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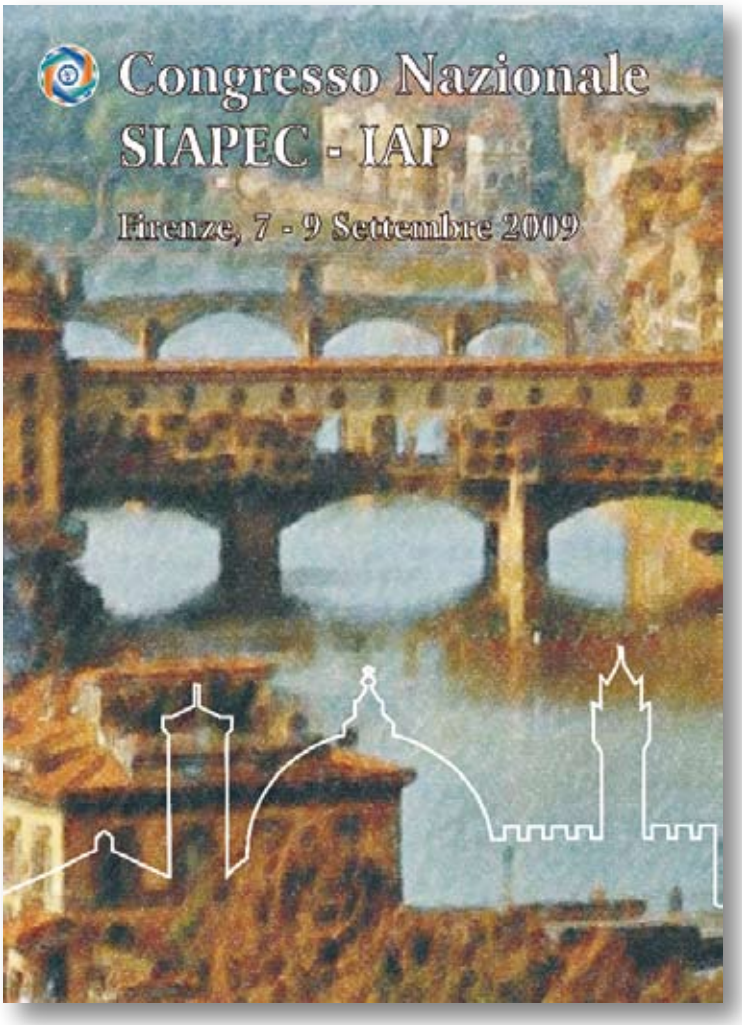

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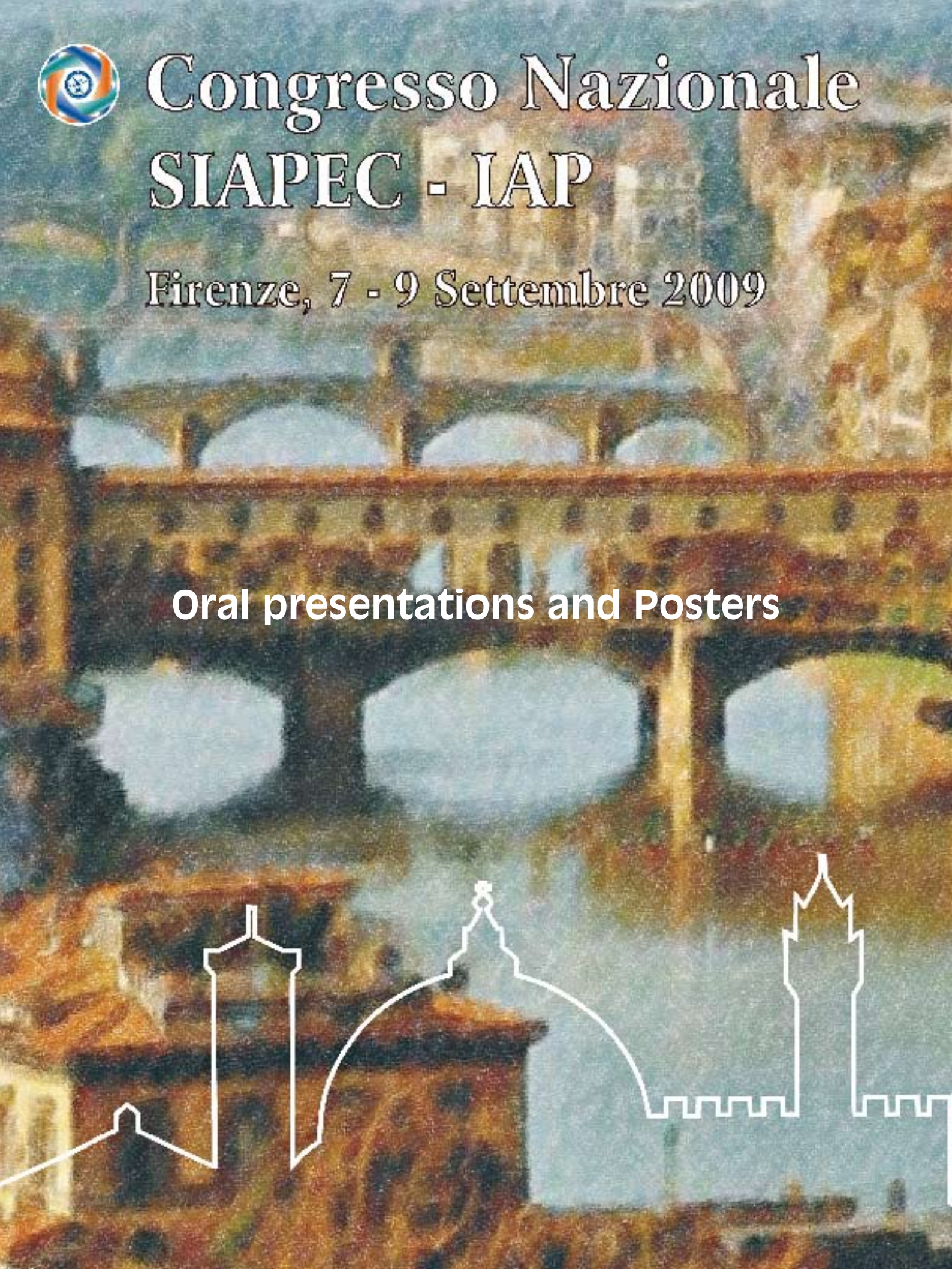
Congresso Nazionale
SIAPEC - IAP
Firenze, 7 - 9 Settembre 2009
Oral presentations and Posters

Congresso Nazionale SIAPEC – IAP
Firenze, 7-9 Settembre 2009

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Oral presentations

CL1.1
Evidence for poliovirus infection in myasthenic thymus: a role of virus-mediated autoimmunity in myasthenia gravis?

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Background. Both genetic and environmental factors are thought to contribute to the etiology of the autoimmune disease myasthenia gravis (MG). Viral involvement has long been suspected but no direct evidence such involvement has been found. We recently reported that Toll-like receptor 4 (TLR4) – one of the key molecule activators of innate immunity – was overexpressed in thymus of MG patients with thymitis and thymic involution, suggesting that thymic infection by virus or other pathogen might be involved in MG pathogenesis.
Methods. We studied thymuses from 29 patients, previously tested for TLR4 expression, to detect cytomegalovirus, varicella-zoster virus, herpes simplex virus types 1 and 2, eubacterial 16S rDNA, respiratory syncytial virus and enteroviruses.
Results. Only the poliovirus (PV) enterovirus was detected: it was present in four (13.8%) thymuses (two thymites and two thymomas) by PCR and immunohistochemistry. No virus was detected in 8 non-pathological control thymuses. A linear correlation ($R2 = 0.96$, $P = 0.02$) between plus- and minus-strand PV RNA levels was observed in all four thymuses, suggesting persistent thymic infection in these cases. The PV capsid protein VP1 was also detected in the cytoplasm of macrophages (CD68+) scattered through the thymic medulla and, in thymitis cases, frequently located around Hassall's corpuscles. By confocal microscopy, VP1 and TLR4 co-localized in some cells.
Conclusions. This is the first demonstration of persistent virus infection in the thymuses of some MG patients, consistent with the hypothesis that persistent TLR4 activation by virus can induce alterations in the thymic microenvironment eventually contributing to autoimmune disease.

