage in cases with cytological predominant features of low grade SIL but with some suspicions but not diagnostic for high grade SIL.

Evaluate the VPP for CIN2+ of these cases in relation to the VPP LSIL diagnostic classes, ASC-H and HSIL.

Methods. Were taken into account in cases of LSIL-H from June 2014 to December 2015 were reviewed double-blind to assess the reproducibility and the correct application of the diagnostic criteria defined by the Bethesda 2014.

It was analyzed the follow-up of LSIL-H cases, LSIL, HSIL and ASC-H in the same period and for each diagnosis was calculated and compared the VPP for CIN2+.

Results. Period: June 2014 - December 2015

<table>
<thead>
<tr>
<th>Pap test triage</th>
<th>N° casi</th>
<th>N° biopsie</th>
<th>Istitologia CIN2+</th>
<th>VPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSIL</td>
<td>66</td>
<td>60</td>
<td>47</td>
<td>78.3%</td>
</tr>
<tr>
<td>LSIL-H</td>
<td>27</td>
<td>20</td>
<td>13</td>
<td>65%</td>
</tr>
<tr>
<td>ASC-H</td>
<td>18</td>
<td>14</td>
<td>7</td>
<td>50%</td>
</tr>
<tr>
<td>LSIL</td>
<td>302</td>
<td>177</td>
<td>30</td>
<td>17%</td>
</tr>
</tbody>
</table>

Conclusions. The VPP cases defined as LSIL-H is 65%, intermediate value between case of ASC-H (50%) and case of HSIL (78.3%); the result seems to justify the combined use of these two diagnoses. The revision of LSIL-H cases not always occurred reproducibility intra and inter observer.

Table I. Depetrini poster (lsil with some features suggestive of the presence of a concurrent hsil: evaluation of positive predictive value (vpp) in cytology triage).

**OVARIAN SMALL CELL CARCINOMA, HYPERCALCAEMIC TYPE, LARGE CELL VARIANT. A CASE REPORT**

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Introduction. Ovarian small cell carcinoma, hypercalcaemic type (OSCCHT) is a rare, highly aggressive tumor mostly affecting young women [1]. Usually presenting as a unilateral ovarian mass, it shows an undifferentiated histology composed predominantly of small cells but it can also include a large cell component. A peculiar feature of this entity is the association with paraneoplastic hypercalcaemia. Here we present a case of OSCCHT with a preponderant large cell population, ie an OSCCHT, large cell variant.

Case report. A 47 year old patient with symptoms of bowel obstruction had US diagnosis of a 14 cm-ovarian solid mass. Intraoperative frozen section examination disclosed an ovarian high grade neoplasia. Radical hysterectomy with lymphadenectomy and multiple biopsies were performed. At gross examination the tumor was solid with an irregular and lobulated surface, gray-yellow on cut surface with hemorrhagic and necrotic foci and scattered cystic areas containing brownish fluid.

Histology showed a diffuse growth of closely packed cells, focally forming roundish structures [Fig. 1A]. Occasional follicle-like structures containing pale eosinophilic fluid were observed [Fig. 1B]. Cells were also arranged in cords, trabeculae and single nests. The majority of the cells were large with moderate to abundant glassy eosinophilic cytoplasm exhibiting a luteinized or rhabdoid appearance [Fig. 1C]. They had eccentric large and vesicular nuclei with prominent nucleoli. A minor component of monotonous cells with scant cytoplasm and small hypercromatic nuclei, with coarse and clumped chromatin and small nucleoli, was also observed.
The final diagnosis was ovarian small cell carcinoma, hypercalcemic type (OSCCHT), large cell variant. Tumor stage was advanced owing to extraovarian involvement (pelvis, several lymph nodes and ascitic fluid). Serum calcium level checked after diagnosis was markedly elevated. After few weeks, the patient developed widespread abdominal involvement and liver metastases. Chemotherapy (Paclitaxel and Carboplatinum) was administered; however, she died from disease 2 months later.

**Discussion.** OSCCHT is a rare, extremely aggressive tumor which arises in young females (with a mean age of about 24 years) [1]. It is composed of solid sheets, nests and focal follicle-like structures. The cells are usually small with scant cytoplasm but in about 40% of cases large rhabdoid cells are present. When the large cells are predominant or exclusive, the tumor is referred to as large cell variant [2]. The histogenesis is unknown. The WHO categorizes this entity among Miscellaneous ovarian tumors. Various origins have been postulated including epithelial, germ-cell, sex cord-stromal and neuroendocrine ones. Immunohistochemistry performed with routinely assessed markers is almost aspecific. The main differential diagnosis is Juvenile granulosa cell tumor (JGCT). Both involve young females and contain follicles and abundant eosinophilic cytoplasm. In OSCCHT large cell variant, however, more cytological atypia and rhabdoid features are observed. Furthermore, JGCT shows Inhibin and SF1 positivity. An extensive sampling is therefore recommended. Other entities to be considered are: Small cell carcinoma, pulmonary type, ovarian or metastatic (generally TTF1 positive); Desmoplastic small round cell tumor (coexpressing Desmin and EMA); Peripheral primitive neuroectodermal tumor (PNET); Malignant melanoma; Undifferentiated carcinoma.

Concerning the molecular features, this entity is described as predominantly diploid. No mutations of well known candidate genes have been identified (KRAS, BRAF, BRCA 1/2 and TP53). Recent studies found that inactivating mutation in the SWI/SNF (Switch/Sucrose non fermentable) chromatin remodeling gene SMARCA4 is a peculiar feature of OSCCHT [3]. Mutation causes loss of expression of the gene product BRG1 protein. Alterations in this gene, or the related SWI/SNF chromatin remodeling gene SMARCB1 encoding for INI1, had been previously reported in Atypical teratoid/rhabdoid tumors and Malignant rhabdoid tumors [4]. For this reason, the terminology “Ovarian malignant rhabdoid tumor” has been proposed for OSCCHT, thus supposing a mesenchymal origin [5]. To note, INI1 expression was observed in our case as well as in previous OSCCHT reports [6]. In conclusion, SMARCA4 is the only recurrently mutated gene in OSCCHT and its loss is a useful and sensitive marker for diagnosis as well as a possible target for therapy.

**References**


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Background. Human papillomavirus (HPV) DNA test showed high sensitivity, but poor specificity in detecting high-grade cervical lesions. However, the high prevalence of papillomavirus infection means that such testing has a low positive predictive value.

So, in these years, another supporter marker in cervical pathology, p16INK4a/ki-67 expression were introduced in clinical practice to mark indirectly viral integration. Diagnostic application of p16INK4a/ki-67 has been investigated in cervical pathology being expressed in HPV- associated lesions, in low–grade cervical intraepithelial neoplasia (CIN) and in a high percentage of high-grade CIN. P16INK4a/ki-67 IIC assay could be more specific but is less sensitive because based on a subjective interpretation. It is known that the development of a malignant phenotype requires continuous expression of the E6 and E7 viral oncoproteins, so E6 and E7 mRNA transcripts could predict disease progression. These findings suggest that RNA-based assays could have a higher prognostic value than DNA-based tests and that they could play an important role in future screening programs. In this study, we investigated: i) the relationship between HPV load, measured using Hybrid Capture 2, and HPV mRNA detection using NucliSENS EasyQ in cervical ASC-US, L-SIL and ASC-H cytology as predicting factor of high grade cervical lesions and ii) we compare the results of mRNA assay with p16INK4a/ki-67 IIC assay.

Methods. One hundred and two LBC PAP cervical smears, with a diagnosis of NILM (n=4), ASC-US (n=50), L-SIL (n=14), ASC-H (n=30) and H-SIL (n= 4, used as control) and were selected (between December 2015 and May 2016, in Unit of Anatomic Histology and Cytopathology of the Bonomo Hospital) among 1 LBC PAP smear.

Thin-prep® liquid based cytology test (LBC) samples were processed for HPV DNA detection with Hybrid Capture 2 (Digene, Gaithersburg, MD) and E6/E7 mRNA detection with NucliSENS EasyQ (Biomérieux). All cytological samples were assessed for p16INK4a/ki-67 expression, using CINtec Plus immunostaining Kit.

Results. Our data showed that 34 out of 50 ASC-US cytological samples had a low/medium HPV load (range 1-200 RLU/CO), whereas 16 out of 50 ASC-US cytological samples had high viral load (> 200 RLU/CO). In the ASC-US cases, mRNA E6/E7 detection resulted negative in 28 out of 34 positive HR-HPV DNA detection with lo/medium viral load, whereas 6/34 ASC-US samples were positive to mRNA E6/E7 detection, 2 cases were positive to HPV 45, 2 cases were positive to HPV 31, one case was positive to HPV 33 and the last cases was positive to HPV 18 and HPV 45 both.

In the series of 16 ASC-US with a high viral load of HPV 9 resulted negative to mRNA E6/E7 detection, whereas 7/16 were positive: 2 cases were positive to HPV 16, 1 case was positive to HPV 18, 1 case was positive to HPV 31, 1 case was positive to HPV 33 and 2 cases were positive to HPV 16 and HPV 31 both. Four out of 14 L-SIL cytological cases had a low/medium HPV load (range 1-200 RLU/CO), whereas 10 out of 14 L-SIL cytological samples had high viral load (> 200 RLU/CO). In the L-SIL cases, mRNA E6/E7 detection resulted negative in two cases out of 4 positive HR-HPV DNA detection with lo/medium viral load, whereas 2/4 L-SIL cases were positive to mRNA E6/E7 detection, 1 case was positive to HPV 31, 1 case was positive to HPV 18 and HPV 33 both. Fourteen out of 30 ASC-H cytological samples had a low/medium HPV load (range 1-200 RLU/CO), whereas 16 out of 30 ASC-US cytological samples had high viral load (> 200 RLU/CO). In the ASC-H cases, mRNA E6/E7 detection resulted negative in 6 out of 14 positive HR-HPV DNA detection with lo/medium viral load, two out of these cases were not valuable for mRNA detection, whereas 6/14 ASC-H samples were positive to mRNA E6/E7 detection, 4 cases were positive to HPV 16, 1 case was positive to HPV 18 and 1 case was positive to HPV 31. In the series of 14 H-SIL cases with high viral load of HPV 3 resulted negative to mRNA E6/E7 detection, whereas 14/16 were positive: 6 cases were positive to HPV 16, 2 cases were positive to HPV 18, 3 cases were positive to HPV 31, 1 case was positive to HPV 33, 1 case was positive to HPV 16 and HPV 45 both, 1 case was positive to HPV 16 and HPV 18 both. At the and we analyzed 4 NILM cases and 4 H-SIL cases. The 4 NILM had had a low/medium HPV load and 2 cases resulted negative to mRNA E6/E7 detection, whereas 2/2 were positive: 1 case was positive to HPV 16 and 1 case was positive to HPV 33. One out of 4 H-SIL cases had a low/medium HPV load and were positive to HPV 18 mRNA detection, whereas 3/4 H-SIL case had high viral load and were positive to E6/E7 mRNA HPV detection: 2 cases were positive to HPV 16 and 1 case was positive to HPV 31 (Table 1). Regarding p16INK4a/ki-67 expression, it was expressed in 41/49 cases positive to HR-HPV DNA test with high viral load, but only 33 out of 49 of these cases had mRNA E6/E7 HPV expression (Table 2). This demonstrate that mRNA E6/E7 detection had best specificity than p16INK4a/ki-67, because it analyzed mRNA of viral oncogenic protein.

Conclusions. Our data showed that HPV viral load and mRNA E6/E7 detection in ASC-US, L-SIL and ASC-H cases significantly correlates with the severity of cervical cancer precursors. These data suggest that high viral load and mRNA E6/E7 detection may have a prognostic value in identifying cytological precancerous lesions with a high risk to progress in cancer. Conclusively, HPV load and mRNA E6/E7 detection could be useful in predicting the severity of HPV-related cervical disease.

References

UTERINE CERVIX POLYP IN A 50 YEARS OLD WOMAN: UNUSUAL SETTING OF “SARCOMA BOTRYOID” (EMBRYONAL Rhabdomyosarcoma)

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Introduction. Sarcoma botryoides is a subtype of rhabdomyosarcoma (1, 2); typical of infants, often occurring in the vagina; less commonly in the cervix or uterine fundus with a peak frequency in the second decade. Cervical rhabdomyosarcoma in patient over 45 years of age are very rare (3, 4, 5). In past, pelvic exenteration was the treatment of choice, while more conservative approach are recommended nowadays (6, 7).

Methods. We report the case of a 50-year-old woman who in September 2015 was referred to our university hospital. Two months earlier she presented to another facility with a 6-months history of extramenstrual spotting. At hysteroscopy, an endocervical polyp was detected, averaging 4 cm. A first biopsy retrieved only scanty material which was reportedly diagnosed as endocervical polyp. However, also given the degree of clinical suspicion, a further biopsy revealed a malignant proliferation which was initially labeled as adenosarcoma. Pelvic MRI and total-body CT confirmed that the tumor was confined to the uterine cervix (Fig. 5). The histology slides were then reviewed at our hospital where the diagnosis was changed into sarcoma botryoides. The patient underwent a robotically assisted type B2 radical hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy.

Results. On gross examination there was a greyish polypoid tumor affecting the cervical region (2.5 cm in length x 2 cm in diameter) and extending into the isthmus. Microscopy confirmed a mesenchymal proliferation covered by endocervical mucosa; the latter showed squamous metaplasia and with focal areas of ulceration and hemorrhage (Fig. 1 H&E 2x). There was infiltration of the cervical stroma. The tumor displayed the merging of two main patterns of growth, with edematous to mixoid stroma containing scattered spindle cells on the one hand, and hypercellular foci with periglandular and sub-epithelial condensation on the other. (Fig. 2 H&E 20x). The mesenchymal elements appeared as primitive and immature cells with cytologic atypia, ranging from small and round to spindle-shaped, with a significant increase both of mitotic activity and proliferation index (Ki67/MIB1) (Fig. 2c SABC 40x). Tumor cells were positive for myogenin (8) (Fig. 2b SABC 40x) and vimentin (9), and only focally for desmin (Fig. 2a SABC 20x). A tiny focus of cartilage differentiation (Fig. 3 H&E 40x) (S100+) (Fig. 3a SABC 40x) was also seen. Surgical resection margins were uninvolved by tumor, and lymphovascular invasion was not observed. As there was no evidence of extrauterine spread, the tumor was staged as clinical group I A(Intergroup RMS Study Group, IRS). As of today, there has been no evidence of recurrence over a 10-month follow-up.

Conclusion. Sarcoma botryoides is a mesenchymal tumor with skeletal muscle differentiation arising beneath a mucosal epithelial surface In adults, extremities and retroperitoneum are the main affected sites, while it preferentially occurs in the neck region and genitourinary tract (especially the vagina) in children (4). Altogether, this tumor is quite rare in the female genital tract (0,2% of all malignant neoplasm of uterus)(11) and only 0,5% of primary rhabdosarcomas originate in the cervix (3). Fanghong et al (4) included in his review only 14

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Table I. Correlation between cytological category and HPV viral load.

<table>
<thead>
<tr>
<th>CYTOLOGICAL CATEGORY</th>
<th>HPV VIRAL LOAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOW/ MEDIUM (1-200 RLU)</td>
</tr>
<tr>
<td>NILM</td>
<td>4</td>
</tr>
<tr>
<td>ASC-US</td>
<td>34</td>
</tr>
<tr>
<td>L-SIL</td>
<td>4</td>
</tr>
<tr>
<td>ASC-H</td>
<td>14</td>
</tr>
<tr>
<td>H-SIL</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>57</td>
</tr>
</tbody>
</table>

Table II. Correlation between High HPV viral Load, E6/E7 mRNA detection and p16INK4a/Ki-67 expression.

<table>
<thead>
<tr>
<th>HIGH LOAD (&gt;200 RLU)</th>
<th>E6/E7 DETECTION</th>
<th>P16INK4a/Ki-67 EXPRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC-US</td>
<td>7/16</td>
<td>10/16</td>
</tr>
<tr>
<td>L-SIL</td>
<td>7/10</td>
<td>8/10</td>
</tr>
<tr>
<td>ASC-H</td>
<td>14/16</td>
<td>16/16</td>
</tr>
<tr>
<td>H-SIL</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>33/45</td>
<td>41/45</td>
</tr>
</tbody>
</table>
patients aged 40 to 59 years and 3 patients older than 60 years age. Most patients present with vaginal bleeding and the tumor form grape-like clusters or single or multiple polyps (10). Irrespective of the age of the patient, it’s important to include sarcoma botryoides in the differential diagnosis of uterine or cervical spindle cell tumors. Indeed, the hypocellular background could wrongly suggest a benign polyp or a low-grade biphasic tumor such as adenosarcoma, and haemorrhagic areas might obscure the hypocellular foci. (12)

In fibroglanular polyps, hypocellular foci are exceedingly uncommon, and the glands in the hypocellular stroma have benign cytological and histological features (4).

More complex was the differential diagnosis between adenosarcoma exhibiting rhabdomyoblastic/chondroid differentiation and sarcoma botryoides. In our case: 1) the very edematous/myxoid nature of the lesion (rarely seen in adenosarcoma); 2) the paucity of endocervical glands randomly distributed throughout the proliferation (more common in adenosarcoma as they are part of the neoplasm) (Fig. 4 H&E 2x); 3) the finding of alternating hyp- and hypocellular areas within the lesion; and finally 4) the “blue”, primitive appearance of the stroma underneath the surface epithelium. All the above-mentioned features are more suggestive of sarcoma botryoides (13-14) but may be focal: in fact, they were more evident in the biopsy than in the hysterectomy specimen in our case. Malignant Mesenchymal Tumor (MMMTs) or carcinosarcoma also has to be considered in the differential, in order to avoid a misdiagnosis. However in sarcoma botryoides there should not be an epithelial component with malignant features (13).

A precise diagnosis of sarcoma botryoides is sometimes difficult because approximately 40% of these lesions lack cross striations typical of rhabdomyoblastic differentiation, while tumor cell often fail to express muscle cell-related markers. In these instances a panel of antibodies is useful for a correct diagnosis, including positivity of MyoD1 (myoblast determination gene number 1) and myogenin, as preferential markers of skeletal differentiation (6,15), lack of hormone receptors expression and an increase of proliferative activity (Ki67/MIB1).

The optimal management of rhabdomyosarcomas of the female genital tract is still uncertain, owing to their rarity and the lack of standard treatment guidelines, with anecdotal experience and largely confined to case reports (2, 11). Patients with favorable prognostic parameters, such as localized disease without deep myometrial invasion, single lesion and patients with favorable prognostic parameters, such as localized disease without deep myometrial invasion, single lesion and embryonal histologic subtype (sarcoma botryoides), can be managed aggressively but with caution, because the high frequency of metastases and the lack of standard treatment guidelines, with anecdotal experience and largely confined to case reports (2, 11). Patients with favorable prognostic parameters, such as localized disease without deep myometrial invasion, single lesion and embryonal histologic subtype (sarcoma botryoides), can be effectively treated by surgery with or without adjunct chemotherapy (14, 16, 17, 18).

References

Disseminated peritoneal leiomyomatosis: behaviour and pathogenesis

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Introduction. Disseminated peritoneal leiomyomatosis (DPL) is a very rare, benign entity characterized by the presence of multiple subperitoneal or peritoneal smooth muscle nodules throughout the peritoneal surface. It occurs in women of childbearing age and it has mostly an asymptomatic course without specific symptoms. The pathogenesis remains unclear although frequently it has been reported to be associated with pregnancy, oral contraceptive use, endometriosis, and previous laparoscopic myometrectomy morcellation. DPL is known to mimic disseminated malignancy clinically and surgically. We present a case of DPL in a young woman and discuss the etiopathogenesis and the behaviour of this pathology.