CL1.2
Bcl10 and MALT1 genes aberrations and Chlamydia psittaci infections in a series of ocular adnexa B cell lymphoma

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Ocular adnexa MALT-type lymphomas (OABLs) have distinct characteristics from those of gastric MALT model, being in specific geographic areas associated to Chlamydia psittaci infection and recognizing different distribution rate of chromosomal aberration described.
44 cases of OABLs with known follow-up data has been selected. A prognostic TMA has been elaborated for evaluation of t(11;18)(q21,q21), t(14;18)(q32;q21), linked to MALT1 gene deregulation and t(1,14), linked to bcl10 gene deregulation. Moreover immunohistochemical bcl10 topographic expression has been assessed. Finally a PCR based study of presence of C. psittaci was performed. Our data demonstrated there were no occurrences of t(11;18), while 6/42 cases exhibited t(14;18) and 3/35 showed bcl10 gene aberrations (1/35 amplification and 2/35 translocation). Only the sample with bcl10 gene amplification showed nuclear positivity. 4/28 cases showed C.psittaci infection, that correlates with presence of chromosomal aberration and/or bcl10 nuclear positivity. Moreover bcl10 nuclear positivity is associated with shorter disease free survival.
Our data confirm that the most frequent translocation observed in OABLs is t(14,18) (q32,q32). Moreover bcl10 gene is rarely involved in amplification, related to bcl10 nuclear expression, and in translocation. Bcl10 shuttling to nucleus is critical to DFS, but pathogenetic mechanism of this phenomenon is partially explained by chromosomal aberration. Infection by C.psittaci is rarely observed in our cases, originated in a restricted geographic area of South Italy. Its presence seems to be strictly associated with chromosomal aberration observed, but no specific aberration is associated with C. infection.

CL1.3
Epstein-Barr virus persistence and reactivation in non-neoplastic myasthenia gravis thymus

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Objective. Increasing evidence supports a link between Epstein-Barr virus (EBV), a ubiquitous B-lymphotropic human herpesvirus, and a number of B-cell related autoimmune diseases. Here, we searched for evidence of EBV infection in the pathological thymus associated with myasthenia gravis (MG), an autoimmune disease characterized by intrathymic B-cell activation and formation of germinal centers.
Methods. Seventeen MG thymi (6 with follicular hyperplasia, 6 with diffuse hyperplasia, 5 involuted) and six controls were analyzed using in situ hybridization for EBV-encoded small RNAs, immunohistochemistry for EBV latent and lytic proteins, and PCR techniques for EBV DNA and mRNA.
Results. Numerous cells expressing EBV-encoded small RNAs, latency membrane protein (LMP)1 and/or LMP2A were present in the medullary infiltrates and germinal centers of all the MG thymi analyzed. Cells expressing the early lytic cycle-associated proteins BFRF1 and BMRF1 were also frequent in each thymic pathology, together with variable numbers of cells expressing proteins of the late lytic phase (p160 and gp350/220). RT-PCR for latency (LMP2A, EBV nuclear antigen 1) and lytic (BZLF1) viral transcripts and/or PCR for

EBV DNA confirmed the presence of EBV in all the MG thymi analyzed. We found no evidence of EBV infection in control thymic tissue. Accumulation of CD8+ T cells, NK cells and plasmacytoid dendritic cells in the immune infiltrates, but not in the germinal centers of MG thymi, suggests an attempt of the immune system to counteract EBV infection.
Interpretation. Dysregulated EBV infection in the pathological thymus is a common feature in MG and could contribute to the immunological alterations initiating and/or perpetuating the disease.

CL1.4
Differences in pathological assed response to radio-chemotherapy between squamous and non-squamous uterine cervix carcinoma

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Differences in response rate and treatment outcome between cervical Squamous Cell Carcinoma (SCC) and Non Squamous Cell Carcinoma (NSCC) are still debated.. 128 patients were treated with neo-adjuvant radio-chemotherapy followed by radical surgery. All the patients received a pre-treatment surgical biopsy: 111 (86,72%) were SCC; 17 (13,28%) were NSCC. Local response to therapy were classificated as pR0: Pathological Complete Response; pR1: Pathological Partial Response; pR2: Pathological No Response. All patients were restaged according to TNM and FIGO staging; FIGO 0 were considered as No Residual Disease (NRD), FIGO I-II as Local Residual Disease (LRD), FIGO III-IV as metastastic disease (MD). 50 patients (39.06%) achieved pR0; 48 were originally SCC, 2 were NSCC. 44 patients (34,38%) achieved pR1; 40 were SCC, 4 were NSCC. 34 patients (26,56%) resulted pR2; 23 were SCC, 11 were NSCC. NSCC showed a significant ($p < 0,001$) worse local response with an higher percentage of pR2 and a lower percentage of both pR1 and pR0. NSCC more frequently showed infiltration of the cervical wall and infiltration of the parametria ($p < 0,005$). No significant difference was observed for total number of metastatic lymphonodes nor for presence of regional lymphonodes metastases (N1) although NSCC showed a significant ($p = 0,018$) propensity for distant metastases (M1). 49 patients (38,28%) achieved NRD, 47 were originally SCC, 2 were NSCC; 62 patients (48,44%) demonstrated a LRD, 51 were SCC, 11 were NSCC; 17 patients (13,28%) had a MD, 13 were SCC, 4 were NSCC. NSCC showed a significant ($p = 0,04$) worse systemic response rate with an higher percentage of MD a lower percentage of both LRD and NRD: NSCC seems less responsive to multi-modality treatment both locally and systemically.

CL1.5
Survivin expression in pre-treatment biopsies of patients treated with neoadjuvant radio-chemoterapy for uterine cervix carcinoma and its correlation with pathological assessed response on surgical specimens

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Radio-chemotherapy followed by surgery represent a relevant therapeutic option for cervical cancer. The identification of potential markers of response to therapy it is crucial for treatment planning. Survivin is a member of the inhibitor of apoptosis (IAP) the product of the gene is re-expressed in the majority of human tumours and apoptosis is the primary mode of cell death induction by therapy. The relationship between survivin immunohistochemical expression on pre-treatment biopsy and pathological assessed response to radio-chemotherapy is investigated. The study included 52 uterine cervix carcinomas. Cells expressing surviving (Hscore) were identified using the polyclonal rabbit anti-survivin antibody. Local response to therapy was classified on the as follows: pR0: Pathological Complete Response; pR1: Pathological Partial Response; pR2: Pathological No Response. All patients were restaged according to TNM and Figo. FIGO 0 were considered as No Residual Disease (NRD); FIGO stages I-II as Local Residual Disease (LRD); FIGO stages III-IV as Metastatic Disease" (MD). 25 cases (48,07%) were pR0, 17 cases (32,69%) were pR1 and 10 cases (19,23%) resulted pR2. pR2 patients showed an higher Hscore compared to both pR0 ($135,2 \pm 76,38$ Vs $200 \pm 58,3$; $p = 0,021$) and pR1 ($124,11 \pm 63,84$ Vs $200 \pm 58,3$; $p = 0,011$). 24 patients (46,16%) demonstrated NRD, 23 patents (44,23%) demonstrated a LD (44,23%) and 5 patients (9,61%) demonstrated a MD. MD patients showed an higher Hscore compared to both NRD ($134,58 \pm 77,96$ Vs $228 \pm 48,68$; $p = 0,01$) and LD ($130,11 \pm 60,23$ Vs $228 \pm 48,68$; $p = 0,004$). These findings seems to confirm the in vitro observation of a possible role of survivin in cancer cell resistance to therapy. Patients with higher Hscore showed a worse response both locally and systemically.