Methods. Clinical case: 42-year-old nulliparous, unmarried, caucasian female presented to our hospital for a ultrasonography due to mild right hypochondrial pain. The ultrasound examination revealed a huge, hyperechoic abdomino-pelvic mass and an enlarged uterus. The patient referred since seven years before a laparoscopy to remove an uterine leiomyoma and an enlarged uterus. The patient was submitted to FNAB, whose histological diagnosis was smooth muscle neoplasia of uncertain malignant potential. She underwent to surgical removal of the mass. The intraoperative view showed the abdominal cavity stuffed with multiple isolated tumoral masses ranging from 30 to 1.7 cm, occasionally enclosed by highly vascularized omental tissue. The uterus did not present relationship with the masses but itself presented 10 cm intramural nodule on the fundus, and three smaller nodules in the ligamentum latum. Hysterectomy and oophorectomy was performed also.

Results. FNAB: The needle biopsy showed a monomorphic bland spindle cells neoplasia with minimal focal nuclear pleomorphism, without mitoses and necrosis; the neoplastic cells showed smooth muscle differentiation by immunohistochemistry (SMA+/desmin+); the proliferation index was low (<10%). The diagnosis of smooth muscle neoplasia of uncertain malignant potential was made.

Macroscopical findings: All the neoplastic masses were irregularly multilobated, withish to dark-red in colour with areas frankly hemorrhagic, hard to spongy in relation to the amount of blood entrapped. The cut surface outlined the irregular aspect of the neoplasm, mainly due to the blood congestion with formation of trabeculae and pseudocysts formation. There were no necrotic areas. The enlarged uterus evidenced a very irregular cut surface due to an intramural nodule and a diffuse leiomyomatosis. The ovariess were normal except on the left where there was a 2.5 cm cyst.

Microscopical findings: All the nodular specimens showed a bland spindle cells proliferation devoid of atypia or mitosis with smooth muscle differentiation evidenced by the presence of cosinophilic fibrillary cytoplasm and blunt ended nuclei, and immunohistochemically by positivity for smooth muscle actin, desmin, and caldesmon; the hormone estrogen and progesterone receptors were also positive. The proliferation index was low. The uterus was modified by extensive leiomiomatosis changes and by a large neoplastic nodule on the fundus. No significant pathological changes of the adnexa was found, but endometriosis foci in the surrounding tissues and on the surface of one of the masses in the pelvis. The diagnosis of disseminated peritoneal leiomyomatosis was made.

Conclusion. Disseminated peritoneal leiomyomatosis is a rare benign neoplasm characterized by multiple nodules located on peritoneal surfaces of the abdominal and pelvic cavities giving the clinical impression of a widespread malignant tumor. It can be usually observed in young women but it has been also reported in men, and occurs without specific symptoms. DPL pathogenesis is still not well established; several theories have been proposed, all of which point toward hyperestrogenic status as a strong causal factor (pregnancy, oral contraceptive use or hormone replacement therapy, and estrogen secreting tumors of the ovary). The smooth muscle metaplasia of subperitoneal mesenchymal cells is a well-known hypothesis too. Reduction of estrogen exposure could be cause of regression; malignant transformation occurs rarely. Herein we report a DPL case occurring seven years after laparoscopic myometrectomy morcellation and we conclude that the current DPL is likely the result of implantation and evolution of leiomyoma cells from the initial procedure. Although this complication is extremely rare, consideration should be given to informing patients about this risk prior to laparoscopic hysterectomy with morcellation.

Dermatopatologia

CO-EXPRESSION OF COX-2 AND PD-L1 IN PRIMARY AND METASTATIC MELANOMA: NOVEL THERAPEUTIC APPROACHES

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Background. The limited clinical responses to immunotherapy utilizing immune checkpoint inhibitors blocking the interaction of PD-1 with PD-L1 in the treatment of melanoma have prompted investigators to implement novel clinical trials which combined immunotherapy with different treatment modalities as well as to investigate the mechanisms which might regulate the dynamic expression of PD-L1 on tumor cells and PD-1 on T cells in order to identify predictive biomarkers of response. COX-2 is an enzyme mainly involved in inflammatory reactions. However it is currently investigated as a major player of tumor progression in several type of malignancies including melanoma. In the present study we investigated the potential relationship between COX-2 and PD-L1 expression in melanoma.

Materials and methods. Tumor samples obtained from 44 primary melanoma and 45 not matched lymph node metastasis were analyzed for both PD-L1 and COX-2 expression by IHC analysis. Status of BRAF and NRAS mutations in metastatic lymph nodes was analyzed by sequencing and PCR. In addition co-localization of PD-L1 and COX-2 expression in melanoma tumor biopsies was analyzed by double fluorescence staining. Lastly the BRAF^{V600E} melanoma cell line A375 and
the NRASQ61R melanoma cell line SK-MEL-2 were used as an in vitro model to evaluate the effect of COX-2 inhibition by celecoxib on the proliferation and expression of PD-L1 by melanoma cells.

**Results.** BRAF (V600E and V600K) and NRAS (Q61R and Q61L) mutations were detected in 57.8% and 8.9%, respectively, of the metastatic melanoma tumor biopsies. PD-L1 and COX-2 expression was heterogeneous expressed in both primary melanoma and not matched lymph node metastasis. PD-L1 expression in melanoma tumors ranged from negative to light positive and positive. A significantly lower number of PD-L1 negative lesions was found in primary tumors as compared to metastatic lesions (P = 0.002). COX-2 expression in melanoma tumors ranged from 0 to 80% of positive cells. No significant correlation was found between PD-L1 or COX-2 expression with the pathological characteristics as well as with the genotype of lesions analyzed. However a significant correlation between COX-2 expression and PD-L1 was found in both primary (P= 0.001) and metastatic (P= 0.048) lesions. Melanoma cells expressing a higher levels of COX-2 also co-expressed a higher level of PD-L1. In addition inhibition of COX-2 by celecoxib inhibited the proliferation and down-regulated the expression of PD-L1 in both A375 and SK-MEL-2 melanoma cell line.

**Conclusions.** COX-2 expression correlates with PD-L1 expression during melanoma progression and immune evasion as well as to enhance the anti-tumor activity of PD-1/PD-L1 based immunotherapy for the treatment of melanoma patients with or without BRAF/ NRAS mutations.

**References**


**SCRAPING EXAMINATION OF RARE CUTANEOUS METASTASES**


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**Introduction.** Cutaneous involvement by internal malignancies is uncommon and has been estimated to occur in 0.7% to 9% of patients with internal cancers.1,2 Skin metastases generally develop in the late, disseminated stage, but may also be the first clinical manifestation of an asymptotically progressing cancer.3 In men, the most common cancers giving skin metastasis are melanomas followed by lung-, colorectal-, oral- and kidney cancers; in women, breast cancers, followed by colorectal-, lung-, kidney-, ovarian cancers and melanomas are the most frequent malignancies leading to cutaneous metastases.4,5 In some cases, it is necessary to differentiate between primary skin tumors and metastatic skin lesions of synchronous primary internal tumors. These types of lesions may have similar morphological patterns and thus immuno-histochemical markers can be very useful for their differentiation.6 Ulceration is an uncommon feature of cutaneous metastases, in which scraping examination is the best procedure to obtain a rapid cytological diagnosis. The study examined four rare cases of cutaneous metastases in unusual sites: a 38 year-old woman, with a cutaneous lesion at the forehead as primary presentation, which revealed to be an atypical lymphocytic proliferation; a 65 year-old woman, with a lesion at pectoral wall, which appeared six years later the initial diagnosis of papillary thyroid carcinoma (PTC); a 67 year-old man, with a simultaneous hip ulceration and a primary small cell lung cancer; a 56 year-old man with a metastasis from colorectal cancer at the tip of the nose. The man also presented a history of melanoma.

**Methods.** Scraping slides were obtained by means of a tip of a needle or a lancet. Smears were either air dried and stained with Diff Quik™ or wet-fixed in 95% ethanol and stained with Papanicolaou (Pap). After the initial cytomorphological evaluation, immunocytochemistry was performed on wet-fixed slides stained with Pap, after coverslip removal in xylene and rehydration in descending alcohols, using a semi-automated immunostainer (Leica Bond Max).

**Results.** Cytological features and the clinical information resulted crucial for the best markers choice. Respectively, CD20 was positive in cutaneous lymphoma; TTF1 was strongly positive in PTC metastasis; CD56 resulted positive in small cell lung cancer metastasis and CDX2 expressed positivity in colorectal metastasis.

**Conclusions.** Clinical information, morphological appearance and pertinent immunocytochemistry led to a correct cytological diagnosis, later confirmed by histological diagnosis.

**References**

**Introduction.** Cutaneous metastases of internal malignancies are uncommon, with a reported frequency of 0.7% to 10.4% and representing about 2% of all skin cancers. They usually occur late in the course of the disease, but rarely they may be the first sign of a hidden internal cancer. Metastatic cutaneous lesions usually arise from breast, lungs, colon, rectum, ovary, head and neck tumors and kidney. Cutaneous involvement may show different clinical features. Lesions are generally painless single or multiple nodules, but can infrequently display a sclerodermoid, inflammatory (erysipelas-like), zosteriform and cellullitates-like pattern, resembling an epidermoid cyst. We describe an unusual clinical-diagnostic sequence of a patient presenting with a rare pattern of cutaneous metastasis over the facial skin. This lesion showed a signet-ring cell (SRC) growth, visible under a microscope, bringing to the forefront a previously undiagnosed gastric carcinoma.

**Methods.** A 82-year-old male patient presented with an insidiously developed skin lesion of almost one month’s duration. Clinical examination revealed a relatively well-defined, not pruritic, erythematous leathery plaque on his face, measuring approximately 6 cm in its maximum diameter. The patient gave a past history of prostatic adenocarcinoma. Subsequently, abdominal computed tomography showed a mural thickening and rigidity of the stomach associated with the presence of peritoneal nodules. On the basis of endoscopic findings, gastric biopsy was carried out; a gastric signet-ring cell adenocarcinoma was detected.

**Results.** At histology, the skin biopsy showed a normal epidermis and infiltration of the dermis by nests, cords and scattered cells, mostly appearing as signet-ring elements, surrounded by dense fibrous stroma, and focally encircling the adnexa. The histochemical staining for Alcian-PAS revealed mucin deposits in neoplastic cells, in the cytoplasmatic areas forming vacuoles, with focally bull’s-eye features. There was a narrow layer beneath the epidermis that was not infiltrated by atypical cells distributed in the deep dermis till the surgical deep margin. Immunohistochemically, SRCs were diffusely positive for AE1-AE3 cytokeratins, CK7 and EMA, with focal expression of CK20. negativity for desmin, PSA, S-100 protein, CD34, CD31, estrogen receptor, chromogranin, GCD-FP-15, CDX-2, TTF-1 and CK 5/6 confirmed the epithelial nature of the neoplastic elements. These pathological results indicated a diagnosis of secondary SRC adenocarcinoma and suggested gastric origin.

**Conclusions.** Cutaneous metastasis from gastric SRC adenocarcinoma is a rare finding; however it should be considered in the differential diagnosis of primary and secondary malignancies of the skin. Cutaneous primary neoplasms that may contain SRCs include squamous cell carcinoma, basal cell carcinoma, cutaneous SRC carcinoma of eccrine or apocrine origin, liposarcoma, melanoma, epithelioid hemangioendothelioma and atrophic variant of mycosis fungoides. Therefore, metastasis from SRC adenocarcinoma to the skin can be a challenging diagnosis [1]. It may alert the clinician to an undiagnosed primary gastric tumor and/or more widespread metastases and, depending on the patient’s symptomatology and overall clinical picture, arrive at the decision making regarding surgical resection. Prognosis for patients with cutaneous metastasis from gastric SRC adenocarcinoma is difficult to predict due to the limited number of cases reported in the literature, but the survival rate is usually low [1].

**References**


**CLASSICAL KAPOSI’S SARCOMA (CKS): CORRELATION BETWEEN MORPHOLOGICAL-IMMUNOHISTOCHEMICAL ANALYSIS AND IMMUNOLOGIC PROFILES IN TUMOR LESIONS. A PIVOTAL STUDY OF 529 CASES**

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**Introduction.** Kaposi’s Sarcoma (KS) is a low-grade vascular tumor associated with Kaposi sarcoma herpesvirus/human herpesvirus 8 (KSHV/HHV8) infection. Kaposi sarcoma lesions predominantly present at mucocutaneous sites, but may involve all organs and anatomic locations. Recognized epidemiologic-clinical forms of KS include classic (mediterranean), African (endemic), AIDS-associated (epidemic) and iatrogenic KS. KS lesions evolve from early (patch stage) macules into plaques (plaque stage) that grow into larger nodules (tumor stage). The microscopic features of KS are the same in all the clinical-pathological forms. They are represented by angiogenesis, oedema, erythrocytes extravasations, lympho-monocytic inflammatory infiltration and spindle cells proliferation; latency-associated viral antigen (HHV8) is the most specific immunohistochemical marker available to help distinguish KS from its mimics. The aim of this pivotal study are the correlation between morphological-immunochemical analysis and immunologic profiles in tumor lesions in 529 histological cases.

**Methods.** We performed a preliminary retrospective analysis, prospective and epidemiologic study of all diagnosed cutaneous classical Kaposi’s sarcoma (CKS) at the Tricase (LE), Gallipoli (LE), Lecce, Brindisi and Taranto Hospitals between 2007-2015 years. 529 cases of CKS were detected and diagnosed in ours five Pathology Units from January 2007 to May 2015. Biopptic specimens were obtained from cases of a skin classical Kaposi sarcoma (CKS). Immunohistochemical staining and cytometric evaluations for endothelial-vascular, lymphocytic and cells T-subset populations, macrophages, stromal cells and viral (HHV8) were made.

**Results.** We investigated the application of morphological and immunohistochemical methods, routinely used in the diagnosis of tissue and blood diseases to the characterization of lymphocyte and inflammatory cellular populations in the Kaposi sarcoma lesions and subsequently observed the correlation between data from morphological and immunohistochemical analyses and data from immunological studies (ELISPOT-CSA), with a focus on specific antiviral (HHV8) T-cells subsets. The expected results are the assessment of HHV8-specific cellular immune response, in particular cytotoxic effector T cells, in order to optimize for the generation of HHV8-specific CTL for in vivo use.

**Conclusion.** Our preliminary study representing a pioneer-
A RARE CLINICAL PRESENTATION OF A PRIMARY CUTANEOUS "CYSTIC" SQUAMOUS CELL CARCINOMA OF THE BREAST

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Introduction. The occurrence of a squamous cell carcinoma (SCC) in the breast is a rare occurrence that can be primarily ascribed to the well-described primary pure SCC of the breast, a rare type of metaplastic tumor, that accounts for 0.04 to 0.1% of all breast carcinomas and shows an aggressive clinical behavior.1 Despite its rarity, this is a well-described entity that needs to be differentiated from a cutaneous conventional SCC and verrucous carcinoma. Cutaneous conventional SCC arises from the overlying skin or from a pre-existing epidermal inclusion cyst2 and may deeply involve the breast parenchyma while verrucous carcinoma of the breast, that shows negligible cellular atypia and may be associated with HPV infection, seem to be exceedingly rare being so far reported only as an anecdotal isolated case.3

We report on an extraordinary case of 54-year-old woman presenting with a rapidly enlarging cystic lump in the breast histopathologically diagnosed as a well-differentiated squamous cell carcinoma (SCC) with an endophytic architecture reminiscent of verrucous carcinoma arising from the skin of the breast and involving the mammary parenchyma. The challenges in the diagnosis and accurate classification of this peculiar tumor, its possible development from a pre-existing epidermal inclusion cyst and its appropriate management are discussed.

Case report. In November 2015, a 54-year-old woman came to our attention for a hypochoeic cystic lesion in the lower outer quadrant of the right breast, 13 mm in diameter, diagnosed during a control breast ultrasound (US). Fine needle aspiration cytology (FNAC) was performed, revealing isolated squamous cells in a background of inflammatory cells and these findings were considered consistent with an epidermal inclusion cyst complicated with supplicative inflammation. The patient underwent antibiotic therapy for 20 days. In December 2015, the patient presented a palpable mass in the same region. Another US was performed, revealing an enhancement of the lesion that measured 27 mm in diameter, with irregular margins, inhomogeneous pattern and enhanced vascularization. FNAC showed similar findings consistent with an inflammatory process surrounding a cyst. The patient was treated with local incisions and medications. In March 2016 the ulceration and enlargement of the lesion up to 56 mm in diameter, led to the request of a CT of chest and abdomen, that described, besides the primary nodule, the presence of omolateral lymph nodes and three subcentimeter pulmonary lesions with undeterminate significance. Two different biopsies of the breast lesion, showing isolated squamous cells with mild cytological atypia in the background of diffuse necrosis and inflammation. The diagnosis was not conclusive and a diagnosis of SCC could not be ruled out. In addition, an axillary lymph node biopsy was made, showing reactive hyperplasia. The patient continued to be treated with antibiotic therapy without recovery until April 2016, when another CT was performed in order to plan the surgical excision. CT scan showed a further growth both of the lesion up to 83 mm in diameter and of the lymph nodes. Surgical excision by quadrantectomy was performed. Histopathological examination showed a cutaneous well-differentiated SCC, with an endophytic growth, infiltrating the mammary parenchyma; the superior and inferior margin were close (0.02 and 1 mm). The tumor displayed clear relationship with the overlying epidermis, supporting its cutaneous origin. Due to the incomplete excision of the tumor, in June 2016 a second surgery was performed, after a treatment with neoadjuvant chemotherapy with carboplatin plus taxol iv q 21. The final histopathological examination confirmed the diagnosis of a cutaneous well-differentiated SCC, with an endophytic growth. In situ hybridization for HPV was negative. The patient underwent adjuvant chemotherapy with the same schedule and radiotherapy as well as breast reconstruction. The patient is alive and under follow-up, four months after the diagnosis.

Discussion. The case herein described was diagnostically challenging on FNAC and biopsy due the clinical appearance of a cystic breast lump in the presence of extensive necrosis or inflammation obscuring diagnostic cells and a sampling error could not be ruled out. Despite negative findings more suggestive of an inflamed cystic lesion, the rapid growth of the lesion reaching large dimension led to close follow-up and subsequent surgery. The complete histopathological examination allowed to achieve the correct diagnosis of a well-differentiated cutaneous SCC of the breast, which, on the basis of the clinical history, might have been developed in relation with the a pre-existing epidermal inclusion cyst. Histopathologically, the main differential diagnosis included pure SCC of the breast (that was excluded due to the relation with the overlying epidermis) and verrucous carcinoma. Verrucous carcinoma is a type of squamous cell carcinoma that is locally invasive and has a low to negligible rate of metastasis4. Although our case displayed an endophytic growth pattern, it lacked other morphological features (exophytic "warty" growth which can have multiple open sinuses on the surface, lack of irregular infiltrative nests or extensions from the tumor). The expected clinical behaviour of a large well-differentiated SCC of the breast skin is less aggressive in comparison with pure SCC of the breast although lack of sufficient clinical information of a sizable series does not allow to reach definite conclusion on the proper treatment and clinical outcome.

References

THE MORE WE KNOW, THE MORE WE ARE BEWILDERED: CHALLENGING DIAGNOSIS OF A CUTANEOUS LYMPHOPROLIFERATIVE LESION WITH DUAL GENOTYPE

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Introduction. Synchronous monoclonal IgH and TCR gene rearrangement (dual genotype) has been rarely described in lymphoproliferative disorders. Dual genotype may underlie actual bigenotypic lymphomas, with the same neoplastic cell bearing both IgH and TCR gene rearrangements, or may represent lymphoproliferative processes harbouring two distinct (T and B) monoclonal populations. In primary cutaneous lymphoproliferative disorders monoclonal birearrangements are less frequently encountered than in nodal lymphomas. The clinico-pathological characteristics and the biologic potential of these lesions have not be fully elucidated. We herein detail a case of a cutaneous lymphoproliferative lesion with dual lineage rearrangement. In addition to the bewildering molecular genetics findings, this case also showed perplexing histological and immunophenotypical features, thus representing a diagnostic challenge.

Materials and methods. A 67-year-old patient presented with a six month history of erytematous, at times hairless, patches and plaques of varying sizes, affecting the trunk and the upper and lower limbs. Raising a suspicion of mycosis fungoides, an incisional biopsy of one of the patch lesions was carried out. Histopathological and immunohistochemical examination, along with molecular biology assays following the EuroClonality (BIOMED-2) guidelines, were performed. Considering the recent observation of this case, data concerning therapy regimens and outcome are not available at this time.