CL1.6
Hepatocellular adenoma of the placenta. Description of a case and revision of the literature

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Background. Hepatocellular adenoma of the placenta is a rare benign placental lesion. This benign lesion may be associated with maternal congenital disease and/or fetal malformations. A case of hepatocellular adenoma of the placenta in a woman with congenital disease is described.
Materials and methods. Our patient, a 28 years old woman, with a congenital disease of the coagulation (defect of VII factor), had an interruption of pregnancy at twelfth week. The

fetus was affected by onphalocele. A placental fragment (cm 11x7x2) with a peduncolated, oval, dark red and fleshy nodule (cm 2,5) was observed. Histological slides for HE and PAS and immunohistochemical tests were performed. The nodule was composed of a uniform population of hepatocytes with a clear cytoplasm, central nuclei with nucleoli. Some foci of extramedullary haematopoiesis, portal tracts, bile ductules and central venous structures were observed. The tissue showed immunoreactivity for Hepar-1, Alpha 1-antitrypsin, AFP, FattVIII, CD31.
Conclusion. Hepatocellular adenoma of the placenta was firstly described by Chen et al in 1986. The histogenesis of this lesion is uncertain. It has been suggested that it represents an heterotopia of embryonic liver tissue. Our case is the ninth described in literature, the presence of portal tracts in the hepatic tissue support this histogenetic hypothesis.

CL1.7
Mixed undifferentiated carcinoma of the cervix (neuroendocrine carcinoma and adenocarcinoma of intestinal type). Description of a case

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Background. Neuroendocrine carcinoma (NEC) is infrequently described in the uterine cervix and usually mixed with an adenocarcinoma of cervical type or squamous cell carcinoma. Here we describe a case of NEC mixed with an intestinal adenocarcinoma.
Materials and methods. We examined cervical biopsies of a 32 years old woman. She had negative historical data for intestinal and ovary neoplasia. HE staining and immunohistochemical tests for Chromogranin, Synaptophysin, Keratin, CDX2, p63 were performed.
Results. In HE sections we observed a carcinoma with two neoplastic cellular components: a) a NEC component with small-medium size cells, scant cytoplasm, round nuclei with finely dispersed chRometin, focally necrotic, arranged in irregular sheets; b) an intestinal adenocarcinoma component. The NEC component was immunoreactive for Chromogranin, Synaptophysin, Keratin, no immunoreactivity for p63 was observed. Next the NEC, a minor intestinal adenocarcinoma component was immunoreactive for keratin and CDX2.
Conclusion. Here we describe a rare case of neuroendocrine carcinoma mixed with an intestinal adenocarcinoma of the cervix. The use of immunohistochemistry may be helpful in the diagnosis.

CL1.8
Could a mucinous adenocarcinoma of the vagina arise from foci of intestinal metaplasia in a urethral-vaginal fistula? A case report

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Primary mucinous adenocarcinoma of the vagina is very rare and it is reported in perimenopausal women. Histologically the tumor may resemble typical endocervical or intestinal adenocarcinomas of the cervix. The origin of this tumor is unknown and major hypothesis are referred to vaginal adenosis. We describe a case of a 53 years old woman positive for HIV and HCV presented with an ulcerated lesion at the anterior wall of vagina. On biopsy the lesion revealed an intestinal adenocarcinoma. Colonoscopy was negative. A radical hysterectomy with cystectomy was performed. Morphologic and immunohistochemical examination of the neoplasm (positivity for CDX2 and CK20; negativity for endocrine markers and CK7, GATA 3, PSA-PSAP) confirmed the diagnosis. Macroscopically a crater-like lesion was found at the anterior wall of the vagina and an urethral-vaginal fistula (UVF) flowing into the neoplasm was identified. The UVF was covered by a pseudostratified squamous epithelium with multiple foci of intestinal metaplasia. One of these foci was next to the adenocarcinoma with a transitional area of dysplastic epithelium. Neoplastic and dysplastic cells were positive for p53. The origin of these neoplasms are referred to vaginal adenosis. Other possibilities considered have been cloacal remnants, GI metaplasia, heterotopic GI epithelium, mesonephric remnants, and arising in endometriosis. This case support the hypothesis that intestinal adenocarcinoma of the vagina could arise from unknown foci of cells with intestinal differentiation.

CL1.9
Small cell undifferentiated carcinoma of cervix presenting with highly malignant behaviour in a 23-year-old woman: a case report with a review of literature

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Less than 3% of cervical carcinoma is classified as undifferentiated some of these are of large cell type, other are of small cell type. Small cell undifferentiated carcinoma of cervix is known to be an aggressive tumour with a mean age of fifty years. Here we report a case of this kind of neoplasia arising in a very young Colombian woman. A 23- year-old woman referred to our hospital complaining of vaginal haemorrhage and with a clinical presentation of hydronefrosis. A computerized tomography scan showed pelvic and para-aortic lymph nodal-adenopathy and bilateral renal obstruction due to "ab extrinseco" cervix compression. The patient revealed that one month earlier it was diagnosed a high-grade squamous intraepithelial lesion seen on pathological examination of a Papanicolaou (Pap) smear. Cervical biopsies were performed. The surgical specimen were formalin fixed, and paraffin

embedded; 3 micron-thick slices were obtained and stained with haematoxylin-eosin. Microscopic examination revealed well-defined nest of basaloid-type cells with hyperchRometic nuclei resembling small cell neuroendocrine carcinoma, but with more cytoplasm, prominent nucleoli, coarser chRometin and mitoses. Immunohistochemistry was performed on paraffin sections. Immunohistochemical examination showed that the neoplastic cells were positive to keratins and the diagnosis was small cell undifferentiated carcinoma of cervix. No medical treatments were planned because the patient died two weeks after diagnosis. In this report we discussed the clinical, histopathological and racial features of this case with a review of the literature aiming to further studies to define genetic and molecular markers that may be predictive of outcome in patient with this extremely aggressive carcinoma.

CL1.10
Chlamydial genital infections in asymptomatic women between 15-24 years old

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Introduction. In our study has been studied the prevalence of C. trachomatis (Ct) in the voluntary women from September 2005 to May 2008.
Methods. Each patient was investigated between the medical history and cervical swabs. The swabs were collected from 371 asymptomatic consecutive patients: 196 italian female patients and 175 immigrant female patients, between 15-24 years old. C. trachomatis was detected by culture. Clinical samples arrived in laboratory at 4°C and in 2SP medium. C. trachomatis was isolated in LLCMK2 cells were grown in EMEM and identified using a UV Zeiss microscope.
Results. The prevalence of chlamydial infection (5.7%, 21/371) was similar in immigrant patients (4.6%, 8/175) and Italian patients (6.6%, 13/196). The patients were between 15-24 years old (mean age 21.8). All the patients were clinically asymptomatics. The positive patients to isolation of Ct showed the following risk factors: in 61.9% (13/21) non-use of barrier contraceptives and in 57.1% (12/21) number of the partners ≥ 3. The population examined had heterosexual orientation in the 85.7% (18/21) of the cases. The mean age of first intercourse was 16.1 y. o. The mean level of instruction was intermediate (76.2% of cases, 16/21).
Conclusions. In this work the chlamydial prevalence is similar than in female patients of other European studies. This infection was dangerous, since all positive patients to isolation of Ct, have been identified as infected between a mass screening and not for the occurred symptoms. It is the confirms that we need to invest more resources in the prevention of this infection the importance, in the chlamydial infection, the "diagnosis space" and the awareness of patients about this infection without creating excessive alarmism.

CL2.1
Clinical implications of KRAS mutations in lung cancer patients treated with tyrosine kinase inhibitors: an important role for mutations in minor clones detected by a highly sensitive method

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Purpose. Mutations inducing resistance to anti-epidermal growth factor receptor (EGFR) therapy may have a clinical impact even if present in minor cell clones which could expand during treatment. We tested this hypothesis in lung cancer patients treated with tyrosine kinase inhibitors (TKI).
Experimental design. Eighty-three patients with lung adenocarcinoma treated with erlotinib or gefitinib were included in this study. The mutational status of KRAS and EGFR was investigated by direct sequencing (DS). KRAS mutations were also assessed by mutant-enriched-sequencing (ME-sequencing).
Results. DS detected KRAS mutations in 16/83 (19%) tumors; ME-sequencing identified all the mutations detected by DS but also mutations in minor clones of 14 additional tumors, for a total of 30/83 (36%). KRAS mutations assessed by DS and ME-sequencing significantly correlated with resistance to TKI ($p = 0.04$ and $p = 0.004$, respectively) and significantly impacted on progression-free survival (PFS) and overall survival (OS). However, the predictive power of mutations assessed by ME-sequencing was higher than that obtained by DS (HR = 2.82, $p = 0.0001$ vs HR = 1.98, $p = 0.04$, respectively for OS; HR = 2.52, $p = 0.0005$ vs HR = 2.21, $p = 0.007$, respectively for PFS). Survival outcome of patients harboring KRAS mutations in minor clones, detected only by ME-sequencing, did not differ from that of patients with KRAS mutations detected by DS. Only KRAS mutations assessed by ME-sequencing remained an independent predictive factor at multivariate analysis.
Conclusions. KRAS mutations in minor clones have an important impact on response and survival of patients with lung adenocarcinoma treated with EGFR-TKI. The use of sensitive detection methods could allow to more effectively identify treatment-resist

CL2.2
Immunohistochemical expression of IMP3 in non small cell lung cancer

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Introduction. IMP3 is a member of the insulin-like growth factor II (IGF-II) mRNA binding protein (IMP) family and

plays a pivotal role in important phases during embryogenesis [1]. IMP3 is associated with an aggressive behaviour in several malignant tumors while in non small cell lung (NSCLC) its significance has not been still investigated. We studied by tissue microarray (TMA) the immunohistochemical expression of IMP3 in NSCLC.