Results. Histopathology examination revealed a band-like lymphocytic infiltrate lying beneath the epidermis, with a grenz zone of uninvolved papillary dermis. Intraepidermal lymphoid proliferation was not noteworthy. Dermal infiltrates consisted of mostly little to medium sized lymphocytes. Immunohistochemically, the prevalent population was of T-lineage, showing a CD3+, CD5-, CD4+, CD8- phenotype. Staining for CD30 was negative. Admixed with the T-cell infiltrate was a somewhat discordant feature, as the B-cell population (CD20+, PAX-5+, CD79a+, BCL-6-, BXL-2+) was present. Meshworks of CD21+ follicular dendritic cells were not detected. Immunofluorescence examination identified a monoclonal pattern of T cell receptor β and γ and immunoglobulin heavy chain in the same specimen. A provisional diagnosis of mycosis fungoides combined with a clonal B-lymphoproliferative disorder of undetermined clinical significance was then suggested.

Conclusions. We observed a cutaneous lymphoproliferative lesion in which pathological, phenotypical and genotypical features were not distinctive for a specific lymphoma histoty. Interestingly, a simultaneous B-cell and T-cell immunophenotype and clonal rearrangements of TCR β/γ and IgH were documented. Crediting the diagnosis of mycosis fungoides, the presence of a clonal B-cell population may be interpreted as a reactive clonal population, although a low-grade lymphoma may not be excluded.

References


CUTANEOUS MUCORMYCOSIS: A CASE REPORT

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Background. Mucormycosis is a fungal infection caused by fungi of the order Mucorales, genera Mucor, Rhizopus, Rhizomucor, and Lichtheimia, mainly affecting immunocompromised patients, and burdened with a high mortality.

Cutaneous mucormycosis is the third most common clinical form of the disease, after pulmonary and rhino-cerebral. The predisposing factors are hematological malignancies, diabetes mellitus and solid organ transplantation. Trauma is the most common predisposing factor leading to mucormycosis in immunocompetent patients. If the patient is immunocompromised, the infection may disseminate.

The agents of mucormycosis are ubiquitous in nature and are transmitted to the skin by direct inoculation, as a result of various types of trauma (needle sticks, bites by animals, motor vehicle accidents, natural disasters, and burn injuries). The typical presentation of mucormycosis is the necrotic eschar, but it can present with various other signs.

Cutaneous form may be localized, may extend to deep underlying tissues, or may be disseminated. Diagnosis is difficult and histological examination and culture of soft tissue are necessary for the diagnosis. Treatment consists of surgical debridement, administration of antifungal agents (amphotericin B formulations and/or posaconazole), reversal of underlying risk factors and, occasionally, hyperbaric oxygen. Mortality rates are significant, ranging from 4% to 10% when the infection is localized.

Methods. A 69-year-old man with diabetes mellitus treated with oral antidiabetic was admitted to the hospital because of severe trauma crush with graft of the palm of his right hand with fracture of the fourth metacarpal and contextual vascular and muscle injuries. Emergency surgical intervention was performed at plastic surgery department. Initially, the patient was treated with empirical chemotherapy with partial improvement. After a few days there has been a deterioration of local conditions of the surgical wound with increase of necrotic area and the appearance of very smelly secretions. Surgical debridement and wound covering, with skin grafts taken from the thigh with zimmer, was carried out.

Results. Fragments taken from the center of the lesion included subcutaneous fat were sent to the Unit of Pathology of A.R.N.A.S. Civico Palermo. Microscopically H&E stain showed infarction of dermis and subcutaneous fat with
lymphohistiocytic and neutrophilic infiltrate, as well as angioinvasion by very large, long, non-septate hyphae, up to 30 millimicron in diameter, positive to PAS staining and Silver Methenamine. The hyphae were also found in the surrounding tissue and were thin, twisted or collapsed and they appeared ring shaped or oval in cross or tangential sections. Histological findings were consistent with mucormycosis. Treatment with amphotericin B (3mg/kg/day e.v.) was prescribed. Antifungal therapy was continued for a total of 14 days, monitoring the renal function, obtaining the progressive objective improvement of the surgical wound and the negativity of the microbiological swab.

Conclusions. Mucormycosis remains a severe infectious disease in diabetic patients and it is characterized by a high mortality rate. Clinical diagnosis is often difficult and delayed. In patients with diabetes or other immuno-comorbidity, the deterioration of a post-traumatic injury despite adequate medical and/or surgical therapy has to suspect the presence of mucormycosis, suspicion to be confirmed by histological examination, required for diagnostic certainty. Early diagnosis with combined medical and surgical treatment is necessary to improve the outcome. Aggressive diagnostic procedures for histo-microbiological studies are required to confirm this disease and to reduce mortality. Providers should consider microwave assisted rapid tissue processing for the H&E staining or evaluation of frozen sections in order to expedite diagnosis.

References

PATOLOGIA MAMMARIA

BREAST TUMOURS RESEMBLING PAPILLARY THYROID CARCINOMAS (BPTC): REPORT OF 15 CASES WITH LONG TERM FOLLOW UP

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Introduction. Breast tumours resembling papillary thyroid carcinomas (BPTC) have been described in 20031. Here, 15 cases are reported with long term follow up.

Materials and Methods. Thirteen cases are from the consultation files of two of us (MPF & VE). Two cases had been previously included in the original series1. Representative blocks were available in 7 cases and the selected sections were stained for ER, PR; mitochondrion; CD 31; CD 34; keratin 7; keratin 14; p63; GCDFP-15; smooth muscle actin; calponin; TTF1; thyroglobulin laminin; collagen IV, AR, Ki67 and HER2 using an automated slide processing platform. The cut-off value for ER, PR and AR positivity was set at >1%. The percentage of Ki67-positive cells was recorded, and the cut-off for dichotomizing tumours with low and high proliferative fractions was established at 10% of positive cells. Clinical data of the 15 patients including age, type of surgery, type of treatment, occurrence and type of relapse and current status were obtained. Pathological data included morphological features, grade2, size of the tumours, peritumoural vascular invasion and stage of tumours3. The study was conducted in compliance with the ethical regulatory issues.

Results. Patients were female, mean age 64 years (range 48-85 years). Cases were discovered at mammography, except for case 3 that had a lump present for ten years. All cases initially underwent conservative surgery. Sentinel nodes obtained in six cases were negative. Tumour size was in mean 1.6 cm (range 0.6-2.5 cm). Thirteen cases were scored G1 and two were G2. Vascular invasion was focal in one case. The majority of the patients (66.7%) had a pT1 and the remaining had pT2 tumours (33.3%). The lymph node status was pN0 in 4 and pN1a in 2 out of 6 cases. All cases displayed neoplastic cells arranged in solid to papillary structures as well as follicles of different size filled by eosinophilic colloid-like material. These structures were closely reminiscent of those seen in thyroid and were observed in all cases. Follicles varied in number being scanty in some cases, numerous in other and were the majority as in case 15. Neoplastic cells evidenced abundant eosinophilic granular cytoplasm polarized mostly towards the basal pole. Nuclei were optically clear with a small nucleolus and a well evident nuclear membrane. Numerous grooves and occasional eosinophilic nuclear pseudo-inclusions were visible. All cases studied for mitochondria in >50% of the neoplastic elements. ER, PgR, AR, HER2, TTF1, Thyroglobulin were all negative. Ki-67 scored less than 10% in all cases. Myoepithelial cells were absent in the 10 cases tested (P63 and smooth muscle actin negative). Laminin and collagen IV antibodies evidenced abundant material surrounding the neoplastic solid clumps as well as within the papillary fronds. This material contained within it numerous small vessels as shown by CD 31 and CD 34 and superficially gave a fallacious feature of basal lamina. Case 2 recurred after 36 months from initial surgery. The patients underwent mastectomy and axillary dissection. One axillary node evidences a metastatic deposit with similar histological features of the primary tumour. Case 3 had an intramammary metastatic lymph node. All patients are alive and well after 77 months (mean) of follow up (range 10-192 months). No fewer than 3 patients had 10 years of follow up.

Conclusions. Breast tumours resembling papillary thyroid carcinomas have to be recognized to avoid misdiagnosis as metastatic PTC from thyroid. BPTC are triple negative carcinomas with indolent clinical behavior. They rarely recur and metastasize. No case of death has been described. This is the reason to retain for these lesions the term “tumour” and avoid that of carcinoma for neoplasms that rarely recur and metastasize. All the present cases were invasive, paralleling the features seen in solid papillary carcinomas of the breast that also have indolent behaviour4. Studies are needed to elucidate the reason why such cases in spite of extensive invasion show a very low grade of malignancy.

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CYTOLOGICAL FEATURES OF DUCTAL ADENOMA OF THE BREAST: CASE SERIES AND HISTOLOGICAL CORRELATION

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Introduction. Ductal adenoma (DA) is a rare benign neoplastic condition of the breast that can simulate malignancy for its clinical, radiological and pathological features. Preoperative diagnosis is of utmost importance to correctly plan the therapeutic approach. Among the preoperative procedures, fine needle aspiration cytology (FNAC) is considered an easy therapeutic approach. Among the preoperative procedures, fine needle aspiration cytology (FNAC) is considered an easy to perform, secure for the patient and cheap method. Nevertheless, FNAC provides a limited amount of pathological material for the diagnosis and, even if rarely, misdiagnoses can occur. Few data are available of the cytological features of ductal adenoma (DA). We present a series of cytological cases of DA with the purpose to underline the importance of a proper identification of this lesion to avoid misdiagnosis.

Materials and methods. We reviewed 3568 cases who underwent a cytological examination between January 2008 and October 2015 at Bellaria Hospital, University of Bologna (Italy) and selected all the cases that received the histological diagnosis of DA. A total of 20 cases with both cytological and histological data were identified. The cytological examination was performed on samples obtained by nipple discharge (6 cases) and ultrasound guided FNAC procedures (14 cases). Cytological smears were stained with the Papanicolau method after being fixed in 95% ethanol. All lesions have been surgically removed, formalin fixed and embedded in paraffin.

Results. All patients were female, ranging in age from 15 to 81 years (mean value 53), with no previous history of breast pathology. Although the majority presented only a palpable breast lump on clinical examination, nine patients exhibited spontaneous nipple discharge, showing serous background in breast occupying medium and large-sized breast ducts. This lesion may occur in women of all ages, although the majority of patients are older than 50. Ductal adenomas usually present clinically as breast lumps which may mimic carcinoma, especially if exhibiting spontaneous hematic nipple discharge. Despite showing worrisome cytology, the histological features together with the immunohistochemical demonstration of a myoepithelial layer were clear evidence of the benign nature of the lesions.

Discussion. The cytological examination is a valid and widely used method to early identify breast lesions. The correct diagnosis of DA may be limited by the lack of knowledge about this lesion due to his uncommon occurrence and to his worrisome cytomorphological features. Major pitfalls in the differential diagnosis consist of hypercellularity and nuclear pleomorphism that may lead to an overdiagnosis of malignancy. The different patterns of growth such as ductal, tubular or finger-like papillary morphology contribute to make this diagnosis problematic on FNAC smears. Good knowledge of DA cytological features can lead to a right identification and ensure a proper treatment of this uncommon lesion.

References

PARP1, BRCA1 AND NHERF1 EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER PATIENTS

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Introduction. Triple-negative breast cancers (TNBCs) are defined by the absence of estrogen and progesterone receptors and the absence of HER2 overexpression. They represent
Conclusions: In agreement with Green et al.1 and our previous study2 on patients with primary operable invasive breast cancer, and we could speculate that nPARP1 in association with NHERF1 represents a possible target in TNBCs. Further studies are needed to clarify this aspect in TNBC patients.

References

PRIMITIVE SQUAMOUS CELL CARCINOMA OF THE BREAST (SCCB): CASE REPORT OF AN UNCOMMON VARIANT OF METAPLASTIC CARCINOMA

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Introduction. Metaplastic carcinoma of the breast comprises a heterogeneous group of neoplasms generally characterized by a close admixture of adenocarcinoma with areas of spindle, squamous, chondroid and osseous differentiation. If the tumor shows a purely squamous differentiation, it is designated squamous carcinoma. SCCB account for significantly less than 1% of all invasive breast carcinoma forms. They seem to derive from epithelial or myoepithelial cells of glandular ducts in squamous metaplasia and can present as cystic lesions, often of considerable size, that can simulate a breast abscess.

Case report. A 75-year-old woman came to our attention with a lesion measuring about 5 cm in the IMQ of the right breast. At mammography in December 2014 it appeared as a 3cm oval opacity with microcalcifications and an irregular isoechogenic US appearance resembling a solid-cystic lesion. Cytology suggested a cystic inflammatory lesion (C2) to be monitored after antibiotic therapy. A subsequent ultrasound check in September 2015 confirmed an increased volume (5 cm), which did not regress after serial evacuative bites, so microhistology sampling was performed for suspected malignancy (B4). In February 2016, the patient underwent excision of the cystic lesion.

Results and conclusions. The final histological examination demonstrated a SCCB with diffuse positivity for Cytokeratin 34βE12 and p63; negative reactions to ER and PR; growth fraction (evaluated with monoclonal antibody Ki67) > 40%; HER2/neu with score 2+ and a subsequent negative FISH examination. The patient underwent radical mastectomy sec. Madden which confirmed the histological diagnosis and the negativity of the lymph nodes. In literature, the prognosis and therapy of SCCB are still much debated. In fact, it shows poor responsiveness to common chemotherapy. In relation to the size, a large quadrantectomy or a radical mastectomy with lymph node dissection must be considered the best treatment for this rare form.

References
SENTINEL NODE "MICROMETASTASES" DETECTED BY OSNA IN BENIGN BREAST LESIONS: WHAT ARE THEY?


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Introduction. Sentinel node (SN) biopsy is generally considered the standard procedure for staging patients with clinically node negative early breast cancer. In this study we present seven patients affected by benign breast lesions who, for different reasons (generally an equivocal histological pattern on the core biopsy or a suspicion for malignancy) were subjected to SN examination by OSNA (One Step Nucleic acid Amplification), resulting positive for micrometastases.

OSNA (One Step Nucleic acid Amplification) is a molecular method which allows to evaluate the whole lymph node during the surgery in a extremely standardized way, in a time of about 50’. OSNA assay is based on detection and amplification of CK19 mRNA and yield a semiquantitative result for detecting clinically relevant nodal metastases. CK19 mRNA copy number detected by the OSNA assay can provide good estimates of macrometastases or metastases (CK19 mRNA copy number >5000) and micrometastases (CK19 mRNA copy number <5000 and >250). With a CK19 mRNA copy number <250 the SN is considered metastasis-free. (1) OSNA revealed a better sensitivity to identify micrometastases than traditional histological examination. (2, 6)

Over the past years some authors stressed (3,4,7) the occurrence of non malignant epithelial cells in axillary lymph node; their presence has been generally ascribed to a displacement due to a passive transport to the SN after trauma caused by core biopsy (CB) or fine needle aspiration cytology (FNAC) similarly to the lymphatic drainage to the SN of a large number of cells (mainly inflammatory and epithelial cells) other then some non cellular substances.

Furthermore axillary nodes, though very rarely (0.1-0.2%), may harbour ectopic glandular breast structures (5) and in such very rare cases ectopic breast gland may be responsible for false positivity of SNs.

The current study was aimed at analysing the possible factors responsible for a SN-positivity by OSNA assay in seven patients harbouring benign mammary lesion.

Material and methods. From January 2013 to December 2015, 1958 breast cancer patients from 3 different Apulian Hospitals (Policlinico and San Paolo Hospital of Bari and Casa Sollievo della Sofferenza of S.Giovanni Rotondo) underwent SN analyses by OSNA assay. Almost all the patients had received preoperative diagnosis by CNB (tru-cut or mammotome) or FNAC. The remaining having had confirmed the diagnosis on frozen section.

In each case axillary lymph nodes were no palpable nor ecographically suspected and SN was always identified by injection of a radioisotope tracer (99Tc-labelled nanocolloid). Before surgery SNs were identified using a handheld γ-ray detection probe. Radioactive nodes were considered the SNs and excised.

OSNA ASSAY: Each excised SN was immediately sent on ice to the pathology laboratory were perinodal fat was removed. Each node was measured (3 dimension) and weighted and subsequently analyzed according to the manufacturer’s instructions (Sysmex, Japan).

All patients in whom macrometastases were detected in the SN underwent immediate and complete axillary dissection (ALND), while only a few patients harbouring micrometastases in the SN were subjected to axillary clearance; the latter was not performed in patients with negative SN. All surgical samples (quadrantectomy or mastectomy) were formalin-fixed, paraffin-embedded and stained with haematoxylin-eosin.

Results. From the 1958 patients enrolled from the 3 participating Apulian Hospitals in the period 2013-2015, a total number of 2.545 SNs was examined by OSNA (mean: 1.3 SNs/patient).

More than 75% of patients had had a preoperative diagnosis on core biopsy, 18% on FNAC and 8% on frozen sections. Metastases were identified in 14.3% and micrometastases in 15.6% of SNs.

Among the cases with micrometastases in SNs, 7 of them (Tab. 1) were diagnosed as benign breast lesions, namely: 3 fibroadenomas, 1 apocrine adenosis, 1 granular cells tumour and 2 papillomas.

In one case a fibroadenoma was associated with an intraductal papilloma and another case also showed atypical ductal hyperplasia.

The 7 patients harbouring such benign lesions were submitted to SN biopsy because of pre-surgical diagnosis (CBN= 4, FNAC= 2, CNB+FNAC= 1 case) of malignancy in two cases (n. 1 and 4), NMR hyper-captation “typical for heteroplasia”, despite previous cytopathological diagnosis of benign lesion, in another case (n. 3) and of “suspicious for malignancy” on CNB in the remaining four cases (n. 2, 5, 6 and 7). Also, 2 patients (n. 3 and n. 5) were affected by synchronous, carcinoma in the other breast associated with micrometastasis (n. 3) or with metastasis (n. 5) to the contro-lateral SN. Only one patient (n. 3) underwent complete axillary lymph node dissection (ALND), which resulted metastases-free, following the identification by OSNA of micrometastasis in the SN at the same site of the benign lesion.

Discussion. The occurrence of non-carcinomatous epithelial cells in axillary nodes has been repeatedly reported and it can be due to ectopic glandular tissue (5), or displacement of (benign or malignant) cells following vessel disruption after biopsy (3,4). The presence of epithelial cells in axillary nodes (7), mainly located in the marginal sinus, may be associated with inflammatory infiltration and, obviously, such mechanism can occur for both malignant and benign lesions, as well as for normal mammary tissue, as a consequence of traumatic events.

The increased accuracy of the examination of SNs (serial histological sections vs. whole-node molecular analysis) certainly facilitated the identification of such “metastatic foci” (3). Carter (3) hypothesised the displacement of both normal or neoplastic cells following pre-surgical diagnostic procedures (FNAC or Core biopsy) as a possible cause of false-positive SNs.

The 7 cases reported herewith certainly are a very rare event (0.35% of the current series) and they can really be considered the result of overdiagnosis. Nevertheless, they strongly support the hypothesis of passive displacement of epithelial cells to the SN, by disruption of lymph vessels possibly caused by pre-operative diagnostic procedures, such as CNB, FNAC or both.

Such mechanism apparently is easier to occur in papillomatous lesions, due to their peculiar structure and rich vasculature (3), as detected in 3 cases of the current series. Complete axillary clearance is becoming increasingly less frequent in patients harbouring micrometastases only to the SNs;
THE USE OF A MOLECULAR TEST FOR BREAST CANCER PROGNOSIS: CLINICAL-PATHOLOGICAL CORRELATIONS AND THERAPEUTIC IMPLICATIONS ON A SELECTED COHORT OF PATIENTS.