Materials and methods. A consecutive series of 151 patients with primary operable NSCLC were investigated. Data on tumor type, histological grade, lymph node status, stage, vascular invasion and some clinical data were available. Lung cancer TMA were prepared with 0.6 mm cores obtained from the most representative areas of the tumours. Immunohistochemical was performed using a mouse monoclonal antibody against IMP3 (clone 69.1; DAKO; dilution 1:100). Cases with more than 10% of positive cells were considered positive.

Results. Immunohistochemical analysis was valuable in 126 cases. IMP3 overexpression was found in 69 cases (55%) and was correlated with advanced stages of disease (p =.0011), lymph nodes metastases (p =.005) and higher histological grades (p =.036). Moreover, in squamous cell carcinoma IMP3 overexpression was observed in 75% of cases, while in bronchioloalveolar carcinoma it was present only in 25% of cases. No statistically significative correlations were observed with the other parameters (age, sex, vascular invasion, performance status).

Discussion. Our results suggest that IMP3 could play a critical role also in NSCLC identifying high-risk patients who might benefit from early and tailored systemic therapy.

[1] *Li L, et al. Hum Pathol 2008;39:1205-11.*

CL2.3
Histology of carotid atherosclerotic plaque and acute myocardial infarction

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Acute myocardial infarction is generally caused by severe coronary arteries atherosclerosis. The imaging techniques used to investigate the status of coronary arteries namely coronary angiography, intravascular ultrasonography, CT and MNR are not routinely used. We studied the morphology of 72 consecutive carotid atherosclerotic plaques to identify a histologic feature able to predict the risk of an acute cardiovascular event. We considered two groups of patients: one with myocardial infarction preceding or following the carotid endoarteriectomy and the other with no cardiovascular symptoms. We examined the following histologic parameters: plaque type, calciosis, inflammation, hemorrage, rupture, stenosis. For calciosis and inflammation we used a semiquantitative score system based on the percentage of area occupied by calcium deposition or flogistic infiltration. Statistical analysis was performed. All patients with an acute cardiovascular event presented moderate (23%) or severe (77%) plaque calcification. In the control group severe calcification was present only in a minority of cases (6%; $p < 0.001$). On the other hand inflammation was more pronounced in the control group. No significant differences were observed between the two groups for the remaining parameters. We conclude the presence of a diffuse calcification in the carotid atherosclerotic plaque could be suggestive of a high risk of myocardial infarction and a careful follow up would be indicated in these patients.

CL2.4
Claudin-1, 7 and galectin-3 in papillary thyroid carcinoma: immunohistochemical correlation with different histological patterns and aggressive clinical behaviour

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Background. Claudins (CLDN), integral membrane proteins, are the major components of tight junction. CLDN1 is expressed at higher levels in papillary thyroid carcinoma (PTC) compared to other histotypes while CLDN7 gene expression is reported in both normal and neoplastic thyroid tissue. Galectin-3 (GAL3) is a well recognized PTC marker and its overexpression may be associated with invasive and metastatic properties.

Aim. To evaluate CLDN1,7 and GAL3 protein expression in different PTC subtypes with aggressive course (persistent disease, locoregional/distant metastases) and to analyze the relationship with BRAFV600E mutation.

Materials and methods. 94 PTC [53 classic (PCV), 25 follicular (PFV), 11 tall cell variant (PTV), and 5 PTC with poorly differentiated (PD) areas] were selected for immunohistochemical analysis. BRAFV600E mutation was examined by SSPC-PCR and DNA sequencing in 58 samples.

Results. Most of PCV and PFV showed strong, diffuse, linear and membranous CLDN1 positivity. CDLN1 staining was decreased in PTV and in PTC with PD areas. CLDN7 immunoreactivity showed high heterogeneity both between and within different tumor subtypes. GAL3 was expressed in 92,5% of PTC with variable intensity from weak to strong. Expression was slightly decreased in PFV including solid growth pattern areas. No relationship was detectable with BRAFV600E mutation.

Conclusions. Our study confirms that CLDN1 is upregulated in PTC and may represent a novel marker for this tumor as well as GAL3. No association between CLDN7 or GAL3 protein expression and histological PTC subtypes was observed. Conversely, decreased CDLN1 reactivity was associated with PTC "aggressive" histological variants ($p = 0.003$), thus suggesting a possible prognostic role of this membrane protein in PTC.

CL2.5
Claudin 1 expression in papillary thyroid cancer: prognostic role and correlation with 1513A/C polymorphism in the P2X7 receptor gene

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Background. Expression of Claudins (CLDN), major components of tight junctions, is frequently altered in several human

cancers, including papillary thyroid carcinoma (PTC). The purinergic receptor P2X7 may be involved in human carcinogenesis and we recently observed a relationship between the 1513A/C polymorphism of P2X7 receptor gene and either the follicular variant of PTC or an aggressive behaviour.
Aim. To analyze the presence of CLDN1 and 1513A/C polymorphism in different PTC variants and to evaluate their potential role as combined markers of the disease.
Materials and methods: 57 PTC [34 classic (PCV), 12 follicular (PFV), 7 tall cell variant (PTV) and 4 with poorly differentiated areas (PD)], with different TNM staging (class I/II: 51%; III: 32%; IV: 17%). The 1513A/C polymorphism was evaluated by PCR amplification and RFLP analysis and CLDN1 by a polyclonal antibody.
Results. CLDN1 expression was significantly reduced in PTV and PD samples ($p = 0.01$). The minor allele frequency for 1513A/C polymorphism resulted significantly increased in PFV, PTV and PD samples ($p = 0.003$). A significant relationship was found between 1513A/C polymorphism and either CLDN1 expression or TNM staging ($p = 0.04$; rho = 0.34, for both). Finally, while considering the joint presence of reduced CLDN1 expression and 1513A/C polymorphism, a significant correlation was found with the histological markers of tumour aggressiveness ($p = 0.02$).
Conclusion. Our preliminary data show an association between both the molecular markers and a more aggressive course, suggesting a promising role in selecting patients with different prognosis. The combined appraisal of CLDN1 expression and 1513A/C polymorphism might become a potential tool for modulating the therapeutic approach to thyroid cancer patient

CL2.6
Immunohistochemical analysis of extracellular matrix metalloproteinase inducer (EMMPRIN/CD147) in papillary thyroid carcinoma (PTC): a marker of lymph node metastasis?