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Goal. Over the last decade, gene signatures of prognosis are emerging for tailoring personalized treatment strategies based on the risk profile of individual patients11. These molecular tests are particularly useful for patients who are affected by estrogen receptor-positive breast cancers of indefinite prognosis12-14. We focused on Endopredict® (Myriad Genetics), a multi-gene test, which gives both a pure molecular fingerprint of the tumors (the “EP score”) and a score obtained by combining the EP score with the tumor size and number of metastatic lymph nodes (the “EPclin score”)15. EPclin score discriminates between patients having “low risk” or “high risk” of relapse within 5 years. The attitude to use gene signatures in routine clinical work still varies in different countries. We decided to test the influence of the EPclin score on the therapeutic decision [adjuvant hormonal therapy (HT) alone or HT + chemotherapy (CT)] related to breast cancer with not univocal prognosis based on standard parameters.

Methods and materials. From July 2014, we prospectively collected 56 cases of breast cancer for which the therapeutic intervention was debated at multidisciplinary meeting. The criteria of case selection were: tumor size < 2 cm; lymph node status negative or from 1 to 3 positive lymph nodes; high expression of hormonal receptors (>50% of cancer cells); intermediate expression of Ki67 (15-30%); HER2 negativity. For each case, clinical and pathological data were recorded [age, tumor size, nodal status, grade according with Elston-Ellis, Estrogen (ER) and Progesterone (PgR) receptors expression rates, Ki67 proliferation rate]. EndoPredict® was performed on each case and EP score and EPclin score were recorded. We then correlated both scores with clinical and pathological data. To investigate whether the molecular test results would have facilitate the agreement on the therapeutic protocols, the data base was submitted to 26 oncologists, who were asked to indicate the therapeutic option (HT versus HT + CT) before and after the Endopredict® results.

Results. EP score was significantly related with lymph nodes status (p 0.008), tumor grade (p < 0.001) and PgR expression (p 0.007); EPclin score was related with tumor grade (p < 0.001), PgR expression (p 0.033) and Ki67 proliferation rate (p 0.009). In 11 cases the risk was assessed as “high” according with EP score and “low” with EPclin score, while in 3 cases the risk was “low” by EP score and “high” by EPclin score. Treatment agreement was low (Cohen’s K: 26%; Z: 26.47) when oncologists were blind to Endopredict® results and improved following the results of the molecular test (Cohen’s K: 58%; Z: 24.32). The therapeutic indication changed from HT to HT + CT for 9 patients and from HT + CT to HT alone for 6 patients.

Conclusions. Both EP score and EPclin score correlate with grade and PgR expression. EPclin score gives a more comprehensive estimation of the risk of relapse and improves the agreement between oncologists in the subgroup of patients for whom the therapeutic decision is not univocal.
PATOLOGIA MOLECOLARE

LIQUID-BASED CYTOLOGICAL SAMPLES (THIN PREP®) IN THE DETERMINATION OF THE MOLECULAR PROFILE IN PATIENTS WITH UNRESECTABLE NSCLC, EGFR TYROSINE AND KINASE INHIBITOR THERAPY CANDIDATES

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Introduction. Mutational status of Epidermal Growth Factor Receptor (EGFR) and ALK gene rearrangements are crucial to identify patients with non-small cell lung cancer (NSCLC) who may respond to treatment with EGFR inhibitors (EGFR-TKis). Most of these patients arrive to diagnosis at an advanced stage of the disease where a surgical excision of the tumor is not recommended and the only source of biological samples for histological diagnosis is often represented by cytological specimens. The aim of this study was to evaluate if the residual volume of PreservCyt vial of cytological NSCLC sample that remain after preparation of liquid base prepared Thin Prep slide, contains enough malignant tumor cells informative for EGFR mutational status and ALK rearrangement.

Methods. Between 2014 and 2015 EGFR mutational status and ALK gene rearrangement have been required for 283 patients with lung carcinoma (113 females, 168 males). For 122 patients cytological specimens were collected and preserved in Thin Prep PreservCyt Solution (Hologic, UK). For cytology diagnosis and ancillary studies slides were prepared by Thin Prep @ 5000 processor with Autoloader. Immunohistochemistry was performed with DAKO Autostainer (reagents by DAKO). From the residual cells remained in PreservCyt vials of 122 cytological specimens (20 effusions, 102 FNA) that showed at least 20% of malignant cancer cells on LBP-TP-slide, DNA was extracted (QIAamp DNA mini kit QIA-GEN) and dosed with Nanodrop. EGFR mutational analysis (exons 18-21) was performed by allele specific Real-Time PCR (Therascreen EGFR RGQ PCR CE-IVD Qiagen). According to the guidelines AIOM-Siapec (2014), samples with mutated EGFR (EGFR-mut) were no further characterized while those with EGFR gene (exons 18-21) wild type (EGFR-wt) were subsequently analyzed for ALK rearrangements by FISH (SPEC ALK/ EML4 Tricheck Probe, Zytovision)

Results. Immunohistochemistry was performed on 103 out of 122 LBP-TP specimens. After morphological evaluation 3 out of 122 patients resulted inadequate for the molecular investigation (2 with squamous cell histology and 1 with necrotic cells) 98% of samples were tested successfully for mutational status of the EGFR gene (exons 18-21).

21 out of 119 samples (17, 6%) were mutated in the EGFR gene (11 del19, 1 G719X, 1 Lns20, 8 L858R). A sample EGFR-wt presented a K-RAS gene mutation (Exon 2 cd12 GGT > GTT pG12V).

81 out of 119 samples EGFR-wt were evaluated for ALK – EML rearrangement.

7 out of 67 cytological specimens resulted positive to FISH analysis (≥15% nucleus rearranged) Conclusions

The frequency of mutations in the EGFR gene found in our cohort of patients with NSCLC is similar to that reported in the literature (17.7%).

Molecular investigation was possible with success on LBP cytological specimens positive for malignant cancer cells as observed in LBP –TP slides. The quality of the DNA obtained from the LBP cytological samples is much higher compared to that obtained from FFPE histological slice. This allows have information on molecular status of the EGFR gene and ALK rearrangement from small amount of sample and more rapidly respect to histological specimens that require prolonged time and effort to be prepared. Our study shows that LBP-cytological samples can be used in determining the molecular profile in patients with unresectable NSCLC.

AGAIN HER-2/NEU AMPLIFICATION IN SQUAMOUS CELL LUNG CANCER.

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Introduction. Human epidermal growth factor 2 (HER-2/neu) activation, either as mutation or amplification, is identified in approximately 2-5% of non–small-cell lung cancers1, predominantly observed in patients with adenocarcinoma subtype. Occasional reported cases of HER-2/neu amplification in squamous cell lung cancers2 raise the question whether this subtype should be evaluated for such molecular alteration and selected for ad hoc clinical trials.

Methods. We assessed HER-2/neu gene amplification and immunohistochemical overexpression in surgically obtained specimens from 34 cases of squamous cell lung cancer (30M, 4F; median age 70 years). Tissue microarray was built with 3 cores for each tumor. The HER-2 expression was evaluated by immunohistochemical staining and scored according ASCO/CAP 2013 guidelines from 0 (absence of staining) to 3+ (strong and complete membranous staining in >10% of tumor cells). Fluorescence in situ hybridization (FISH) was used to find HER-2/neu amplification (ratio of HER-2/neu gene copies to chromosome 17 centromere copies >2 or HER-2/neu gene copies >6 in >10% of tumor cells). Silver in situ hybridization (SISH) was also performed.

Results. HER-2 expression was found in three tumors (3+ in two tumors and 2+ in one tumor). HER-2/neu gene amplification was observed in the two 3+ immunoreactive tumors (overall, 2/34, 6%). No heterogeneity has been observed in these tumors. In both cases (1M, 1F), the prognosis was poor. Three tumors showed scattered clearly amplified cells in a background of disomic cells (intermixed single cells heterogenous pattern). No protein overexpression was present in this subset of tumors.

Conclusion. Strong HER-2/neu amplification was found in 6% of squamous cell lung cancer, with high concordance between immunohistochemistry and FISH/SISH techniques. These selected patients should be enrolled in prospective clinical trials targeting such genetic alteration.

References

"EPITELIOID MYOFIBROBLASTOMA WITH ATYPICAL CELLS OF MALE BREAST: A CASE REPORT"

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Background. Myofibroblastoma (MFB) of the breast is an unusual benign tumor that belongs to the family of the "benign spindle cell tumors of the mammary stroma". The name MFB reflects its cellular composition, comprising mainly stromal cells with fibromyofibroblastic and, less frequently, myoid differentiation. Although classic MFB is typically a bland-looking spindle cell tumor, some unusual morphologic variants may show worrisome malignant-looking cells. Recognition of MFB variants and its wide variety of mimickers is very important to arrive at the correct diagnosis, and avoid misdiagnosis of malignancy.

Materials and methods. In september of 2015 a 82 year-old man presented to the clinical breast examination complaining of an indolent swelling in the right breast. Anamnesis clinic documented nor gynecomastia, nor a previous local trauma, nor a local radiation therapy, nor any previous breast malignant neoplasm such as invasive or in situ carcinoma. Physical examination discloses a solitary, unilateral, painless, freely movable, firm in consistency, round in shape, medial inferior nodule of right breast measuring approximately 5 cm in size, that has been growing slowly during the course of several months, from 12 to 18 months. There were no signs of inflammation and not associated with axillary lymph nodes. Ultrasoundography confirms the solid nature of the tumor, showing a well-circumscribed, homogeneous, slightly hypoechoic mass. A clinical diagnosis of fibroadenoma was offered. Subsequently, core biopsy was performed with diagnostic category B3 (sec. European Breast Guidelines, 2006) or benign lesion of uncertain biological behavior to the presence of desmoplastic spindle cell mesenchymal proliferation with low mitotic index and bland atypia. So lumpectomy was performed.

Results. On gross examination the tumor was well circumscribed, nodular and well delineated and measured 5x3,5x3 cm. The cut section reveals a homogeneous, bulging pink whirled surface. Necrosis, cystic degeneration and hemorrhage are not evident. Histological examination revealed an unencapsulated tumor with a pushing, lobulated growth pattern least composed of uniform bland-looking spindle-shaped myofibroblast-like cells with scanty cytoplasm, round or oval nucleus with 1-2 small nucleoli, arranged short haphazardly intersecting fascicles of cohesive cells, interrupted by thick bundles of hyalinized collagen and devoid of mammary ducts and lobules, mainly (> 50%) of larger cells with epithelioid morphology. Epithelioid cells are medium-size mononucleated, binucleated or multinucleated neoplastic cells with abundant eosinophilic cytoplasm with eccentrically located nuclei containing small evident nucleoli, and they are arranged in clusters or alveolar, solid or trabecular growth pattern. Also it is observed the presence of a variable number of atypical mononucleated or multinucleated cells showing a variable degree (mild to severe) of nuclear pleomorphism. Mitotic activity is very low (< 2/10 HPF) in both cellular components. Heterologous mesenchymal differentiation is not observed. On immunohistochemistry, these neoplastic cells were positive for vimentin, CD34, actin HHF35, caldesmon, desmin, SMA, Bcl2, MyoD1, estrogen and progesterone receptors and focally CD68 (multinucleated cells). Cyto-keratins AE1/AE3, CD10, CD117, EMA, S100, HMB45 are negative. Ki67 expression was very low (= 5%). Was made diagnosis of Epiteloid Myofibroblastoma with atypical cells of male breast.

Discussion. Myofibroblastoma of breast is a rare benign spindle cell tumor of mammary stroma, with morphologic features similar to spindle cell lipoma of soft tissue, composed of neoplastic cells showing fibromyofibroblastic differentiation. The term myofibroblastoma of breast was first coined by Wargotz et al. in 1987. The original report demonstrated that MFB had a male predominance. However, later studies illustrated that it can occur in both sexes, mainly older men and postmenopausal women. MFB derive from CD34+/vimentin+ uncommitted mammary stromal cells with ability of multidirectional mesenchimal differentiation along several lines, including fibroblastic, myofibroblastic, adipocytic, leiomiomatous, osseus and cartilaginous. So, MFB encompasses, in addition to classic-type, a wide variety in histologic variants including cellular, infiltrating, epithelioid, decidual-like, lipomatous, collagenized/fibrous and myxoid. In some cases, two or more variants coexist within the same tumor. Sometimes MFB, specially in cellular, epithelioid, myxoid and decidual-like variants, may contain a variable number of atypical bizarre mononucleated or multinucleated cells. This alarming feature may cause a misdiagnosis of malignancy. These atypical cells have been regarded as degenerative in nature, similar to what was observed in other benign soft tissue tumors (ie, atypical/symplastic leiomyoma, ancient schanoma).

Conclusion. Due to the broad morphologic spectrum of MFB, this uncommon benign tumor may mimic a wide variety of both benign and malignant breast spindle cell lesions (solitary fibrous tumor, benign spindle cell lipoma, meta-plastic spindle cell carcinoma, low grade myofibroblastic sarcoma, phyllodes tumor and others) causing a potential diagnostic pitfall. Immunohistochemistry and even cytogenetic analysis may be necessary to arrive at a correct diagnosis in some difficult cases.

References

Figure 1a. EE, 20X.
USEFULNESS OF DETERMINING THE METHYLATION OF MGMT GENE PROMOTER IN PATIENTS WITH ADVANCED METASTATIC COLORECTAL CANCER NOT RESPONDING TO MEDICAL TREATMENT

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Background. The development of colorectal carcinoma (CRC) covers about 5-10 years, making prevention and early diagnosis advantageous for patients survival (1). The O6-methylguanine DNA methyltransferase (MGMT) is involved in DNA damage repair and methylation of MGMT gene promoter and it can predict the benefit of alkylating agents, such as Tomozolomide (2). Methylation of MGMT gene plays an important role in Colorectal carcinogenesis, which occurs approximately in 30-40% of metastatic colorectal cancer (3). Its prognostic role has not been defined yet. The overall survival of patients with colorectal cancer has not been significantly associated with methylation of MGMT (1). The results of biomarker analysis have showed that patients with wild-type KRAS, BRAF and NRAS respond more fully to treatment (44%), than patients with RAS or BRAF mutation (0% P = 0.004) (4). The purpose of this study is to evaluate the pos-
sible integration of biomolecular analysis of routine use with the determination of methylation of MGMT gene.

Methods. From January to December 2015, at the Department of Pathology of “L. Bonomo” hospital of Andria, 125 cases of colorectal surgical resections were analyzed: only 41 cases with metastatic colon carcinoma were taken into consideration. Initially, we analyzed the mutational status of RAS gene, with the following results: 15 RAS wild-type cases and 26 RAS mutated cases. RAS gene mutation analysis was conducted using the pyrosequencing technique. DNA extraction was done using the QIAGEN QIAamp® Mini kit QIAGEN according to standard protocol. The extracted amount of DNA was determined by spectrophotometer QIAxpert® (Qiagen). DNA amplification was performed with the Ther-ascreen RAS RGQ through Polymerase Chain Reaction (PCR) in RealTime on the Rotor-Gene tool (Qiagen). All the amplicons were analyzed by pyrosequencing (PicoMark-Q24 MDX; QIAGEN). Successively, all cases, RAS mutated and RAS wild-type both, were subjected to methylation analysis, conducted through the pyrosequencing technique. Initially, the extracted samples were converted using bisulfite, using the kit EpiTect Bisulfite Kit. The method used to detect and quantify the DNA methylation is based on the DNA treatment with sodium bisulfite, through which the non-methylated cytosine bases are deaminized and sulphated to be converted into uracil, while the 5’-methyl-cytosine bases remain unaffected (Fig. 1). So the treatment allows to discriminate non-methylated cytosine bases from the methylated ones. Successively, the methylated DNA is amplified using the PCR kit Ther-ascreen RAS RGQ through the Polymerase Chain Reaction (PCR) in real time on the Rotor-Gene tool (Qiagen). Finally, the amplicons are sequenced using pyrosequencing (PicoMark-Q24 MDX; QIAGEN). (Fig. 2)

Results. The biomelecular analysis of the data related to the 41 histological findings has revealed that 5 cases out of 26 are moderately methylated (19%), 5 cases out of 26 are hypomethylated (19%) and 16 (62%) cases are not methylated. While, among the 15 RAS wild-type cases, 2 cases are moderately methylated (13%), 3 cases are hypomethylated (20%), and 10 cases are not methylated (67%). Tabella 1

Conclusion. Based on our study, albeit limited to clinical records, it comes to light that in mutated and wild-type RAS cases both, the percentage of methylated cases is equivalent. Then, this data shows that they have to be treated with temozolomide, not only patients with RAS mutation, but also patients with wild-type (20% + 13% = 33% in our series) in order to extend the survival of patients, considering also the low toxicity of the drug.

In conclusion, in patients with metastatic CRC and MGMT methylation, after failure of approved treatments, we consider useful to propose to MGMT test as a protocol for mutated RAS and wildtype cases.

References

Prognostic value of MGMT methylation in colorectal cancer: a meta-analysis and literature review.

Table I. Biomolecular characterization in 41 cases di mCRC.

<table>
<thead>
<tr>
<th>TOTALE CASI</th>
<th>RAS</th>
<th>MGMT IPOMETILIATI</th>
<th>MGMT MEDIAMENTE METILATI</th>
<th>NON METILATI</th>
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<td>41</td>
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<tr>
<td>15</td>
<td>WYLDE-TYPE</td>
<td>3 (20%)</td>
<td>2 (13%)</td>
<td>10 (67%)</td>
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<td>26</td>
<td>MUTATI</td>
<td>5 (19%)</td>
<td>5 (19%)</td>
<td>16 (62%)</td>
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<td>TOT. 41</td>
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<td>8</td>
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<td>26</td>
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Figure 1. KRAS codon 12/13 wilde-type by therascreen® KRAS Pyro Kit – QIAGEN. MGMT unmethylated and methylated by therascreen® MGMT Pyro Kit – QIAGEN

Figure 2. KRAS codon 12/13 wilde-type by therascreen® KRAS Pyro Kit – QIAGEN. MGMT unmethylated and methylated by therascreen® MGMT Pyro Kit – QIAGEN.
MICORRNA PROFILE IN LUNG ADENOCARCINOMA: DIFFERENTIAL EXPRESSION IN YOUNG AND OLD PATIENTS*

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Background. In the last decade, age of patients with diagnosis of lung cancer has been decreased, with an occurrence of approximately 13.4% in patients under 50 years (1). Lung cancer in young adults seems to have several unique characteristics: a high percentage of patients are female, more adenocarcinoma, more advanced stage at the time of diagnosis, and more patients receiving aggressive treatment (2-4). To date, it is still controversial whether younger patients have a different outcome compared with their older counterparts. MicroRNAs (miRNAs) have recently been defined to play a key role in cancer pathogenesis and their aberrant expression has been suggested as a potential biomarker of prognosis in lung adenocarcinoma (5). However, studies about age-related miRNAs in adenocarcinoma (ADC) patients remain limited. To understand the molecular features of young and old adenocarcinoma patients, we investigated the expression levels of a panel of miRNAs selected from recent literature.

Methods. We isolated total RNA including microRNAs from formalin-fixed and paraffin-embedded tumor tissues of forty ADC patients under 50 years old and forty ADC patients older than 50. The expression levels of 30 miRNAs were analyzed by NanoString technology and compared between the two groups. Survival data were used to assess the prognostic impact of miRNAs. To predict the putative pathways targeted by miRNAs we employed the software mirGator v3.0. (6)

Results. The analysis revealed that 7 miRNAs (miR-25-3p, miR-29c-3p, miR-33a-5p, miR-144-3p, miR-153-3p, miR-342-5p and miR-485-3p) were differently expressed in the two groups (Mann-Whitney U test, p<0.05). All these miRNAs showed higher expression levels in young compared to old patients, and their predicted targets included EGF, MET, VEGF-A, TP53 and PDGFRα. miR-144-3p had an opposite influence on overall survival, since its upregulation was associated with a better outcome in young patients (p= 0.01) and a worse prognosis in the old group (p= 0.03).

Conclusion. Our study suggest a differential microRNA expression profile between young and old adenocarcinoma patients. We hypothesized that different regulatory mechanisms may influence the two age-subgroup of patients since we found 7 miRNAs as upregulated in the younger group, probably due to distinct age-related genetic and epigenetic alterations. Moreover, one of the deregulated miRNAs showed a different prognostic impact in the two groups thus supporting that young and old patients deserve a specific clinical approach.