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Introduction. CD147 is involved in regulating the expression of matrix metalloproteinase (MMP), has an high expression in malignant tumour and may facilitate tumour metastasis by activating MMP production. Few reports have investigate its expression in well-differentiate thyroid carcinoma [1] but PTC has not been investigated alone. Aim of this study was to analyze the expression of CD147 in PTC and its association with clinico-pathological characteristic.
Materials and methods. 50 PTC with mutational analysis of the BRAF gene, complete clinico-pathologic parameters and follow-up were enrolled. A monoclonal antibody to CD147 (clone AB1843) was used. Scoring of the staining was done according to the intensity (grades 0-3) and extent (0: negative; 1: ≤ 33%; 2: 34-66%; 3: 67-100%). The results obtained were multiplying together: points 0 – negative staining (NS), 1 or 2 – low (LS), 3 or 4 – medium (MS), 6 or 9 – high (HS).
Results. CD147 expression was detected in the cytoplasm and cell membrane of PTC with different expression: HS in 18 cases, MS in 10, LS in 20 and NS only in 2 cases. CD147 expression was not related with gender, tumour size, histologic variant, extrathyroidal invasion and BRAF mutation. Cases with high CD147 expression (HS vs MS-LS-NS) had a frequency of lymph node metastasis significantly higher (66,7% vs 33,3%; $p = 0,01$); in addition HS tended to be associated with multifocality of tumour ($p = 0,057$). No correlation was found with persistence or recurrence of disease (14%) or with distant metastasis (6%). No cancer-related death occurred.
Conclusions. Our data show an overexpression of CD147 in a subgroup of PTC and suggest that its detection may be useful to predict the lymph node metastasis.

[1] Tan H, et al. Transl Res 2008;152:143-9.

CL2.7
Intrahepatic biliary tract carcinoid, prostate adenocarcinoma, and bronchioloalveolar carcinoma: a rare case report of multiple metachronous primary malignancies

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Prostate adenocarcinoma Gleason 9 (5+4) was diagnosed on sextant biopsies in a 66 year old man later staged as pT-4NxM1, after radical prostatectomy. After one month he was diagnosed with bronchioloalveolar carcinoma. Histological examination of lobectomy specimen also disclosed a focal lung metastasis of prostate cancer. Due to its general conditions, no further therapy was programmed. Twenty-seven months later the follow-up evaluation revealed a diffusely unhomogeneous liver at CT, MRI and PET scans, suggesting either multifocal steatosis or neoplasm. Microscopic examination of percutaneous liver biopsy showed multiple foci of small round to ovoid cells arranged in a trabecular and insular pattern, which were immunohistochemically positive for chromogranin, synaptophysin, neuron-specific enolase and, focally, cytokeratin 7. Slides of the two previous cases were reviewed, but no neuroendocrine cells were found in either primary. Intrahepatic carcinoid tumours are very uncommon neoplasms, and may arise as part of a familial syndrome, which is not the case. To our knowledge, this is the first documented case with this association of primary tumours, traditionally considered as belonging to different histogenetic and molecular pathways.

CL2.8
Lipomas and lipomatosis of the head and neck district: report of 88 cases

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Background. Adipocytic neoplasms are common and usually benign lesions, of mesenchymal origin, which generally occur in adult patients, with a male sex predominance.

Clinically, the first sign is represented by a small, poorly circumscribed, softy nodule, rarely exceeding 10 cm in-diameter. Even though they could arise in any site throughout the body, the head and neck district represents an unusual site of involvement.
Objectives. To determinate clinical, histological and surgical approaches of 88 cases of lipomatous tumours presented to the Department of Maxillofacial Surgery of Naples between 1988 and 2008.
Methods. We analysed 88 specimens from 87 patients with lipomatous tumours of the head and neck district presented in the last 20 years to our Department.
Results. Of 87 patients, 52 patients were males (60, 2%) and 35 were females (39, 8%). At the time of diagnosis, our patients ranged in age from 11 to 80, with a mean age 48 years. The lesions varied in size from 1 to 44 cm in-diameter, with a mean size of 5,32 cm. Three groups, according with the primary site of involvement, could be identified: group A (lipomatous tumours localized inside the oral cavity), 21 patient; mean age 52,8 years; lesions' mean size 3,7 cm; group B (lipomatous tumours localized outside the oral cavity), 65 patients; mean age 47,3 years; lesions' mean size 6 cm; group C (lipomatous tumours of the mandible), 2 patients; mean age 33,5 years; lesions' mean size 5,5 cm.
Conclusions. Because of their rare localization in head and neck district and because of their numerous histological variants, adipocytic neoplasms could represent a diagnostic problem, even though surgical treatment is usually considered the mainstay of therapy, leading to an excellent outcome.

CL2.9
Primary intra nodal mucoepidermoid carcinoma

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Occasionally, salivary gland tumors arise in heterotopic salivary tissue outside the salivary glands. The occurrence of mucoepidermoid carcinoma in cervical lymph nodes in absence of a primary lesion elsewhere is exceedingly rare. In the current report, a fifty-three year-old female affected by a well differentiated mucoepidermoid carcinoma that was confined to, and arose primarily within, a submandibular lymph node is described. Histologically, the lesion had a multicystic appearance, with epidermoid and mucous cell lining. Solid islands composed of tumour cells with intermediate, epidermoid and clear appearance were also identified. We also review the cases of primary mucoepidermoid carcinoma arisen within cervical lymph nodes previously reported in the literature. Review of the literature revealed 15 patients previously reported, 4 with tumour arising in an intra parotid lymph node and 8 with tumour arising in neck lymph nodes (site was not specified in 3 patients). The diagnosis of a primary mucoepidermoid carcinoma within a lymph node requires thorough examination of major and minor salivary glands to exclude the possibility of a metastasis. Warthin's tumour with squamous and mucous metaplasia, as well as lymphoepithelial cyst should also be considered in the differential diagnosis. In conclusion, the possibility of a primary intra nodal MEC should be considered when examining a multicystic lesion with squamous lining, and areas with mucous production and solid nodules of intermediate and mucous cells should be searched for.

CL2.10
Intraoral benign mesenchymoma: report of a case and review of literature

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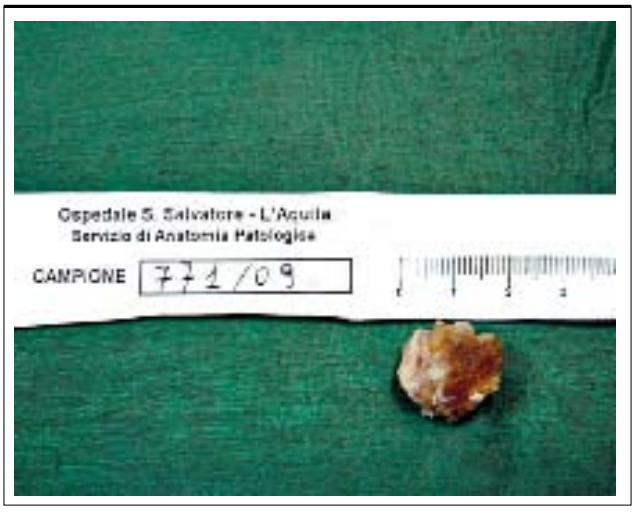
Background. Benign mesenchymoma (BM) is a rare condition, probably due to a proliferation of pluripotent mesenchymal cells, which usually occurs in adults of any age (even though rare in children), with a male sex prevalence. BM is histopathologically defined as a tumour composed by two or more mature mesenchymal elements in addition to fibrous tissue. The upper extremities, kidneys, trunck and perineal region are the most common sites of onset, whereas the oral cavity involvement represents a rare condition. Clinically, intraoral BM usually presents as a solitary mass, with an average size of 3 to 4 cm, covered by normal mucosa and soft in consistency.
Case report. A 68-years-old man presented to the Oral and Maxillofacial Surgery with a firm, asymptomatic mass of the floor of the mouth, of 2 cm in-diameter. Histological examination revealed an unencapsulated mass composed of fibrous connettive tissue interspersed with three well-differentiated mesenchymal tissues, such as adipose tissue, blood vessels and bone. The tumour was seen extending into adjacent normal regional striated muscle bundles and salivary gland loblules. No cellular pleomorphism, necrosis or mitotic activity have been observed.
Conclusions. First described by Klein and Stout (1932 and 1948), the WHO stated MB as a new entity in 1994. No more than 10 cases of benign intraoral mesenchymoma have been reported so far. The diagnostic aid given in the present case by the histologic examination needs to be underlined.

Jones AC, et al. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:67-76.