Further validations are needed in a larger cohort of patients to establish if an age-based genomic signature could be used as prognostic marker in lung cancer.

References

ANALYSIS OF CIRCULATING CELL-FREE TUMOUR DNA BY MASS SPECTROMETRY

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Introduction. The diagnostic value of circulating cell-free tumour (ct) DNA for the detection of somatic mutations in plasma of cancer patients has been widely proved [1, 2]. In fact, whenever tumour tissue is inadequate or unavailable, ctDNA screening can be crucial to predict and monitor response to therapy. However, until now only few methodologies have been validated for ctDNA analysis and they all consist of real-time (RT) PCR assays, which generally evaluate a limited number of mutational sites and require high input of DNA [3]. In some cases a more extensive molecular characterization could be essential for patients’ management, and RT-PCR techniques might not be adequate according to the low amount of ctDNA in plasma [4].

In this study we prospectively evaluated the applicability of a multitarget mass spectrometry (MS) technique on ctDNA from lung and colorectal cancer patients by comparing the results with the standard RT-PCR.

Methods. Lung cancer: EGFR mutational status was investigated in plasma from 76 patients by a validated Scorpion/LNA RT-PCR evaluating 30 EGFR mutations and by a routine MS test detecting 307 hot-spots in EGFR and other 9 genes, such as KRAS, BRAF, HER2 and PIK3CA.

Colorectal cancer: mutational status was screened in plasma from 33 patients by a RT-PCR kit evaluating 22 KRAS mutations and by a MS test used in routine practice detecting 216 hot-spots in KRAS and other 3 genes (BRAF, NRAS, PIK3CA).

In all cases ctDNA was purified from 4 ml of plasma using a commercial kit. For all patients molecular status of tumour tissue was available.

Results. Lung Cancer: on tissue there were 59 EGFR mutations, 10 BRAF mutation and 10 KRAS mutations. On ctDNA, RT-PCR was able to detect EGFR mutations in 42 cases (71%), among which 5 were detected only on plasma. MS analysis allowed to detect 28 mutations in EGFR, 2 of which not detected on tissue, with a concordance for EGF of 67% between RT-PCR and MS. In addition, 2 mutations in KRAS were found by MS.

Colorectal Cancer: on tissue 19 cases were mutated in KRAS, 3 in BRAF and 1 in NRAS. On ctDNA, RT-PCR found a KRAS mutation in 15 cases (78.9%). Of these, MS analysis confirmed 12 mutations, thus concordance for KRAS between RT-PCR and MS was 80%. Moreover, MS genotyping confirmed 1 of the 3 mutations in BRAF and the unique case mutated in NRAS; MS found also 2 mutations not previously detected on tissue in PIK3CA.

Conclusions. The molecular characterization of lung and colorectal cancers has become a routine approach aimed to stratify patients for targeted therapies and to monitor their
response. Recently, ctDNA analysis has been extensively investigated and it is increasingly utilized as a substrate to detect clinically relevant molecular alterations in cancer. Indeed, ctDNA analysis is less invasive than tumour biopsies, and can provide a whole representation of the cancer patient.

In this study, we demonstrated the adequacy of ctDNA for a MS analysis, both in terms of quantity and quality of nucleic acids. The concordance between RT-PCR and MS was satisfactory either in lung and colorectal cancer, raising 67% and 80% respectively for their main oncogenes. Moreover, in both tumour models, MS was also able to detect molecular alterations in genes other than \textit{EGFR} and \textit{KRAS}, some of which were never detected in tissue samples. These results suggest that a multitarget methodology such as MS performed on ctDNA might improve tumour characterization and monitoring of response, being able to mirror tumour heterogeneity. Besides these promising findings, a validation cohort of patients is needed to better explore MS applicability on ctDNA, evaluating its sensitivity and its real clinical utility.

References

GENE EXPRESSION PROFILING OF FOLLICULAR THYROID ADENOMA, MINIMALLY INVASIVE AND WIDELY INVASIVE FOLLICULAR THYROID CARCINOMA

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Background. Fine-needle aspiration cytology is often unable to distinguish between follicular thyroid carcinoma (FTC) and follicular adenoma (FA) [1]. Moreover, a significant clinical difference between minimally invasive (MI-FTC) and widely invasive (WI-FTC) tumors was highlighted. Patients with MI-FTC have generally a good prognosis, with few exceptions, whereas WI-FTC is associated with a higher risk of metastasis and a worse outcome [2]. Although many diagnostic and prognostic molecular markers have been proposed, none of them has been found to be conclusive [3] [4]. In the present study we performed a deep molecular characterization of FAs, MI-FTCs and WI-FTCs, in order to find molecular biomarkers which could improve the diagnostic and prognostic delineation of these tumors.

Materials and methods. A panel of 75 genes previously described as highly differentially expressed between FA and thyroid carcinomas was tested on 18 FAs and 20 FTCs, including 14 MI-FTCs and 6 WI-FTCs. In addition, mutational status of \textit{NRAS} (exons 2 and 3), \textit{HRAS} (exons 2 and 3), \textit{KRAS} (exons 2 and 3) and \textit{TERT} promoter was investigated. The expression profiles were evaluated with an unsupervised hierarchical clustering, performed on normalized data using Pearson correlation [5].

Results. Hierarchical clustering showed two clusters (Fig. 1): cluster 1 (C1) contained 22 samples (15 FAs and 7 MI-FTCs), cluster 2 (C2) enclosed 16 samples (3 FAs, 7 MI-FTCs and 6 WI-FTCs). Samples of cluster 2 were in turn differentiated in two sub-clusters (C2.1 and C2.2). Sub-cluster C2.1 consisted of 2 FAs, 5 MI-FTCs and 2 WI-FTCs, whereas sub-cluster C2.2 was composed of 1 FAs, 2 MI-FTCs and 4 WI-FTCs. Overall, 8 mutations were detected in 4 WI-FTCs (4 \textit{NRAS} mutations) and 2 MI-FTCs (1 \textit{NRAS}, 1 \textit{KRAS} and 2 \textit{TERT} promoter mutations). \textit{TERT} promoter mutations always coexisted with \textit{NRAS} mutations. Interestingly, all mutated samples were grouped in sub-cluster C2.2.

Conclusions. The expression profile of WI-FTCs is considerably different from the majority of FAs. However, despite WI-FTCs have a well-defined gene expression profile, MI-FTCs seem to be a heterogeneous group: some of them have a FA-like profile whereas others markedly resemble to WI-FTCs. In particular, mutated MI-FTCs have a gene expression profile overlapping to that of WI-FTCs. These results appear to be promising in a potential diagnostic application. Further studies investigating associations between gene expression profiles and clinical outcome of patients will help in defining a specific molecular signature thus improving the prognostic definition of these tumors.

References


Introduction. Malignant pleural mesothelioma (MPM) is an asbestos-related malignancy affecting the pleura. It is a rare tumour with a poor prognosis and it is widely unresponsive to therapies [1]. According to the World Health Organization classification, there are three main histological subtypes: epithelioid (E), sarcomatoid (S) and biphasic (B), among which S- and B- MPMs are rarer and have a worse prognosis [2]. In this study we evaluated differences in the expression levels of 117 genes belonging to cancer pathways among MPM subtypes.

Methods. Gene expression analysis was performed by nanoString System, which allows a direct counting of mRNA molecules, without any retro-transcription steps, thus reducing the potential errors associated with multiplex PCR assays. This study was retrospectively conducted on RNA from formalin-fixed and paraffin-embedded tissues of 25 E-MPM, 13 S-MPM and 5 B-MPM patients. A non-parametric Kruskal-Wallis test (p-value < 0.05) was executed and the Dunn’s test and Bonferroni correction were used for multiple comparisons. In addition, to model gene expression profiles we performed a cluster analysis based on Pearson correlation, including only genes differentially expressed in the three groups.

Results. 39 genes were deregulated among MPM subtypes. In detail, 20 genes were up and 12 down regulated in E-compared to S-MPMs. Two genes were up and 9 down regulated in E- compared to B-MPMs. Five genes showed a down regulation in S- compared to B-MPMs (Table 1). The cluster analysis revealed 2 groups, one of which composed only of E-MPM samples (21 out of the 25 E-MPM samples) (Figure 1).

Conclusions. MPM is an extremely heterogeneous tumour including several histotypes with different clinical outcomes [3]. The molecular characterization of MPM subtypes and the evaluation of specific gene expression profiles could lead to the identification of new and effective biomarkers. In this study we found that E-MPMs have a distinct and clear gene profile: almost all of the differentially expressed genes were specifically deregulated in this subtype, and the majority of E-samples were grouped in the same cluster on the basis of gene expression levels. On the other hand, only 5 genes resulted deregulated between S- and B-MPMs, thus indicating their greater molecular similarity.

Most of the genes deregulated among subtypes encode for proteins involved in cell adhesion, cell proliferation, angiogenesis and signalling pathways. Interestingly, some of the down regulated genes in E-MPM are implicated in Hedgehog signalling pathway, which maintains tumour growth in MPM stroma [4]. Among Hedgehog genes, Gli1 and Gli2 deserve a particular mention since they have already been investigated as potential therapeutic targets for MPM [5].

In this study we identified genes deregulated in a histotype-specific manner, but further investigation on a larger number of samples is needed to clarify their role as biomarkers, in order to improve the clinical approach to MPM.

References

Table I. Differentially expressed genes in MPM subtypes.

<table>
<thead>
<tr>
<th>E-PMPM vs S-PMPM</th>
<th>E-PMPM vs B-PMPM</th>
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<tbody>
<tr>
<td>E-PMPM up regulated genes</td>
<td>E-PMPM down regulated genes</td>
</tr>
<tr>
<td>ADAMTS8</td>
<td>CCNO</td>
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<tr>
<td>CDH1</td>
<td>CDK7</td>
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<tr>
<td>CFB</td>
<td>CLDN15</td>
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<tr>
<td>CTNNB1</td>
<td>CXADR</td>
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<tr>
<td>DSP</td>
<td>EGFR</td>
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<tr>
<td>IFITM1</td>
<td>KRT5</td>
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<tr>
<td>LAMA3</td>
<td>NME2</td>
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<tr>
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<tr>
<td>NMU</td>
<td>RAD21</td>
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<td>PTGIS</td>
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<td>SDHB</td>
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</tbody>
</table>

Table I: Differentially expressed genes in MPM subtypes.

Figure 1. Unsupervised cluster analysis of deregulated genes and samples. Each column represents a single sample and each row a single gene. MM: sarcomatoid-MPM, MB: biphasic-MPM, ME: epithelioid-MPM. Red indicates a high level of expression relative to the mean expression, and green indicates a low level of expression relative to the mean expression.
**GENE EXPRESSION PROFILING OF LUNG SARCOMATOID CARCINOMAS**


Azienda Ospedaliero Universitaria Pisana

**Introduction.** Lung sarcomatoid carcinomas (PSCs) are rare types of non-small cell lung carcinomas (NSCLCs) with a sarcoma-like component, accounting for 2-3% of all lung malignancies [1]. The World Health Organization (WHO) classification identifies three histopathologic subtypes: subtype-1 includes pleomorphic, spindle cell, and giant cell carcinomas (PSCGCs), subtype-2 carcinosarcoma and subtype-3 blastoma. PSCs have a worse prognosis than other NSCLCs, however, the clinical impact of different subtypes and their molecular characteristics are still unclear [2]. The aim of this study is to widely investigate the gene expression profiles of PSCs, by a high-throughput sequencing of RNA (RNA-seq), in order to shed light into their cancerogenesis mechanisms.

**Methods.** In this study 5 pleomorphic, 2 spindle cell, 2 giant cell carcinoma and 4 carcinosarcoma patients were retrospectively enrolled. A whole-transcriptome targeted gene quantification analysis was performed on RNA from formalin-fixed paraffin-embedded tumour tissues. RNA-seq reads were normalized and quantified using alignment algorithms. Gene expression levels were compared between subtype-1 and 2 by a non-parametric Mann-Whitney U-test (p-value < 0.01) with linearity correction.

**Results.** 216 genes resulted down-regulated and 15 up-regulated in PSCGCs compared to carcinosarcoma (Table 1).

**Conclusions.** A deep molecular characterization of PSCs could either improve our knowledge of this class of tumours and suggest diagnostic, predictive and prognostic markers, thus allowing a better patients’ management. Until the new WHO classification in 2015 the lung sarcomatoid carcinomas were considered as a unique group, and only recently PSCGCs and carcinosarcoma have been classified as two separate entities [3]. Our results are in agreement with the current classification, since we identified two distinct gene expression profiles between subtype 1 and 2. As expected, most of the deregulated genes are involved in cancer pathways such as VEGF, WNT and Hedgehog, and further studies should clarify their role in PSC subtypes. This study implied a whole-transcriptome analysis of scarcely characterized tumours, and revealed specific genes, which, after appropriate validation, could help to delineate the PSCs genetic and molecular structure.

**Table I.** deregulated genes in PSCGCs compared to carcinosarcoma (p-value<0.01).

<table>
<thead>
<tr>
<th>DOWN REGULATED GENES</th>
<th>UP REGULATED GENES</th>
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<tbody>
<tr>
<td>ABC5</td>
<td>CDKN11</td>
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<tr>
<td>AC5P</td>
<td>CNP4</td>
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<td>DLX5</td>
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<td>FADS2</td>
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<tr>
<td>CKAP4</td>
<td>FAM109B</td>
</tr>
</tbody>
</table>

References


ALK REARRANGEMENT AND MUTATIONAL PROFILING OF EGFR AND KRAS ONCOGENES IN LUNG ADENOCARCINOMA. THE EXPERIENCE OF THE UNIT OF PATHOLOGY OF A.R.N.A.S. CIVICO HOSPITAL (PALERMO, ITALY)

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¹ARNAS Civico Palermo - Unit of Pathology; *University of Palermo, Unit of Pathology

Background. The present study aims to describe the experience of implementing the discovery of ALK rearrangement by immunohistochemistry technique and to investigate the association between receptor ALK (Anaplastic Lymphoma Kinase) rearrangement, Epidermal Growth Factor Receptor (EGFR) and Kirsten Rat Sarcoma viral oncogene homolog (K-RAS) mutations, in a group of patients with primary lung adenocarcinoma (LAC). The morphological characteristics of LAC have been detected according to the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification.

Methods. We have selected 97 cases of invasive LAC, including 27 surgical specimens and 70 biopsies (7 pleural biopsies, 52 EBUS TBB/bronchial biopsies, 1 pericardic biopsy, 1 cerebral biopsy, 8 bone biopsies, 1 limphnode biopsy), collected at the A.R.N.A.S. CIVICO Hospital (Palermo, Italy) between January 2015 and June 2016. Clinical data were retrieved including gender, age, tumor size, lymph-node metastases and stage. Tumor staging was decided according to the 7th edition of the American Joint Committee for Cancer staging system.

Samples were formalin-fixed, paraffin-embedded (FFPE) and stained with hematoxylin and eosin. FFPE blocks were cut into 5-10 sections of 5-μm thickness. The detection of the ALK rearrangement was performed using Ventana immunohistochemistry on a Benchmark XT autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) and with monoclonal rabbit anti-human ALK antibody (ready to use; clone D5F3; catalog no. 790-4794; Ventana Medical Systems, Inc.). The Optiview DAB IHC detection kit (catalog no. 860-099; Ventana Medical Systems, Inc.) was used according to the manufacturer’s protocols. Positive staining was indicated by strong granular staining in the tumor cell cytoplasm, in any percentage of positive tumor cells; otherwise the expression of ALK was considered negative.

Mutational analysis of EGFR and K-Ras genes was carried out by Pyrosequencing method (system PyroMark Q24). The therascreen EGFR Pyro Kit and K-Ras Pyro kit (QIAGEN GmbH, Hilden) is in vitro acid sequence-based detection test based on Pyrosequencing for the quantitative detection of mutations in exons 18, 19, 20 and 21 of the human EGFR gene and in codons 12 and 13 of the human K-RAS gene, in KRAS mutations in exons 18, 19, 20 and 21 of the human EGFR gene and in codons 12 and 13 of the human K-RAS gene, in genomic DNA derived from human tissue samples.

The predominant subtypes were as follows: lepidic predominant, 1 patient; acinar predominant, 5 patients; papillary predominant, 2 patients.

KRAS mutations (codons 12-13) were detected in 2 male patients (2%), 70 and 73 years old respectively. The subtypes were solid predominant subtype, with mucin production, and acinar predominant.

Conclusions. Detecting the rearrangement of ALK gene by immunohistochemistry was reliable and fast, allowing a saving of kits and technical staff. Also considering the reliability of this method, it has never been necessary to perform an additional test by FISH. The present data revealed that EGFR and KRAS mutation status and ALK rearrangement were mutually exclusive. The ALK fusion gene was associated with an average age of 51 years and was characterized by the solid predominant subtype, with mucin production. The frequency of EGFR mutations was increased in women compared with men (7 women) and was associated with an average age of 76 years (considering both women and men). EGFR mutations were associated with the acinar predominant subtypes; whereas KRAS mutations were associated with solid predominant subtype with mucin production and with acinar predominant. However being a relatively small number of patients analyzed, we propose to extend the series with the aim to verify and confirm the data obtained until now.

References
Association between the histological subtype of lung adenocarcinoma, EGFR/KRAS mutation status and the ALK rearrangement according to the novel IASLC/ATS/ERS classification.

KRAS DETECTION ON ARCHIVAL CYTOLOGICAL SMEARS BY THE NOVEL FULLY AUTOMATED PCR-BASED IDyllA MUTATION TEST


Dipartimento di Sanità Pubblica, Università degli Studi di Napoli “Federico II”, Napoli.

Background. Molecular techniques are relevant to modern cytopathology, but their implementation is difficult without molecular expertise and infrastructure. The assessment of KRAS mutational status on cytological preparations may be useful either to refine uncertain diagnoses on pancreatic
aspirates or to yield predictive information to plan targeted treatment of metastatic colorectal cancer (mCRC). The novel test Idylla enables fully automated KRAS genotyping in approximately 2 hours, even in less experienced hands.

**Design.** This study aims to validate this methodology to detect KRAS mutations on archival cytological preparations of pancreatic cancer (n = 9) and of mCRC (n = 9) by comparing the Idylla performance to that of standard real time (RT)-PCR.


**Conclusions.** Even in less experienced laboratories, the cytopathologist may easily integrate morphological diagnostic report with accurate KRAS mutation detection, which is relevant for diagnostic and treatment decisions.

**References**


**AXL DEREGULATION IN PLEURAL MESTHESIOLEIMA**


**Introduction.** Malignant pleural mesothelioma is a rare cancer arising from the pleura, highly associated with asbestos exposure. It is one of the most lethal cancers with limited treatment options, so median survival is of 9–18 months even with tri-modality therapy of chemotherapy, surgical resection and thoracic radiation. Thus, the development of new therapeutic strategies for malignant pleural mesothelioma are urgently required. In recent years extensive research focused on the identification of serum and tissue predictive markers and prognostic factors to select patients subsets for targeted therapy in the future.

Axl is a receptor protein kinase implicated in cell survival and epithelial-to-mesenchymal transition. Recently, Axl overexpression has been associated with adverse prognosis in several neoplasms like hepatocellular carcinoma, esophageal carcinoma, pancreatic carcinoma, thyroid carcinoma and colon carcinoma. It has been rarely described in mesothelioma.

**Materials and methods.** A Tissue Micro Array of pleural mesothelioma was built, including cases of biopsies with available tissue material. Axl expression in primary specimens of malignant pleural mesothelioma, correlating their expression levels with tumour phenotype and clinical outcomes. Furthermore, we performed fluorescence in situ hybridization analysis of AXL gene to define gene status.

**Results.** Our series was constituted by 31 cases, 90% male patients. Epithelioid phenotype was observed in 75% of cases, sarcomatoid in 17% and mixed in 8%. Axl overexpression was observed in 36% of cases, while altered gene status detected through FISH was observed in 12% of cases, particularly gene amplification in 4% of cases and polisomy in 8% of cases. Axl expression related significantly (p<0.05) with epithelioid phenotype and gene amplification, but not with polisomy.

**Conclusion.** Axl overexpression is a relatively common alteration in mesothelioma, mainly in epithelioid phenotype. Differently from other cancers, its deregulation could be due also to amplification, but other genetic unknown mechanisms seem to be more relevant.

**References**


**PATOLOGIA DELL’APPARATO DIGERENTE, FEGATO E PANCREAS**

**GALLBLADDER METASTASIS FROM SMALL CELL LUNG CANCER: REPORT OF AN UNEXPECTED CASE AFTER LAPAROSCOPIC CHOLECYSTECTOMY**

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**Pathology Unit, G. Salvini Hospital (ASSST-Rhodense), Garbagnate Milanese (Milan), Italy.**

**Introduction.** Small Cell Lung Cancers (SCLCs) are the most common subtype of neuroendocrine malignant tumours of the lung and account for about 13% of all newly diagnosed lung carcinomas worldwide. SCLC can spread everywhere in the body, and metastatic spread is a common finding at clinical presentation. The most frequent sites of local spread are intrathoracic lymph nodes (often as bulky disease potentially leading to superior vena cava syndrome) and supraclavicular lymph nodes. Extrathoracic metastatic spread include liver, bone and brain. The report of our case is justified by its rarity and its atypical clinical presentation along with non specific radiological imaging, emphasizing that it is an incidental detection and that an accurate differential diagnosis with an appropriate use of immunohistochemical tools is required in order to exclude the primitivity of the tumor. The correct diagnosis helps the clinician to choose the best therapeutic strategy for the patient’s treatment.

**Materials and methods.** The surgical specimens were fixed in 10% buffered formaldehyde and then routinely processed. Sections were stained with H&E. Immunohistochemical studies were performed using the following panel of antisera: Chromogranine, Synaptophysine, NSE, CK7, EMA, CEA and TTF-1. CK20, Ca 19.9 and CK19.

**Results.** A 83-year-old smoker man presented in October 2015 with a 3-day history of progressively worsening, abdominal pain in the right upper quadrant, nausea and vomiting. He had no relevant previous medical history. On physical
Conclusions.

Guided fine-needle aspiration cytology of the lung nodules.

In favor of a stable disease before performing an ultrasound-patient, the case was discussed in a weekly multidisciplinary and cough. For these reasons and due to the elderly age of the had a progressively worsening with weight loss, dyspnoea results are all foundamental for the correct diagnosis.

Unfortunately, a month later the clinical status of the patient had a progressively worsening with weight loss, dyspnoea and cough. For these reasons and due to the elderly age of the patient, the case was discussed in a weekly multidisciplinary consultation meeting, and clinicians decided to wait and see in favor of a stable disease before performing an ultrasound-guided fine-needle aspiration cytology of the lung nodules.

Conclusions. Gallbladder is an uncommon site for metastases. In a review of one large autopsy series metastases to the gallbladder were found in 5.8% of cancer patients. Primary tumors can metastasize to the gallbladder either by direct invasion of the porta hepatitis or by hematogenous spread. Hepatocellular carcinoma and pancreatic tumors have been reported to invade the gallbladder by direct invasion. Hematogenous metastases to the gallbladder include melanoma, breast cancer, lung adenocarcinoma, renal cell cancer, gastric cancer, and hepatocellular carcinoma. Most gallbladder metastases are often asymptomatic. In patients who do have symptoms, the most frequent symptomatic presentations are biliary colic, acute cholecystitis, biliary obstruction and abdominal pain. Metastases initially occur as small, flat nodules below the mucosal layer and then grow as pedunculated nodules, rarely surpassing several millimeters in size.

For this reason it is difficult to grossly differentiate between cancer and cholesterolosis, especially when neoplastic lesion are solitary, small and located in the thickness of the wall. On microscopic examination, the differentiation of primary SCC of the gallbladder from metastatic SCCs is not possible by morphological features only. In fact the histopathologic findings of our case were undistinguishable from those of any other SCC case. In this context, immunohistochemistry was useful to exclude possible diagnoses of metastases, being an essential tool but not the only one to distinguish between a primitive or secondary neoplasm. Our case demonstrated positivity with neuroendocrine markers and TTF-1. The latter is a useful marker that has been identified in respiratory epithelial cells and it is expressed in the early stages of the thyroid. In the routine diagnostic pathology practice, it is widely used as an immunohistochemical marker for benign or malignant primary or metastatic lung and thyroid tumors. Its positivity has also been observed in primary adenoscarcinomas of the gastrointestinal tract. Pegolo et al reported two cases, the first one was a gastric adenocarcinoma, the other one was a gallbladder adenocarcinoma. The last one was a poorly differentiated adenoscarcinoma with an immunohistochemical profile of a gallbladder carcinoma as it was positive for CK7, CK19, EMA, CEA and Ca19.9 immunostainings. Kaufmann et al and Yadav et al tested the expression of TTF-1 in pulmonary and extrapulmonary SCCs and other neuroendocrine carcinomas of various primary sites and concluded that TTF-1 cannot be used to distinguish between extrapulmonary and pulmonary SCCs. In our case the diagnosis of a metastatic neoplasm was confirmed by these aspects: negativity for CK19 and Ca19.9 and positivity for TTF-1, absence of mucosal dysplasia, presence of neoplastic vascular invasion and presence of pulmonary nodules. In addition to our immunohistochemical results, the clinical history and the radiologic findings were all necessary to hypothesize the lung origin. Our patient was thoroughly investigated to exclude any other malignancy via complete workup that included CT scans of the chest and abdomen and PET. CT scan of the chest showed multiple small nodules localized in the left lower lobe of the lung and for this reason our histological diagnosis was supported. Considering that TTF-1 alone may not be used for the differentiation of gallbladder SCCs from SCLCs, histopathologic examination, immunohistochemistry results, clinical features, and radiologic findings are all foundamental for the correct diagnosis.

We would like to stress that TTF-1 is an important immuno-histochemical marker and despite its high specificity in lung and thyroid neoplasms, its expression is not uncommon in extrapulmonary sites as documented in some reports. TTF-1 expression has not previously been described in gallbladder SCCs and in this setting we document the first case of TTF-1 expression in a SCC localized at gallbladder in a patient with pulmonary nodules. We highlight the importance of a holistic diagnostic approach especially in the presence of a rare case as ours because it pose a problem of misdiagnosis.

References


MESENCHYMAL GASTRIC TUMOR: NOT ONLY GIST. EUS GUIDE-FNAB DIAGNOSIS OF GASTRIC SCHWANNOMAS

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Introduction. Gastrointestinal stromal tumors (GISTs) are the most common gastric mesenchymal tumors, representing approximately 80% of them. They are frequently diagnosed during endoscopic ultrasonography (EUS). Although they are the principle mesenchymal gastric pathological entity, it is important to differentiate them from other spindle cells neoplasms like schwannomas. Our purpose is also to highlight that it is important to include schwannomas within the differential diagnosis of mesenchymal gastric tumors because intramural gastric masses are not only GISTs. EUS with fine needle aspiration biopsy (FNAB) could be the first line and the best diagnostic choice to differentiate GISTs from schwannomas to avoid a surgical overtreatment approach. In fact, schwannomas have an excellent prognosis because of their benign nature whereas 10 to 30% of GISTs can have malignant behavior, needing appropriate surgical treatment. FNAB is fundamental for a first immunohistochemical evaluation of the lesion, even if sometimes it can fail to confirm the diagnosis because of insufficient tissue biopsy for immunohistochemistry. Herein we describe four peculiar cases, clinical settings that allow a more precise classification among stromal tumors of the gastrointestinal tract. Schwannomas are benign, slow-growing tumors, originating from Schwann cells of the neural sheath and were first described in the gastrointestinal tract by Daimaru et al. in 1988. The most common gastrointestinal site is the stomach. Schwannomas are rare among the mesenchymal tumors and constitute only 0.2% of all gastric tumors. They arise from the fundus, body or antrum of the stomach and are commonly intramural. The incidence rates of schwannomas are different in male and female, being more common in females and arising in the fifth to sixth decades of life. They are usually misdiagnosed as other submucosal tumors preoperatively. They are asymptomatic, solitary lesions, usually arising from the lesser curvature of the stomach and can be discovered incidentally during clinical exams for other reasons. In fact were referred to our hospital and were firstly evaluated by FNAB under EUS, with the diagnosis confirmed after surgery. Two cases were detected incidentally during radiological examination for colo-rectal carcinoma and urothelial carcinoma of the urinary bladder. Two patients had symptoms including epigastric pain and weight loss without other related clinical conditions. The patients were three female and one male and their age ranged from 53 to 85 years. The mass lesions were located on the lesser and greater curvature of the stomach. Endoscopic ultrasound evaluation showed round, well-circumscribed tumors with an heterogeneous hypoechoic aspect and an internal high-echoic area. The lesions had a connection with the proper muscle layer of the stomach, were covered by a smooth surface and were homogeneous without cystic spaces, lobulations or calcifications. All patients underwent numerous clinical examinations before surgery but the lesions were clinically misdiagnosed as GISTs and for three patients the preoperative diagnosis was achieved only with the histological examination of the material obtained by endoscopic ultrasound-guided FNAB. In one patient this technique failed to establish the exact diagnosis because tissue was insufficient for immunohistochemistry. Three patients underwent laparoscopic wedge resection and one patient underwent subtotal gastrectomy. Macroscopically, the lesions had an exophytic, oval, solid, intramural aspect (maximum diameter ranging from 1 cm to 5 cm) and were covered by normal mucosa. On cut surface they revealed a fasciculate and whorling arrangement. Histological examination of the resected specimens sustained the first FNAB diagnosis showing lesions mainly constituted by spindle cells with a fasciculate or storiform growth pattern. The cells had an eosinophilic cytoplasm with dark nuclei. Necrosis was absent, mitotic activity rate was very low and the Ki67 proliferative labeling index did not exceed 3% in all tumors. Peripherically, a cuff of small lymphocytes confluent in lymphoid aggregates with some germinal center was present. Immunohistochemistry showed a strong cellular positivity for p-S100 while neoplastic cells were totally negative for CD117/c-Kit, DOG-1, CD34, SMA, Desmin, Vimentin and CKA1/E/AE3. According to the microscopic features and to the immunohistochemical results a histological diagnosis of gastric schwannoma was made in all patients and in two cases a combination of synchronous colo-rectal adenocarcinoma and co-occurent urothelial cell carcinoma of the urinary bladder was observed. To date patients are still alive and healthy without evidence of recurrence of the gastric schwannoma.

Conclusions. In the past, spindle cell stromal tumors of the gastrointestinal tract were considered to originate only from smooth muscle. With the development of immunohistochemistry and electron microscopy various distinct origins have been reported. These have allowed a more precise classification among stromal tumors of the gastrointestinal tract. Schwannomas are benign, slow-growing tumors, originating from Schwann cells of the neural sheath and were first described in the gastrointestinal tract by Daimaru et al. in 1988. The most common gastrointestinal site is the stomach. Schwannomas are rare among the mesenchymal tumors and constitute only 0.2% of all gastric tumors. They arise from the fundus, body or antrum of the stomach and are commonly intramural. The incidence rates of schwannomas are different in male and female, being more common in females and arising in the fifth to sixth decades of life. They are usually misdiagnosed as other submucosal tumors preoperatively. They are asymptomatic, solitary lesions, usually arising from the lesser curvature of the stomach and can be discovered incidentally during clinical exams for other reasons. In fact...
two of our cases were detected during exams for colorectal adenocarcinoma and urothelial carcinoma of the urinary bladder. Only few cases of occurrence of carcinoma from other sites and schwannoma have been reported. Whether or not such a co-occurrence is a simple incidental association or the lesions are connected by a causal relationship needs further study. According to the extremely low occurrence rate of the gastric cancer and schwannomas, we suppose as other authors that the co-occurrence is incidental. Moreover, the typical endoscopic appearance of gastric schwannoma is that of a round, protruding intramural mass. On pathological examination the tumors are covered by intact mucosa and they involve the submucosa and muscularis propria. These tumors have spindle-shaped nuclei and have a fascicular arrangement. Gastric schwannomas are clinically misdiagnosed as other gastric submucosal tumors like GISTs and various types of sarcoma, which leads to unnecessarily excessive surgical treatments. EUS followed by FNAB is an important imaging tool to diagnose and evaluate gastric mesenchymal tumors because endoscopic ultrasound images together with histological and immunohistochemical results are valid and useful diagnostic techniques to attend the precise and correct diagnosis. EUS alone cannot solve the dilemma about the cellular origin of a mesenchymal tumor because the ultrasound images are similar across the stromal lesions and for this reason is fundamental the multidisciplinary approach, engaging radiologists, gastroenterologists, pathologists and surgeons.

Lastly, although gastric schwannomas are rare, clinicians must keep in mind that gastric mesenchymal tumors are not only GIST and that gastric schwannomas do exist.

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**ENTEROPATHY ASSOCIATED T-CELL LYMPHOMA TYPE 2: A CASE REPORT**

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**Introduction.** A 69-year-old man presented with complaints of fever, abdominal pain and distension. The patient had a history of abdominal aortic aneurysm. Laboratory tests were negative for celiac disease. Abdominal x-ray and CT scan were done and showed features suggestive of intestinal obstruction and perforation. Jejunal resection was done following a clinical diagnosis of intestinal adhesions and perforation related to previous surgery. Two weeks later, the patient died of surgical complications.

**Methods.** On gross examination the mucosal surface of small intestine showed multiple foci of ulceration with thickening and perforation of wall (figure 1). Microscopic examination revealed a lymphoid neoplasm comprising of medium sized abnormal cells with coarse chromatin, irregular nuclear contours, large nucleoli and abundant eosinophilic cytoplasm (figure 2a). Minimal inflammatory background with necrosis was also documented. Tumor cells were seen diffusely infiltrating into the intestinal smooth muscle fibres of muscularis propria and serosal adipose tissue with mucosal involvement (figure 2b). The evaluable villi showed admixed reactive and neoplastic infiltrate along with plasma cells.

Immunohistochemistry revealed that neoplastic cells were positive for CD3 (figure 2c), CD8 (figure 2d), CD56 (figure 2e), bcl-2 and negative for CD20, CD5, CD34, c-kit and bcl-6. Ki67 index was more 80% (figure 2f).

**Results.** The histology and immunophenotype supported the diagnosis of enteropathy associated T-cell lymphoma (EATL) type 2.

**Conclusions.** EATL is a rare primary intestinal lymphoma, accounting for fewer than 5% of all gastrointestinal tract lymphomas. It’s an aggressive disease with a poor prognosis and low survival rate. Most patients present with bowel obstruction, small intestinal perforation, abdominal pain and diarrhea. The jejunum is most frequently involved followed by other parts of the small intestine (1).

The WHO classification of tumors of hematopoietic and lymphoid tissue distinguishes between 2 types of EATL, based on morphological, immunophenotype and relationship to celiac disease. Type 1 is a large cell lymphoma which is more common EATL and is commonly associated with celiac disease. Histologically it shows medium sized to large pleomorphic cells with conspicuous nucleoli accompanied by prominent mixed inflammatory infiltrate composed of histiocytes, small lymphocytes, plasma cells and eosinophils along mitotic figures and necrosis. Immunoprofile of neoplastic cells is CD3+, CD5−, CD7+, CD8+, CD4−, CD56−, TCRB+, with variable CD30 expression. Type 2 accounts for 10% to 20% of all cases. Typically, it occurs sporadically, but up to one-quarter of patients have a histi...
tory of celiac disease. Histologically it shows monomorphic small to medium sized lymphocytes with slightly irregular nuclei and small nucleoli surrounded by scant pale cytoplasm, infrequent mitosis and sparse inflammatory background. Tumor cells are CD3+, CD4+, CD8+, CD56+ on immunohistochemistry and express TCRβ F1 or TCRγδ (2).

The differential diagnosis of EATL includes other T-cell lymphomas including anaplastic large cell lymphoma (positive for CD30 and ALK), extranodal NK/T cell lymphoma of nasal type (positive for CD56 and EBER), and peripheral T-cell lymphoma, NOS.

References

SEGMENTAL ATROPHY OF THE LIVER: A CASE REPORT OF THIS UNCOMMON AND UNRECOGNIZED ENTITY FROM OUR INSTITUTE

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Goal. Segmental atrophy of the liver is a rare entity that could clinically and radiologically mimic a neoplasia or other diseases, representing an important diagnostic challenge. This
lesion is not completely described in literature and Singhi et al. proved that it presented multiple evolutive stages, a feature that lead very difficulties in recognize and correctly diagnose this rare entity even on histological evaluation, whereas a correct diagnosis could imply an appropriate prognostic and therapeutic stratification.

Aim of the present study is to present a case of this rare entity in a fibroelastosis nodular stage (stage III-IV sec. Shinghi et al.) with relative clinical and radiological background.

Methods and materials. A 67-year-old man, presented with a lesion of the left hemi-liver (measured 9 cm in major diameter) discovered at ultrasound ecography, during a panel of investigations for high blood pressure and confirmed by TAC and RMN, with abnormal vascularization and dilatation of left bile ducts. Blood exams showed increased in Transaminases and Gamma-GT. For these reason the patient made a percutaneous liver biopsy with a diagnosis of “hepatic parenchyma free from neoplasia”. Nevertheless, on the basis of this clinical-radiological background a suspect of hilar cholangiocarcinoma was rendered and the patient underwent an operation of left-hepatectomy with removal of the hepatic artery lymph nodes and retroduodenal lymph nodes.

Results. On the macroscopic examination there was a white and hard consistency hilar and subcapsular lesion of 8x6x5 cm, with irregular margin and an infiltrative growth pattern, an appearance very suspect for a malignant neoplastic lesion and for these reason, largely handled. On the microscopic examination the perihilar and subcapsular hepatic parenchyma was almost entirely replaced by diffuse and dense areas of fibrotic appearance with a partially nodular arrangement, well circumscribed and with an abrupt interface with the background liver. The fibrotic areas were composed by a dense matrix entirely made up of collagen, reticulin and elastic fibers, as shown by special staining (Masson’s Trichromic Stain, Mallory’s Trichromic Stain, Veihoff Van Gieson’s Stain, Sirius Red). In the fibroelastotic areas there was rare scattered islands of hepatocytes and residual entrapped portal tracts showing high ductular proliferation but minimal ductular metaplasia of the hepatocytes, associated with an hyperplasia of bile glandular component and a very mild chronic inflammation. An important and distinctive histological features is the “histological abnormality of the vascular component” that consist in the partially subocclusion of the artery lumen, elastosis of the arteriolar wall, thrombosis with aspects of recanalization of the venous vases and myointimal and arteriolar hyperplasia. These aspects is very interesting, because is clearly known and documented how and why vascular injury is associated with aspect of atrophy and compensatory hyperplasia of the hepatic parenchyma, not only in the “Primary Segmental Atrophy of the liver” but also in the lobar/segmental atrophy of the liver related with other diseases that imply a decreasing in the normal blood flow to the hepatic parenchyma. We have carefully excluded, according with clinical, laboratory, radiological and immunohistological aspect, other diseases related to hepatic fibrosis and atrophy as cholangiocarcinoma, cirrhosis, chronic active hepatitis with cirrhosis, hydatid disease, hepatic failure and, on the basis of peculiar histological appearance (hepatic parenchyma almost entirely replaced by a dense fibroelastotic matrix with partially nodular arrangement, scattered residual portal space, very mild chronic inflammation and histological signs of vascular injury) we made a conclusive diagnosis of “Segmental Atrophy of the Liver in a fibroelastosis nodular stage (stage III-IV sec. Shinghi et al.”).

Conclusion. “Segmental Atrophy of the Liver” is a rare, benign pseudotumor of the liver that can clinically and radiologically mimic a variety of lesion, included benign and malignant neoplasia. Recognize this rare and little known entity could determine an improvement in the treatment of these patients and could help us in understanding the physiopathology at the base of the hepatic processes of atrophy and hyperplasia.

References


Figure 1. Clear abrupt interface between residual hepatic parenchyma and fibrotic area composed of dense collagen matrix. Note very mild inflammation, hyperplastic aspect of bile glandular component and subocclusion of the artery lumen (Hematoxilin and Eosin Stain. Magnification at 10x).