CL3.1
Kaposi Sarcoma (KS): an interesting model for studying clinical-pathological relationship in tumors

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Introduction. Kaposi Sarcoma (KS), an angioproliferative disease of possible vascular origin, is an interesting model for studying endothelial cells (EC) and related immune cells behaviours. The KS histopathological aspects are characterized by the coexistence of stRomel cells, EC and inflammatory cells. The aim of this study is to detect each cell type, its distribution, reactivity and role.
Materials and methods. Bioptic specimens were obtained from 20 cases of a skin classical Kaposi Sarcoma (CKS). Immunohistochemical staining for endothelial, T-subsets populations, macrophages and stRomel cells were performed. Cytometric evaluations were done on endothelial cells, the stRomel or immature components, T-lymphocyte populations and macrophages.
Results. Endothelial markers staining intensity, which was assessed per unit area, was irregularly distributed between endothelial and spindle stRomel cells. The volumetric densities of T-cell lymphocytes subpopulations were detected. Th and Tc-s demonstrated an equal volumetric densities. The same evaluations were done for macrophages, which appear to be irregularly distributed.
Discussion and conclusions. The histopathological similarities of KS lesions in the different forms, suggest a possible common pathogenetic evolution. EC and stRomel cells may origin from different unrelated cell types or from a common developing cytotype. Anyway from our study, it appears that the stRomel component has a different origin, presumably macrophagic. Immune T-cell response has an uniform expression in KS. It appears that EC lymphocityes and macrophages may play different roles in a unique process which is tumor angiogenesis.

CL3.2
The fluorescence in situ hybridization (FISH) in melanocytic lesions; preliminary results

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Introduction. The videodermatoscopy allowing an early diagnosis of the melanocytic suspicious lesions has caused an increase of the number of thin melanomas (< 1 mm of thickness). Besides it made to emerge melanocytic lesions with atypia to uncertain malignant potential (LMA). This lesions presents same but not all morphological characteristics of melanoma. Studies of genomic comparative hybridization (CGH) has founded in melanomas the loss or amplification of specific locus. In this locus are been mapped some genes like to: RREB1 (6p25), MYB (6q23) and CCND1 (11q13). The FISH could be help to interpret the malignancy potentialities of LMAs.
Materials and methods. 25 melanocytic skin lesions have been selected: 10 melanomas; 10 LMAs and 5 nevi. The FISH analysis have been made on paraffin sections. We are been used the following probes for genes: REB1; MYB and CCND1 (Abbott melanoma kit test). The cases have been valued as suggested by the manufacturing firm.
Results. The FISH test resulted positive in 9/10 melanomas and negative in nevi. The LMAs often presented gene copy alterations (7/10), but only some of this (2/7) reached the parameters of positive test to suggested by the manufacturing firm.
Conclusions. The FISH test is valid in study of melanocytic lesions formalin fixed and paraffin-embedded. The positive tests in melanomas has confirmed that the genes studied have involved in melanocytic carcinogenesis. The gene copy alteration founded in some LMAs supports the hypothesis that some of these cases can be the melanoma precursors, even if the test validity in these lesions needs a verification on a vast population.

CL3.3
Prognostic significance of immunohistochemical IMP3 expression in cutaneous melanoma

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Introduction. It has been proved that IMP3 is a prognostic marker in several tumours. Only a previous work has demonstrated that IMP3 is involved in the biological behaviour of melanocytic neoplasms [1].
The aim of our study is to investigate the possible role of IMP3 in identifying more aggressive melanomas that could metastatize to sentinel lymph node (SLN).
Materials and methods. 21 cases of cutaneous melanoma with metastasis to sentinel lymph node (SLN+) were enrolled and matched with a group of 21 cases without SLN metastasis (SLN-). A monoclonal antibody against L523S/IMP3 (clone 69.1) was used. Cases were considered positive when at least 10% of cells showed cytoplasmic immunostaing (IMP3+). The results were correlated with the clinicopathological characteristics.
Results. IMP3+ was expressed in 61.5% of cases with SLN+ and in 38.5% of SLN-. Melanoma with IPM3+ was related with Breslow's thickness > 1 mm ($p = 0.01$), lymphocytic infiltrate "non brisk" ($p = 0.04$) and regression ($p = 0.008$). In addition, IMP3+ tended to be associated with Clark's levels III, IV and V ($p = 0.059$). The cases with IMP3+ and SLN+ were associated especially with macrometastasis (> 2 mm) ($p = 0.005$).
Conclusions. Our results suggest that IMP3 is expressed in melanoma with a more aggressive behaviour. Moreover it can predict macrometastasis to SLN. Additional studies are necessary to confirm these data.

[1] *Pryor JF et al.Mod Pathol 2008;21:431-7.*

CL3.4
Non-linear imaging of connective skin tissue

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Non-linear imaging represents a novel powerful tool to image biological tissues up to 150 mm depth with sub-cellular resolution and without any exogenously added contrast agent or probe. In the last twenty years several non-linear microscopy techniques have been exploited and applied to tissue imaging. Despite them, second-harmonic generation microscopy is particularly useful to investigate molecules with large hyperpolarizability as such collagen, whereas two-photon fluorescence is useful to image isotropic molecules as such elastin. In this work we have used second-harmonic generation microscopy to investigate collagen remodeling and reorganization in connective tissue ex-vivo samples in some particular pathologic conditions. In particular, we have examined collagen in healthy dermis, as well as in scars, keloids, and at the tumour-stRome interface. A morphological image pattern analysis, based on the grey-level co-occurrence matrix, has allowed to classify different tissues having different morphological arrangement of collagen fibers. Further analysis has been performed by combining two-photon fluorescence and second-harmonic generation microscopy in order to assess the collagen-elastin content of the tissue. This connective tissue characterization method, based on non-linear imaging, represents a promising tool to be extended to other collagen disorders, as well as to be used in in-vivo skin imaging applications.

CL3.5
Cutaneous melanocytic lesions: detection of RREB1, MYB, CCND1 markers and centromere 6 by FISH analysis on paraffin-embedded tissue sections

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Introduction. Cytogenetic abnormalities as aneuploidy, deletions, overexpressions and other chromosomal rearrangements, have long been associated with many tumors types and can be monitored by using fluorescence in situ hybridization (FISH) probes specific for the sought abnormalities. Recently, a multicolor FISH probe has been introduced to aid in the differentiation of benign melanocytic nevi from conventional malignant melanoma.
Materials and methods. We performed FISH analyses with the Vysis LSI® RREB1/LSI MYB/ LSI CCND1/ CEP® 6 probes CE Kit on 55 paraffin-embedded benign and malignant melanocytic lesions and correlated FISH analysis results to clinical follow up data. Specimens included histologically ambiguous diagnoses, including atypical spitzoid tumors. A specimen was considered FISH-positive if the average signals per nucleus of CCND1 or MYB was ≥ 2.5 or if the percent loss of MYB against CEP6 was ≥ 31% or if the percentage of nuclei with RREB1 signals greater or lesser than 2 was ≥ 63%.
Results. FISH test demonstrated genetic alterations in 100% of melanomas while all common acquired nevi were FISH negative. Among atypical lesions, 4/25 "dysplastic" nevi showed single copy number alterations and 2/5 cellular blue nevi showed multiple genetic aberrations. Atypical spitzoid tumors associated with sentinel lymph nodes micrometastatic deposits (without subsequent distant metastases) did not show chromosomal abnormalities.
Conclusions. The FISH probe has demonstrated high specificity and sensitivity in the evaluation of conventional melanocytic nevi and melanomas. However, the clinical value of FISH test in non conventional, atypical, spitzoid and non spitzoid melanocytic lesions, needs to be further evaluated in larger series with long follow up data.

CL3.6
IMP3 protein expression in triple negative breast carcinoma

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Introduction. Insulin-like growth factor-II mRNA binding protein 3 (IMP3) is a newly identified oncofetal RNA-binding protein that is involved in cell growth and cell migration during embryogenesis and seems to be an emerging biomarker of multiple different systemic malignancies and may potentially provide a molecular target for directed therapies. The expression of IMP3 has not been reported in breast cancers, and in particular in the "triple-negative" (TN) subtype, which is characterized by an aggressive behaviour and lacks of targeted treatments.
Methods. We examined the immunohistochemical expression of IMP3 (clone 69.1, dilution 1:100, Dako) in 61 TN breast cancers and in a control group of 48 breast carcinomas and correlated it with the main clinicopathological features.
Results. The most relevant finding consisted in the total absence of IMP3 expression in the control group whereas the cancers with TN phenotype were positive in 25 (41%) cases. Interestingly, we observed the major indexes of biological aggressiveness in the TN-IMP3 positive cases and, in particular, the co-expression of "basal" cytokeratins (CK 5/6, CK 14) and the oncofetal protein IMP3. This latter aspect is in agreement with a more aggressive behaviour reported in patients with TN cancers expressing a basal phenotype.
Conclusion. Our results demonstrate, for the first time, that IMP3 is preferentially expressed in a subgroup of TN cancers which exhibit a more aggressive profile. This observation seems to suggest that TN carcinomas are an heterogeneous category in which the positivity for IMP3 could help to identify a subgroup with a more unfavourable outcome.