Figure 2. Fibrotic area is composed of a mixture of a dense collagen (red) and elastic fibers (black). Note very mild inflammation, clear abrupt interface with residual hepatic parenchyma and subocclusion of the artery lumen (Elastic Van Gieson Stain. Magnification at 10x).
FOLLICULAR DENDRITIC CELL SARCOMA, AN UNCOMMON TUMOR WITH UNPREDICTABLE BEHAVIOR: STUDY ON DIAGNOSTIC AND PROGNOSTIC MARKERS FROM AN INTERNATIONAL MULTICENTRIC COHORT OF 41 CASES

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Introduction. Follicular dendritic cell sarcoma (FDC-S) is an extremely rare neoplasm, it is a disease of adulthood, with no gender predilection, and may occur in any site of the body, both nodal and extranodal. Its clinical presentation is often vague and symptoms are related to the site of occurrence. Histologically, FDC-S is a neoplastic proliferation of spindled to oval cells with morphologic and immunophenotypic features of follicular dendritic cells. The histological variant Inflammatory pseudotumor-like (IPT-like) FDC-S is associated with Epstein-Barr virus infection, occurs exclusively in intra-abdominal sites, such as spleen and liver, and is associated to a female prevalence.

FDC-S diagnosis can be challenging due to its rarity, its highly variable tumor cell morphology and the heterogeneity of FDC markers expression.

Notably, FDC-S can show no recurrence after complete surgical resection or may recur numerous times and lead to patient’s exitus; in a large review study local recurrences and surgical resection or may recur numerous times and lead to patient’s exitus. 1; in a large review study local recurrences and surgical resection or may recur numerous times and lead to patient’s exitus. 1; in a large review study local recurrences and surgical resection or may recur numerous times and lead to patient’s exitus.

Studies with large cohorts of patients are still lacking and little is known about sensitivity and specificity of FDC markers in FDC-S diagnosis; furthermore, the microenvironment of this tumor was never explored in a large, clinically characterized cohort of cases. In order to answer these questions and correlate them with clinical data we gathered FDC-S cases from different European and extra-European institutions and performed a large morphological and immunohistochemical study.

Materials and methods. Formalin-fixed, paraffin-embedded tissue of follicular dendritic cell sarcomas and the available clinical data were collected from six different pathological institutions situated in Europe and India. Seventy-four cases of soft tissue tumors were collected from the archives of the Institute of Pathology of the Spedali Civili, Brescia and they included twenty-three cases of solitary fibrous tumors (SFT), twenty gastrointestinal stromal tumors (GIST), thirteen Synovial Sarcomas (SS), six inflammatory myofibroblastic tumors (IMT), five dermatofibrosarcomas protuberans (DFSP), four desmoids type fibromatosis (DTF) and three low grade fibromyxoid sarcomas (LGFM). Twenty-two FDC-S and all soft tissue tumors cases were arranged in six Tissue Microarrays using an automated tissue microarrayer (TMA Master, 3DHistech). Three representative tumor areas were identified on hematoxylin and eosin (H&E) stained sections. For each area, a 1 or 1.5 mm core was obtained by punching the original tissue block. All cases were stained for CD21, CD23, CD25, CXCL13, Clusterin, Podoplanin and Claudin 4. The phenotype of each case was evaluated with a two-tier scoring system considering 50% of stained tumor cells as threshold. Tumor microenvironment was evaluated by CD3 and CD20 stainings for T and B cells respectively and TdT was used to identify immature lymphocytes.

Results. We collected forty-one cases of follicular dendritic cell sarcoma; patients median age was 66 years (ranging from 14 to 84) and the majority were men (M:F=1.4:1). Extranodal cases outnumbered nodal ones (27/39, 69% and 12/39, 31%, respectively) in particular, their majority was intrabdominal (12/27, 44%), including retroperitoneum, 6 cases were from the head and neck district (6/27, 22%) and the same number of cases were intrathoracic (6/27, 22%). Among nodal diseases one third was from the latero-cervical district (4/12, 33%). Considering the stage at presentation the vast majority of our cases (23/27, 85%) presented as monofocal disease, only 4 (14.8%) were multifocal or metastatic at presentation.

Morphologically, 3 cases belonged to the IPT-like variant (2 male, 1 female; 1 liver, 2 spleen). Notably, we found Castleman’s disease-like features in numerous cases (8/34, 23.5%) although a previous history of Castleman’s disease was not reported in any case.

When applying FDC markers to our cohort we could verify that CXCL13 and Clusterin were the markers with best sensitivity (85.00% and 80.49%, respectively) and CD21, CD23 and CD35 those with best specificity (100%). Albeit, Clusterin showed a low specificity (85.78%) since it stained more than half of extra-gastric GIST tumors (6/10, 60%), two SS (2/12, 17%), two SFT (2/23, 9%) and one LGFMS (1/3, 33%) and CD23 was little sensitive since it was expressed only by 24/41 of FDC-S cases (58.54%).

We evaluated and characterized the lymphoid infiltrate in 32 and 20 FDC-S cases, respectively. The inflammatory infiltrate was abundant in 17 (53.12%) cases (including the 3 IPT-like variant cases) moderate in 10 (31.25 %) and scarce in 5 (15.62%). Half of the cases showed a mixed B- and T-cell (10/20, 50%), numerous had a prevalently T cell-rich background (7/20, 35%). Notably, when analyzing the influence of the inflammatory infiltrate to the prognosis we found a worse prognosis in cases with scarce lymphoid infiltrate (p=0.018). In addition, we found immature TdT+ lymphocytes in 5/20 (25%) cases and their presence correlated with a younger age (p=0.035) in line with previous reports.
SECONDARY PANCREATIC DIFFUSE LARGE B-CELL LYMPHOMA: A CYTLOGICAL DIAGNOSIS OF A RARE CAUSE OF PANCREATIC MASS

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Introduction. Lymphomatous involvement of the pancreas is a rather unusual event: in fact, primary pancreatic lymphoma represents a rarity, accounting for less than 2% of all lymphomas. Secondary pancreatic involvement during systemic lymphoproliferative diseases can occur but cytological diagnosis is very rarely performed considering the multorgan lymphomatous dissemination. Lymphomas can show different pattern of pancreatic involvement, the most common being, at radiological investigations, the nodular, hypodense one. Lymphomatous involvement of the pancreas is usually of the non Hodgkin’s or B-cell type with diffuse large B-cell lymphoma being the most common histotype.

Therefore, pancreatic involvement by lymphoma may be misdiagnosed as pancreatic cancer thus leading to incorrect therapeutic management. In the last decade, to address the issue of clinic-radiological-pathological diagnosis of pancreatic masses, the Endoscopic Ultrasound (EUS) has emerged as the most cost-effective and safe procedure and it became the gold standard. From a pathological point of view, EUS enables the execution of Fine Needle Aspiration Cytology (FNAC) and therefore to reach a cytological diagnosis.

Herein we report an unusual case of secondary, pancreatic, nodular involvement diagnosed by EUS-FNAC in patient suffering from a diffuse large B-cell lymphoma.

Materials and methods. The FNAC was performed using a 22 and 25 Gauge (G) needles. Direct smears were stained with May-Gruenwald-Giensa and with Papanicolaou stain. The 22 G needle and syringe were rinsed and the liquid obtained was later centrifugated. The sediment obtained was fixed in 10% buffered formaldehyde, routinely processed and paraffin-embedded as a cell block. Two–micrometer–thick sections were cut, stained with hematoxylin and eosin (H&E). Other sections were cut and used for ancillary studies. Immunophenotypic profiles were determined according to standard immunoperoxidase methods. The panel of antisera for the cell block sections included CD20, CD79a, CD3, cytokeratin AE1/AE3.

Case report. An asymptomatic 68-ys-old male patient was referred to our Hospital for radiological follow-up for lymphoma. In fact, in the past year, the patient was diagnosed with a diffuse large B-cell lymphoma involving the hepatic hilum (diagnosed at another center) and therefore received chemotherapy resulting in complete remission. Peripheral blood leukocyte count was normal and, at clinical evaluation, there was no evidence of palpable superficial lymph nodes. During the last total body Computed Tomography (CT) scan no lymphadenopathies and no other neoplastic lesions at thoracic organs were detected. However a solid, unique, nodular lesion of the pancreatic head was discovered. The patient underwent EUS showing, within the pancreatic head, a hypoechoic mass measuring 17 mm in its greatest dimension. The lesion was characterized by infiltrative margins and by a central area of necrosis. Moreover, a peripheral rim of oedematous pancreatic parenchyma was detected. The Endoscopist performed FNAC (3 passes with a 25 G needle for direct smears and 3 passes with a 22 G needle to obtain a cell block) in order to confirm or rule out pancreatic involvement.
by the known lymphoproliferative disease. Both the direct smears and the cell block sections displayed an abundant, scattered population composed by monomorphous large cells with round nuclei, with multiple nucleoli and with a rim of cytoplasm. On morphological examination, these cells were interpreted as lymphoid centroblasts. The immunocytochemistry analysis, performed on the cell block, confirmed the cytological hypothesis showing expression of CD20 and CD79a and negativity for CD3 and cytokeratin AE1/AE3. Moreover necrotic background was detected.

Therefore, integration of clinical, endoscopic-ultrasound, cyto logical and immunocytochemical data led us to a cytological final diagnosis of secondary pancreatic localization of diffuse large B-cell lymphoma.

Conclusions. Malignant lymphomas of the pancreas are unusual, solid tumors categorized as non-epithelial neoplasms. Primary pancreatic lymphoma is an extremely rare entity representing less than 1% of pancreatic tumors and less than 2% of extranodal lymphomas. On the contrary, secondary pancreatic involvement during systemic lymphoproliferative disease can occur in up to 30% of patients even if in this setting a predominant involvement of the pancreatic parenchyma is uncommon. However our patient was affected only by an asymptomatic pancreatic lesion discovered during follow-up radiological procedures for a diffuse large B-cell lymphoma previously treated with chemotherapy. From an epidemiological point of view, diffuse large B-cell represents the most common non Hodgkin lymphoma and is characterized by frequent extranodal involvement, which occurred in our case. Dissemination of lymphoma to the pancreas may display several patterns: nodular, diffuse and multinodular, with the nodular one being the most common form. In this context, lymphomatous involvement of the pancreas is characterized by a nodular lesion visualized as hypodense at CT scan or hypoechoic at Ultrasound (US) evaluation. This kind of presentation poses major diagnostic challenges, especially if devoid of anamnestic information, since it may easily mimic the most common histotype of pancreatic neoplasm, adenocarcinoma. The distinction between pancreatic adenocarcinoma and primary or secondary pancreatic involvement by lymphoma is crucial to plan an appropriate therapeutic treatment: in fact, while for pancreatic adenocarcinoma surgery represents the most important strategy, in the setting of lymphomas chemotherapy alone can be administered. In the last decade, to address the issue of clinic-radiological-pathological diagnosis of pancreatic masses EUS has emerged as the most cost-effective and safe procedure. EUS provides detailed radiological information, especially about vascular and lymph nodes involvement by pancreatic malignancies particularly for lesions measuring < 20 mm. Moreover it enables the Endoscopist to perform FNAC in order to reach a cytological diagnosis: in this setting FNAC carried out by EUS has achieved a sensitivity and a specificity for malignant cytology respectively of 85-91% and 94-98%. Despite its invasiveness this technique is usually safe with a complication rate (perforation, pancreatitis, infections, tumor seeding and bleeding) of 0,28%–0,85% according to recent studies.

In our case the cytological material was adequate both qualitatively and quantitatively to formulate a diagnosis. The cytological smears were composed by large lymphoid cells resembling centroblasts. Therefore immunocytochemistry was applied on cell block and centroblasts displayed positivity for B-cell markers as CD20 and CD79a and negativity for cytokeratin and T-cell marker CD3. Therefore, integration of previous anamnestic, endoscopic-ultrasound, cytological and immunocytochemical data helped us to define this uncommon occurrence and a final cytological diagnosis of secondary pancreatic localization of diffuse large B-cell lymphoma was performed.

We can firmly state that, in the setting of rare and unusual cases with significant diagnostic pitfalls, medical malignancy history and multidisciplinary approach are of paramount importance, since they may be able to influence the diagnostic algorithm and to guide forward the correct diagnosis.

In conclusion, despite the abovementioned variables, we want to stress that a fundamental role is played by the Cytopathologist’s expertise because it is imperative to render an accurate diagnosis and the cytological examination is considered an effective tool to achieve it.

Furthermore, it is an appreciable benefit to perform a cell block whenever possible in order to better apply ancillary techniques like immunocytochemistry that can be helpful to reach a specific diagnosis within a correct diagnostic algorithm and to differentiate between primary and secondary neoplasms, especially in deceiving cases.

References


EVALUATION OF P53 PROTEIN EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS): PROGNOSTIC IMPACT

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Introduction. MDS are disorders of the hematopoietic stem cell, which has a complex pathogenesis and risks to progress into acute leukemia. It is therefore essential a good prediction of the risk of evolution into acute leukemia (IPSS - R). Strong nuclear immune staining of the p53 protein has been used as a surrogate marker for TP53 gene mutations in hematologic and other malignancies; a strong correlation of p53 nuclear expression with TP53 mutation has also been demonstrated.

Methods. 74 cases of bone marrow biopsies, with a clinical diagnosis of MDS have been examined. From the paraffin material 4 µm-thick sections were obtained, dehydrated, subjected to antigen retrieval with EDTA in a waterbath at 98 °C, and immunohistochemistry with the D0-7 antibody.

Results. An experienced pathologist evaluated the intensity (poor, moderate and strong) and the percentage of immunostained cells.
The cases, with moderate / strong intensity staining, were 28:
• 14 showed hypercellularity of single series (erythroid or myeloid);
• 11 showed hypercellularity of two series;
• 3 showed increase of all series.
In 90% of cases increase medullary fibrosis was observed.

Conclusions. p53 expression is present in bone marrow biopsies of patients, with alterations of a single or more series, while patients with single alterations of the megakaryocytic series were immuno-negative;
TP53 positivity, although not replacing the prognostic score systems, predicts a worse prognosis and a higher risk class; the increase of medullary fibrosis associated to p53 immuno-positivity indicates reduced survival.
The expression of p53 in immunohistochemistry is useful to assess the risk and the evolution of MDS but is not enough to impact overall survival.

UNVEILING ANOTHER MISSING PIECE IN EBV-DRIVEN LYMPHOMAGENESIS: EBV-ENCODED microRNAs EXPRESSION IN EBER-NEGATIVE BURKITT LYMPHOMA CASES

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Introduction. Burkitt lymphoma (BL) is an aggressive B-cell malignancy currently classified as endemic (eBL), immunodeficiency associated (ID-BL) and sporadic (sBL). It is the first tumor for which an association with EBV has been demonstrated. The virus is found in 30% of sBL, 50% of ID-BL and virtually all eBL. The possible contribution of EBV to BL pathogenesis is largely unknown and it is unclear how directly infection and disease are linked. EBV infected cancers in immunocompetent hosts tend to express the same viral genes as non-transformed cells. They differ in carrying oncogenic host mutations; indeed, BL is associated more strongly with MYC translocation than with EBV infection. Viral genes seem mostly to have triggering or accessory roles in disease, with host oncogenes being the main drivers. The hit-and-run hypothesis proposes that viral genomes initiating disease can be lost entirely to obscure a cancer’s viral origin. Early on, viral genes are likely to be essential for cancer-cell survival. Every population of proliferating EBV-positive cells loses 8% of the viral genomes each cell cycle; after 8 cycles, only 50% of the viral DNA will remain; after 50 cycles, only 1% will remain in the population. Following, cancers accumulate vast numbers of host mutations, some of which will inevitably promote more autonomous growth. Thus, it seems inevitable that a cancer will, with time, evolve increasing independence from viral gene functions that could allow viral genome loss. In fact, an inverse correlation emerges between the number of viral genes expressed in these tumor cells and their associated mutations. The main problem with the hit-and-run hypothesis has been a lack of evidence in primary tumors; therefore, there is the needing to track the fate of viral genomes in transformed cells. To assess the presence of the virus in a specific sample, different approaches can be used, most of them are characterized by a high sensitivity. To date, the most employed methods for diagnostic purposes are immunohistochemistry and EBER-in situ hybridization (ISH). However, they have a low specificity and the accuracy of such assays has been recently called into question by molecular studies that showed the presence of the virus in samples previously diagnosed as EBER negative. The aim of this study is to identify the most suitable method to detect EBV vestiges in pathology samples, shedding new light on “EBV-negative” tumors.

Material and methods. The cases cohort was represented by 10 formalin-fixed and paraffin-embedded BL samples characterized by clinic, morphology, immunohistochemistry (IHC) and cytogenetic consistent with WHO diagnosis of BL; all patients were VCA IgG, EBNA-1 IgG positive and VCA IgM negative. On these specimens we applied different conventional and non-conventional methods to detect the presence of the virus. First of all, IHC for EBNA-1, LMP-1, LMP-2A and ISH for EBER. Then laser capture microdissection was used to isolate areas composed by EBER-negative neoplastic cells only, avoiding the risk to include reactive lymphocytes eventually positive. Following, real-time quantitative PCR (q-PCR) was employed to detect EBNA-1 gene and portion of conserved BamHIW segment, both very sensitive assays. The EBNA-1 targets a single copy highly conserved gene, essential for maintaining the virus long term in dividing cells. The BamH W1 assay binds a reiterated sequence that is present at approximately 10 copies per EBV genome. As microRNA (miRNA) expression has been shown to be a quiet sensitive and specific tool to characterize normal and neoplastic cells, even for pathogens detection, we explored the expression values of EBV-encoded miRNAs in BL samples by performing EBV-encoded miRNAs profiling on Illumina platform. To validate our findings and to test whether they were specific for BL or not, Taqman primers and probes specific for each selected miRNA were applied to analyse by qRT-PCR the EBV-negative BL cases. Three T-LLs samples, currently considered not to be affected by EBV were used as negative control. RNU6B was used as and endogenous control (Apllied Biosystems, Applera, Italy) and the absolute expression was calculated using the 2-Δ ΔCT formula. Kruskal-Wallis Test was applied for statistical analysis.

Results. Four cases showed EBNA-1 expression by IHC and were EBER positive by ISH in 70-95% of neoplastic cells. Six cases were EBNA-1, LMP-1/LMP-2A and EBER (neither in neoplastic cells nor in reactive small lymphocytes) negative. EBV viral load measurement demonstrated that almost all the samples present few copies number of viral genome, EBNA-1 and BAMHIW-1. microRNA profiling showed a clear expression of viral miRNAs in EBER-positive BLs and detected some degree of expression also in the EBER-negative ones. Moreover, it clearly differentiated (by unsupervised hierarchical clustering) the two groups (positive versus negative), and ascertained a variable expression of EBV-miRNAs in EBER-negative samples. qRT-PCR analysis detected a significant differential expression for all the tested miRNAs, namely ebv-miR-BART9-5p, ebv-miR-BART10-3p, and ebv-miR-BART19-3p (p<0.05), viral miRNAs being expressed in all tested BLs but not in T-LL cases.

Conclusions. The findings presented support the possibility that EBV might have contributed to lymphomagenesis in our samples, even when no more detected by conventional methods, but they do not represent a definitive proof of its presence inside the neoplastic cells. In immunocompetent hosts, EBV genome loss may be required for cancers to evolve, the few remaining EBV genome in neoplastic cells be responsible
for the production of the detected miRNAs. Cells driven to proliferate by the EBV growth program are normally killed by antiviral T-cells, so EBV-driven cancers are limited to the immunocompromised patients. In contrast, host mutations drive non-immunogenic cell proliferation even when the viral growth program is turned off. This creates a new balance: viral genes are required only for accessory roles, allowing viral antigen recognition to be reduced. The current study raises the point that, using anti-EBV vaccines, one could potentially prevent not only EBV-associated neoplasms but also clinically virus-negative tumors, thus affecting the worldwide epidemiology of EBV-linked tumors. Finally, miRNA detection might be proposed as the most specific and sensitive tool to identify even EBV vestiges (e.g. EBV exosomes) and diagnose a previous EBV infection in “virus-negative” patients.