Reis-Filho JS, et al. Histopathology 2008;52:108-18.
Jiang Z, et al. Cancer 2008;112:2676-82.

CL3.7
Radial scar on core needle biopsy: correlations with subsequent surgery

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Background and aim. Radial scar lesions (RS) are rare finding on core needle biopsy (CNB), thus limited data are available in literature about the management of these patients. We want to retrospectively look for the diagnosis of RS on CNB and check the correlation with surgical excision (SE).
Material and methods. From May 1999 to December 2008 at Ospedale Civile Maggiore (Verona) were performed 2142 CNB with vacuum-assisted procedure (mammotome, 11 gauge needle). The diagnosis of RS was made in 29 cases; two cases of RS with concomitant ductal carcinoma in situ (RS+DCIS) were excluded. The mammographic alterations leading to CNB were spiculated lesions in 21/29 cases and calcifications in the remaining 8 cases. Subsequent SE data were available in 17 cases.
Results. At CNB we found 10 case of RS without atypia; in 7 cases we observed the coexistence of RS and atypical lesions: 3 cases of RS were associated to atypical ductal hyperplasia (RS+ADH), 1 case to flat epithelial atypia (FEA) and 3 cases to lobular intraepithelial neoplasia (NLI). Subsequent SE showed more advanced lesions in two cases (RS+DCIS in 1/10 cases of RS without atypia and in 1/3 cases of RS+ADH); none of the CNB diagnosis of RS associated to NLI or FEA were upgraded in the SE.
Discussion. Although limited by the small number, our results confirm that a CNB diagnosis of RS+ADH present a significant risk (33%) of subsequent upgrading to carcinomatous lesions. A CNB finding of RS without atypia has a lower risk (10%) of upgrading at SE: our patient upgraded presented a large lesion (25 mm), only partially excised by CNB. Therefore, we suggest that a CNB finding of RS without atypia, especially if related to a small radiologic target, could be spared surgery and managed with close follow-up.

CL3.8
The potential role of chemokine receptor CXCR4 in breast pathology: a progressive tissue microarray study

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Purpose. The biological axis of chemokines and chemokine receptors, such as CXCR4, are involved in the migration, invasiveness, metastasis and proliferation of breast carcinoma (K) cells.
Experimental design. A large cohort of breast K and pre-invasive breast samples was collected.
Our series included 20 typical hyperplasia, 20 atypical ductal hyperplasia, 30 ductal in situ carcinomas (DCIS), 10 in situ lobular K, 60 invasive ductal K, 30 invasive lobular K, 30 axillary lymph-node metastasis (LNM), 10 extra-axillary LNM, 20 cutaneous M, 40 visceral M.
Main clinic-pathological data, hormone receptor status, HER2-neu amplification by FISH,were recorded. Progressive tissue microarrays has been constructed by obtaining triplicate 0.6 mm cores from available paraffin blocks from each one cases; slides cut from the tissue microarrays were immunostained for CXCR4 receptor.
Results. The percentage of CXCR4 staining increased from normal breast tissue (20%) to DCIS (43%) to invasive K(67%). Furthermore the level of CXCR4 expression in lymph- node positive group was higher than that in lymph node-negative group ($p < 0.005$). Moreover only cytoplasmic expression correlated with the number of positive lymph node involved by metastasis, TNM tumor stage, histologic grade, tumor dimension. CXCR4 positivity in the cytoplasm was associated with HER2 amplification as well as with hormone receptor negativity (both ER and PR).
Conclusions. Thus targeting CXCR4 in breast K could be a new therapeutic tool:examining the expression of CXCR4 should provide insights into breast K therapies and improved successful treatments for breast K. Moreover the difference in localization and staining patterns may also carry different significance. However, these results warrant further investigation.

CL3.9
HER2 testing in breast cancer: towards common recommendations in Sicily?

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Many factors such as type and duration of fixation, choice of antibody, threshold for interpretation of positive immunostaining can largely modify the accuracy and reproducibility in the management of immunohistochemical prognostic and predictive markers in breast carcinomas. We report herein the regional experience of Sicily addressed to increase the accuracy and reproducibility of HER2 testing in breast cancer, achieving also more accurate informations about the pre-analytic and analytic procedures utilized. Sixteen public and private anatomopathological institutions made available their breast cancer casuistry collected in the period 2007-08 consisting in 3669 cases. Samples have been fixed in 10% neutral thickμbuffered formalin for 12-72 hrs and included in paraffin at 56°C. On 4 m sections, HER2 determinations were immunohistochemically performed mainly utilizing DAKO HER2 neu-Herceptest (K 5204). The FISH validation was available only in three laboratories located in the main Sicilian cities; only one institution was confident with CISH procedure. Analyzing HER2 scores, as regional values, negative cases (0,1+) were 2540 (69,22% - range from 43 to 86,75%), equivocal cases (2+) were 523 (14,25% - range from 5,6 to 55%), positive cases were 606 (16,51% - range from 5,55 to 24,40%). On the light of these data, it appears the percentage of HER2 positive cases (3+) is clearly underappreciated, with a negative impact on clinical therapy with trastuzumab and overall survival of patients. Consequently, the enrolled sicilian pathologists will attend to further regional meetings in HER2 proficiency testing to obtain a more rigorous interpretation and reproducible results.

CL3.10
Silver in situ hybridization (S.I.S.H.): an alternative method for identification of HER2 gene status in breast carcinoma

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Background. A promising high-sensitivity in Situ Hybridization (ISH) based on silver deposition (SISH) has recently been introduced to establish HER2 gene status in breast carcinoma (K) by light field microscopy.
Methods. 40 infiltrating Ks with HER2 overexpression were evaluated by SISH. Moreover each one case has been assessed for HER2 status by Fluorescence ISH (FISH), in order to define specificity, sensitivity and SISH/FISH concordance for SISH validation. SISH results were assessed by 2 independent observers.
Results. Our series was constituted by 23 cases with immunohistochemical 3+ FDA score (IHC3+) and 17 cases 2+ FDA score (IHC2+).Out of 40 cases,28 showed SISH HER2 amplification.In particular 22/23 (96%) has been previously scored as IHC3+ and 6/17 (35%) as IHC2+.Three different degrees of amplification were recorded: high amplification (ratio > 10) in 43% (12/28), an intermediate amplification (5 < ratio ‹ 10) in 33% (9/28), low amplification (ratio < 5) in 25% (7/28). Amplification, as correlated to hystomorphologic parameters, neither with hystological degree ($p > 0.05$),nor with lymphonodal status($p > 0.05$),nor with tumour size ($p > 0.05$) instead was significantly associated to absence of estrogen receptor ($p < 0.05$) and high proliferative index,ki67 ($p < 0.01$). Concordance SISH/FISH was 95% ($k = 0.87$):FISH+/SISH- discrepancy was recorded only in 2 cases, due to high intratumoral heterogeneity and minimal rate of HER2 amplified cells (20%). Moreover sensitivity of SISH testing was 93% and specificity was 100%.
Conclusions. A multi-step testing algorhythm for determination of HER2 is suggested. After IHC, in cases with 2+ or that are unreliable or inconsistent with IHC,SISH is employed for amplification search;only in borderline or low amplification cases, FISH may be resorted to.