**Material and methods.** A 82-year-old female presented to our hospital complaining of generalized weakness. On physical examination moderate splenomegaly without lymphadenopathy was revealed. Laboratory tests detected pancytopenia with neutropenia and a haemoglobin level of 8 g/ml. Abdomen ultrasound examination revealed enlarged spleen (18 cm in maximum diameter) with non focal lesions neither splenic hilum and abdominal adenopathy. Bone marrow biopsy demonstrated normal cellularity for the age of the patient, with a slight erythroid predominance and normal maturation of myeloid and megakaryocytic series. There was no evidence of infiltration by non-resident cells. Due to splenomegaly, persistent pancytopenia and normal bone marrow, a diagnosis of hypersplenism was made; accordingly, the patient underwent splenectomy.

**Results.** The surgical specimen consisted of spleen and hylar lymph nodes. The spleen measured 17x11x6 cm and weighed 337 g. The cut surface showed numerous whitish nodules, ranging in size from 0.5 to 1 cm and localized in the sub-capsular parenchyma. On microscopic examination, a polymorphous infiltrate spanning the periaerital lymphoid sheath and the marginal zones was observed. At higher power, the infiltrate was mainly composed by a mixture of lymphocytes, plasma cells, histiocytes, immunoblasts, and scattered eosinophils, admixing to large mono-binucleated cells with prominent nucleoli resembling Reed-Sternberg (RS) cells. The neoplastic cells were positive for CD30, CD15, LMP-2A; they were negative for CD45, CD20, PAX5/BSAP, CD79a, IRF-4/MUM-1, CD3, ALK-1, Oct-2, Bob-1. Characterization of latency type of EBV, evidenced positivity for EBNA-1, LMP-1, Ea-D, Ea-R, ZEBRA and negativity for gp350, thus suggesting activation of lytic cycle. In situ hybridization (ISH) analysis for EBER was positive. The histologic, immunohistochemical, and ISH findings supported the diagnosis of classical HL. The patient started chemotherapy with the mustargen, oncovin, procarbazine, prednisone protocol. At present (10 months after the initial diagnosis), the CT scan is negative, and the patient is well.

**Conclusions.** Although HD has been found to involve the spleen from the onset in up to third of patient undergoing explorative laparotomy, primary splenic localization has been observed in very few cases and most of them are questionable. The largest series of PSL reported so far, are those of Ahmann and Kraemer both analyzing 49 cases of PSL. Ahmann in 1966 identified 12 cases of primary HD; our post—hoc analysis revealed that most of the patients suffered from large cell lymphoma, probably confused with HD due to the absence of useful immunohistochemical markers. Kraemer in his review reported one patient affected by refractory thrombocytopenia, whose splenectomy showed only one focus diagnostic of HD. However, no imaging study was carried out and the follow up was very short (only two months). More recently, two cases of primary splenic HD have been reported. In one, the patients was affected also by a chronic granulomatous disease, but neither whole body CT scan nor bone marrow biopsy or follow-up data have been presented. The second case described seems to fulfill the criteria for PSL; however, the follow-up confirmation is missed. Therefore, herein we present the first demonstrated case of primary splenic HD. More interestingly, we carried out the immunohistochemical evaluation of EBV latency type, revealing activation of the lytic cycle of the virus. Our data are against the few findings of the literature reporting that RS cells are protected from entry to the virus lytic cycle, being the virus-replicative cycle in B-cells an event presumably incompatible with lymphomagenesis.

**PRIMARY HODGKIN LYMPHOMA OF THE SPLEEN CHARACTERIZED BY ACTIVATION OF EPSTEIN-BARR VIRUS LYTIC CYCLE: REPORT OF A CASE AND REVIEW OF THE LITERATURE**

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**Introduction.** The spleen may be the primary site of lymphoma or it may be an element of disseminated malignancy. Primary splenic lymphomas (PLS) are very rare, with a reported incidence of less than 1%. It is well known that diagnosing as primary in usual sites a neoplasm that commonly arises elsewhere or is already diffuse when discovered, is strictly related to the accuracy of available diagnostic means. At present, the definition of PSL is still used ambiguously in the literature, so that inaccurate observations and series reviews adopting loose selection criteria are found. This is undoubtedly due to the absolute rarity of this presentation. Moreover, half of all patients with Hodgkin disease (HD) and one third of those with non Hodgkin lymphoma (NHL) have splenic involvement. Different diagnostic criteria have been proposed to achieve a diagnosis of PSL. Some Authors consider PSL to be an entity which is limited to the involvement of spleen and the splenic hilum. Others consider it to be a phenomenon that develops in the spleen and that has got the potential for spreading to other organs but in which splenomegaly is the dominant feature. The most strict criteria are those of Das Gupta et al. which proposed that the diagnosis of PSL can be correctly achieved only when the disease is confined to the spleen or the hylar lymph nodes, and no dissemination is evident for at least 6 months after splenectomy. PSL are usually NHL type, originating from the B-cell and represented by small cell lymphomas. The actual frequency of PSL, HD type is difficult to estimate due to the lack of a standard set of diagnostic criteria. In fact our close scrutiny of the cases reported in the literature revealed in most instances the lack of a complete staging (by bone marrow biopsy and whole body CT-scan), immunohistochemical evaluation and follow-up. This raises doubt on the primitivity and probably the diagnosis has been posed as the tumor burden was predominantly localized in the spleen. We report the first case of primary splenic HD with a complete staging and immunohistochemical evaluation, and long-term follow-up. Moreover, since the canonical latency type of Epstein-Barr virus (EBV) has been recently put into doubt in other types of EBV-associated tumors, a study of virus latency has been carried out.

**Results.** In situ hybridization (ISH) analysis for EBER was positive. The histologic, immunohistochemical, and ISH findings supported the diagnosis of classical HL. The patient started chemotherapy with the mustargen, oncovin, procarbazine, prednisone protocol. At present (10 months after the initial diagnosis), the CT scan is negative, and the patient is well.

**Conclusions.** Although HD has been found to involve the spleen from the onset in up to third of patient undergoing explorative laparotomy, primary splenic localization has been observed in very few cases and most of them are questionable. The largest series of PSL reported so far, are those of Ahmann and Kraemer both analyzing 49 cases of PSL. Ahmann in 1966 identified 12 cases of primary HD; our post—hoc analysis revealed that most of the patients suffered from large cell lymphoma, probably confused with HD due to the absence of useful immunohistochemical markers. Kraemer in his review reported one patient affected by refractory thrombocytopenia, whose splenectomy showed only one focus diagnostic of HD. However, no imaging study was carried out and the follow up was very short (only two months). More recently, two cases of primary splenic HD have been reported. In one, the patients was affected also by a chronic granulomatous disease, but neither whole body CT scan nor bone marrow biopsy or follow-up data have been presented. The second case described seems to fulfill the criteria for PSL; however, the follow-up confirmation is missed. Therefore, herein we present the first demonstrated case of primary splenic HD. More interestingly, we carried out the immunohistochemical evaluation of EBV latency type, revealing activation of the lytic cycle of the virus. Our data are against the few findings of the literature reporting that RS cells are protected from entry to the virus lytic cycle, being the virus-replicative cycle in B-cells an event presumably incompatible with lymphomagenesis.
However, a recent paper by Abate and Ambrosio in Burkitt lymphoma demonstrated that also the lytic cycle activation might be of pathogenetic relevance by increasing the number of potentially infected cells by which the tumor may originate. The survival of the PSL patients significantly correlates to the stage of the disease. Mustara et al. suggested that the best therapeutic choice is early splenectomy along with combination chemotherapy (as in our case), due to the higher rates of remission, a more prolonged duration of the remission and the better overall survival.

In conclusion, a thorough clinical, histologic, and radiologic examination is necessary to achieve a correct diagnosis in organs not commonly affected by HD, essential to decide the best therapeutic approach. A deeper investigation of the latency type of EBV on a larger series of HD is warranted to shed new light on the pathogenetic role of the virus in the disease.

**SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION OF THE SPLEEN AND IgG4+ PLASMA CELLS.**


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**Introduction.** Sclerosing Angiomatoid Nodular Transformation of the Spleen (SANT) was first described by Martel et al (1) in 2004 as a distinctive splenic process having a striking multinodular quality that can simulate the appearance of granuloma on low power examination. Some authors (2) believe it is a vascular neoplasm that entrapped the red pulp with a nonneoplastic stromal proliferation. Most lesions are asymptomatic and found incidentally on imaging. Although SANT has a specific imaging findings, the differential diagnosis from other splenic tumors or malignant lesions is very difficult (2). We illustrate a case report of a 61 years old male with a 50-55 mm ovalar mass of the spleen. Two years before he was diagnosed with seminoma. The patient underwent an orchiecetomy and was treated with carboplatin as adjuvant therapy. CT/MRI scan showed a solitary, round, lobulated mass which was centrally hypodense with peripheral-enhancing portions. Considering the diagnosis of a seminoma a metastatic disease was suspected, thus suggesting a splenectomy. Gross pathology examination of the spleen showed a well circumscribed but non encapsulated mass with multiple dark brown nodules, interspersed with a whitish fibrotic stroma (Fig 1). Microscopically, the lesion showed a multinodular growth pattern composed of slitlike, round, or irregularly shaped vascular spaces often surrounded by dense collagen fibrosis or fibrinoid rims (Fig 2). In the core, the nodules were populated by vascular channels of varying caliber lined with plump endothelium with scattered ovoid or spindle cells. Numerous erythrocytes were extravasated and the intervening stroma was fibrosclerotic with a consistent myofibroblastic proliferation, plasma cells, siderophages, and inflammatory cell infiltrates (Fig 3). No evidence of mitotic figures, necrosis or nuclear atypia was found.

**Methods.** Resected tissue was fixed in buffered formalin, embedded in paraffin, cut and treated with hematoxylin and eosin and Masson’s trichrome staining. Immunohistochemistry was performed using the following mouse monoclonal antibodies: Anti-CD8 (e8/144b dilution 1:100 DAKO), anti-CD31 (JC70a dilution 1:100 DAKO), anti-CD34 (QBEnd-10 dilution 1:100 DAKO). Smooth muscle actine (1A4 dilution 1:400 DAKO); CD 68 (clone KP1 dilution 1:5000 DAKO); CD138 (MI15 dilution 1: 100 DAKO); IgG4 (EP4420 dilution 1:2000 ABCAM); CKpool (AE1/AE3 dilution 1:100 DAKO). Flex Target Retrieval Solution Ptlink low pH (DAKO) were used for visualization. Immunofluorescence was performed in paraffin tissue with IgG antibody (polyclonal dilution 1:40 DAKO).

**Result.** At immunohistochemical analysis the nodular component was (CD34 +; CD31+ CD8-; CKAЕ1/AЕ3-) which indicated a vascular proliferation derived from capillary-like elements (1) (Fig 4a, b, c). Smooth muscle actin was positive in the myofibroblasts within the nodules. CD68 was positive in histiocytes within the splenic cord and scattered on sinusoidal macrophages. These findings and the characteristic macroscopic features led to the diagnosis of SANT. We evaluated the expression of IgG4 and IgG in plasma cells component revealing a positivity in 40-50% of the cells (5). In 2009, Kuo et al (4) reviewed the clinicopathological features of 10 cases of SANT and performed immunohistochemistry using IgG4 and IgG antibodies. They reported that the densities of IgG4 + and IgG + cells and the IgG4/IgG ratios were significantly higher in SANT than in control spleens, which suggests that SANT might be related to IgG4 associated sclerosing disease.

**Conclusions.** In conclusion, we report a rare case of SANT expressing IgG4 in plasma cells component. This tumor-like lesion has an unclear pathogenesis that needs to be differentiated from other vasoformative lesions or tumors of intermediate malignant potential; however, the case we reported seems to support the hypothesis that SANT of the spleen could be a reactive process (6) that shares a number of feature with...
inflammatory pseudotumor (5) rather than a true vascular neoplasm and it could be related to IgG4 associated disease. Further research based on a large number of cases are needed to clarify the pathogenesis of this tumor.

References

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EXTREMEDULLARY HAEMATOPOIESIS IN ECTOPIC ADRENAL TISSUE: A CASE REPORT OF AN INCIDENTAL FINDING DURING COLONIC RESECTION

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Background. Extramedullary haematopoiesis (EMH) occurs most commonly in the reticuloendothelial system such as spleen and liver, but it may also be seen in organs, such as bowel, breast, brain, pleura and adrenal glands. It is a response to the insufficient blood cell production by producing blood elements outside of the marrow cavity. It occurs most often because of hemolytic anemias such as thalassemia. It also could be seen in prolonged iron deficiency anemia, myelofibrosis, polycythemia, leukemia and lymphoma. Aberrant adrenal tissue is not a rare finding near the adrenal gland proper, but the occurrence of ectopic adrenal tissue in structures around the bowel is rather rare. In 1740, Morgagni first described yellowish nodules resembling adrenal tissue adjacent to the main glands. Since then, several accounts have been published locating ectopic adrenal tissue in various sites, most frequently in relation to the kidney. EMH in the adrenal glands is rare, with fewer than 10 cases reported, and nobody never reported it in heterotopic adrenal cortical tissue.

We relate a case of 66-years-old man of extramedullary haematopoiesis involving the ectopic adrenal tissue.

Methods. A 66-years-old man was resected for adenocarcinoma of the large bowel, moderately differentiated, with lymph-node metastases and infiltration of soft tissues of the abdominal wall. There was no hepatomegaly and splenomegaly. Laboratory investigations showed an iron-deficiency anemia. Serum cortisol and 24-hour urinary metanephrines were within normal limits. In perivisceral adipose tissue has been found a nodule of mm 4 size with characters of ectopic adrenal gland. EMH was present in the adrenal gland in all cases and nobody never reported it in heterotopic adrenal cortical tissue.

Results. Gross examination revealed a well-circumscribed yellowish mass measuring cm 0,4 in maximum dimension. Histologically this was composed of well-defined layers of adrenal cortex, with predominance of the fasciculate zone, surrounded by a fibrous capsule and none contained any medulla. The immunohistochemical tests were positive for...
alpha inhibin, chromogranin, synaptophysin, negative for pan-keratin, vimentin and calretinin. The central hemopoietic tissue was composed of nests of hematopoietic precursor cells including erythro-myeloid, megakaryocytic cells with hemosiderin deposit similar to those found in normal bone marrow normocellular. The patient did not show any evidence of hormone imbalance pre- or postoperatively.

Conclusions. Hemopoiesis normally occurs in the marrow of long bones, the ribs, and the vertebrae of the adult, in contradistinction to the fetus, in which the principal sites of hemopoiesis are yolk sac, spleen, and liver. Extramedullary hemopoiesis favors certain sites such as the liver, the spleen, and the paraspinal regions of the thorax. However, in addition to these common sites of extramedullary hemopoiesis, the process can involve virtually any organ or tissue. The adrenal heterotopia is usually discovered incidentally in autopsies and surgical specimens and are of no clinical significance.

True adrenal heterotopia is a rare condition and arises as a failure of separation of the developing cortex from the coelomic mesothelium, thus allowing it to become partly or wholly incorporated into adjacent organs. Pathological conditions may develop in this ectopic tissue similar to those seen in normally situated glands. Adenomatous hyperplasia can occur as an independent phenomenon or in association with similar changes in the adrenals proper. Compensatory functional hypertrophy after destruction or removal of normal adrenal glands has been reported, and may rarely account for relapse after adrenalectomy for breast cancer or Cushings’s syndrome. This is the first report of extramedullary hemopoiesis in ectopic adrenal cortical tissue, sited in large bowel perivisceral adipose tissue, in patient with iron-deficiency anemia and adenocarcinoma of the large bowel.

References

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM OF THE TESTIS: A CASE REPORT

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Introduction. Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare entity that was initially identified in 1995. However, the lack of knowledge regarding the histogenesis of BPDCN led to a succession of different classifications of this disease. According to the 2008 World Health Organization (WHO) classification, BPDCN is included in the same class as the acute myeloid leukemia-associated precursor neoplasms. Accumulating evidences show that the normal cellular counterpart resides in the plasmacytoid dendritic cell (pDC) lineage. In fact, the BPDCN tumor cells express the highly specific pDC markers, blood dendritic cell antigen (BDCA)-2, cluster of differentiation (CD)303 and BDCA-4/CD304. The disease usually occurs in elderly patients, with a mean age between 60 and 70 years; however, it can present at any age, even in children. It more often affects men (male to female ratio, 3:1) but has no known racial or ethnic predilection. Clinically, the tumor is characterized by a high frequency of skin lesions at diagnosis, accompanied by extracutaneous spread to the bone marrow, peripheral blood, and lymph nodes. It is extremely rare for BPDCN patients to present without skin involvement. Testicular localization in absence of cutaneous lesions is uncommon and, to the best of pure knowledge, only two previous of such cases have been described in a review series, both affecting male children.

Material and methods. A 51-year-old male presented for a right testicular mass. Physical examination did not identify any evident superficial or deep lesion other than the testicular enlargement. Laboratory tests revealed only a mild thrombocytopenia. Testicular ultrasound examination showed diffuse testicular enlargement with hypervascularity and hypechoic, solid nodules. No inguinal lymphadenopathy was demonstrated. Whole body CT-scan was unremarkable. In the suspicious of a testicular neoplasm, orchietomy was carried out. The surgical specimen consisted of right testis and epididymis. The sample underwent standard procedures for sampling and processing.

Results. Macroscopically, a white-tan-pink, fleshy lesion, resembling seminoma, was observed. The nodule presented extra-testicular involvement. Microscopically a diffuse, mononuclear infiltrate of medium-sized blast cells with irregular nuclei, fine chromatin and numerous small nucleoli was observed. Mitoses were prominent. The proliferation extended into the rete testis, the epididymis and the spermatic cord. Immunohistochemical staining with leukocyte common antigen (LCA), CD4, CD56, CD68 and CD123 was positive; staining with CD2, CD3, CD5, CD7, CD8, CD20, CD30, CD34, myeloperoxidase, CD38, CD138, terminal deoxynucleo-tidyl transferase (TdT) and PAX-5 was negative, with a proliferative index (Ki-67 labeling) of 80-90%. In situ hybridization for Epstein-Barr virus-encoded RNA detected no positive elements. These findings fulfilled the criteria for the diagnosis of BPDCN. Following, the patient underwent bone marrow biopsy that demonstrated an interstitial infiltration by a population of atypical element morphologically and immunohistochemically diagnostic of BPDCN. Thus, the patient was treated with the CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) protocol. Following an initial good response, the disease relapsed and was resistant to second-line chemotherapeutic agents. The patient succumbed one months after the first course of chemotherapy had ended for the development of a leukemic syndrome. The case herein described increases the number of tumors affecting the testis and points to the importance of a correct differential diagnosis in challenging cases. The knowledge of rare entity, especially when presenting in an uncommon way, is the foundation of correct diagnosis and treatment.

Conclusions. BPDCN is a rare but very aggressive disease whose nomenclature, ontogeny and underlying biology have evolved over the years, since its first description by Brody et al. in 1995. Despite the number of cases reported in recent years has increased, as well as our knowledge about the biology of its postulated normal cellular counterpart (i.e. pDC lineage cells), the diagnosis of the disease still remains a challenge. This is probably due to several reasons including its overlapping features with other entities, the lack of recurrent and specific chromosomal abnormalities, and its heteroge-
neous clinical presentation with multiple and variable tissue localizations. In addition, diagnosis of BPDCN can be difficult to achieve, particularly when blast cells do not completely fit the typical immunophenotypic profile. Such heterogeneity is also observed at the genetic and molecular level, since multiple and diverse, but unspecific, chromosomal and molecular alterations have been identified. Therefore, BPDCNs are not only clinically heterogeneous but they also show a considerable biologic diversity which in some cases makes diagnosis difficult. Therefore study of large series of patients, including those (as in our case) with an atypical clinical presentation, are warranted to further sub-classify this entity, similarly to what happens with other neoplasms, such as B and T-cell malignancies or even AML. Deciphering the molecular landscape of the BPDCN according to the different clinic and immunophenotypic presentations, may help to improve the treatment of the disease for which, to date, a standard of care does not exist.