CL3.11
HER-2/neu assessment in infiltrative lobular breast carcinoma using the ASCO/CAP guideline recommendations

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Background. Little is known in regard to Her-2 assessment of lobular breast carcinomas by using the new American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP)scoring systems. We evaluated HER-2/neu status in 32 consecutive infiltrative lobular breast carcinomas.
Methods. Immunohistochemistry by Hercep Test and fluorescence in situ hybridization (FISH) by PathVysion analysis have been performed.
Results. Scores were 3+ in 3/32, 2+ in 1/32, and 0 or 1+ in 28/32 cases. All 3+ and 2+ cases showed high grade Her-2/neu gene amplification (ratio > 4). One case out of 28 showed high grade gene amplification. Three out of 28 cases showed polysomy of chromosome 17 without gene amplification.
Conclusion. About 19% of infiltrative lobular breast carcinoma show Her-2 positive immunoexpression or gene amplification; all cases with amplification show high grade level of amplification; a low rate of polysomy of chromosome 17 without gene amplification is observed among cases with negative Her-2 immunoexpression. The new ASCO/CAP criteria confirm the low rate of Her-2 molecular rearrangements among infiltrative lobular breast carcinomas.

CL3.12
Claudin 4 represents a very useful marker for carcinoma versus mesothelioma diagnosis in serosal effusions

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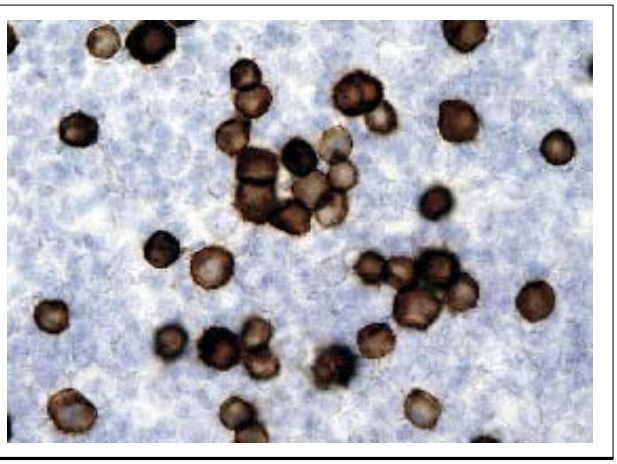
Claudin 4 (CL4), a member of tight junctions proteins, was recently found to be a powerful marker for carcinoma versus mesothelioma diagnosis, being positive in the vast majority of epithelial neoplasms, but regularly negative in mesotheliomas (Virchows Arch 2007;451:669). In this study we evaluated the diagnostic usefulness of CL4 on cytological specimens. Two-hundreds and seventy-seven specimens (pleural, 124; peritoneal, 133; pericardial, 20) were used in this study; all samples were fixed in 95% ethanol and stained with Papanicolau technique. Diagnoses were established by morphological evaluation, clinical information, and, when required, by a panel of antibodies against classical carcinoma and mesothelioma antigens; in most cases serosal biopsies were also available. After coverslip removal, smears were treated by microwave heating in EDTA buffer (pH 8.0, 3x5 min. at 750 W) and subsequently stained with the monoclonal antibody anti-CL4 (clone 3E2C1, Zymed). The results are reported in Table 1. All cases of reactive (52) or malignant (35) mesothelial proliferation were CL4-negative, while 188/190 (98.9%) metastatic carcinomas of different origin



resulted strongly positive. CL4 was very useful in detecting rare metastatic cells on a reactive background (Figure). CL4 represents a robust pan-carcinoma marker, with a very high sensitivity (99.0%) and specificity (100%) for distinction from mesothelioma. CL4 has several advantages compared with other pan-carcinoma markers: it is regularly negative in mesotheliomas and reactive mesothelium, it demonstrates similar diagnostic usefulness in pleural and peritoneal pathology, and it reacts on a wide range of epithelial neoplasms. CL4 represents an ideal "one shot" reagent to detect epithelial metastatic cells in effusions.

CL3.13
E-cadherin cyto-immunoexpression on FNA from breast carcinoma in selecting appropriate IORT-boost radiation

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Background. Intraoperative radiotherapy (IORT) refers to the delivery of irradiation at surgery. Preliminary data suggest that IORT given as a boost during breast-conserving surgery, in patients affected by breast cancer, could be a reliable alternative to conventional long-term post-operative boost radiation. Significant different amount of Gy are given to patients affected by ductal versus lobular breast carcinoma. There is an increasing request in the knowledge of the accurate subtype of breast cancer before planning the appropriate amount of Gy dose during surgery. We sought to evaluate the accuracy of subtyping of breast carcinoma on material from fine needle aspiration (FNA).
Methods. We selected 20 pre-operative FNA cytology, 10 subsequently histologically-confirmed ductal and 10 lobular breast carcinomas. We compared cyto-smeared cells to histological sections. Immunolabelling was performed by using the E-cadherin marker. A positive case was considered when a carcinoma showed immunoexpression in > 80% of neoplastic cells.
Results. None of the lobular carcinomas expressed E-cadherin on cytologic material whereas all of the ductal breast carcinomas were positive. The tissue sections from the matched tumours showed similar immunoexpression.
Conclusions. 1) E-cadherin cyto-immunoexpression on FNA pre-operative cytology have a role in pre-operative correct subtyping in differentiating ductal from lobular breast carcinomas; 2) this pre-operative analysis is increasing as a request from radiologist in order to apply the appropriate Gy boost radiation to patients during surgery and substitutes long post-operative radiation time; 3) further analysis are needed to study heterogeneous morphological cases.

CL3.14
Comparison between fine needle aspiration cytology and ultrasound features in thyroid nodules: an US score to select thyroid nodules requiring FNA

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Introduction. Ultrasound (US) examination of thyroid does not offer precise criteria between benign and malignant nodules because no single parameter has a clinically relevant sensitivity and specificity. Recently we described a US score including more risk parameters [1]. This score was constructed comparing US features with cytologic diagnosis in a large casistic. The discrimination power of each US parameter (number of nodules, echostructure, echogenicity, halo, microcalcifications and ratio between antero-posterior and transversal diameters (AP/TR)) was used to construct a 10 point score. The aim of this paper was to confirm the accuracy of US score in another series of nodules.
Materials and methods. Among all FNA of thyroid nodules received between April 2006 to December 2008 we selected 1,741 cases where all US parameters were available. The cytologic diagnoses were made in accordance with the 5 diagnostic groups of British Thyroid Associations (2002).
Results. The cytologic diagnosis revealed 1,644 Thy2 cases, 34 Thy3, 63 Thy4-5 cases respectively. To evaluate the frequency distribution of thyroid malignancy with each US parameter comparison was done for all Thy2 and Thy4-5 lesions (n. 1,707). Nodules with US score ≤ 2.4, those between 2.5-5.4 and ≥ 5.5 had a frequency of malignity of 0.6%, 1.6% and 13.6% respectively ($p = 0.000$). Nodules with US score ≥ 5.5 were characterized by a 71.4% sensitivity and 82.5% specificity compared to the values of single parameters which were either sensitive or specific.
Conclusion. These data confirm the reproducibility and the accuracy of our US score in identify malignant lesion especially in iodine deficient areas where US Score can reduce unnecessary FNA.

[1] Cavaliere A, et al. J Endocrinol Invest (in press).

CL3.15
A new morphological-molecular combined test: perspectives of implementation in the cervicocarcinoma screening and therapy

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The new test represents a way overcome the limits of the conventional or liquid- based pap-test, morphological analysis,

and other molecular tests for HPV-DNA (PCR). The first one shows a short sensitivity and specificity in the initial stages of the HPV infection when the typical cellular alterations aren't still evident or in case of benign alterations or in atypical cells (ASC-US, ASC-H). The second one shows the presence of some types of HPV (6-11 and 16-18) without having the possibility of displaying all the main oncogenic types and they don't normally provide information about the stages of the infection (the RNA test are very expensive inside a screening). The test, here, suggested, gives the possibility of trying different oncogenic types of HPV and stages where the infection takes place. In this way, it is possible to discriminate the simple presence of HPV, cytoplasmic infection or a virus integrated into the host DNA. The combined morphological-molecular test provides the possibility of reducing the costs in the reading (now possible in only one stage), avoiding the repetition of exams and saving times and costs of normal test PCR. Many researches (FUTURE II) have shown how the elimination, through the vaccination, of the types more analyzed, has caused the proliferation of other oncogenic types not subjected to vaccination, producing a moderate total reduction of intraepithelial lesions or malignancy. It is supposed that it could also happen in Europe and in Italy soon; here, the vaccination vs HPV has been introduced recently. It follows that it is necessary a revision of the diagnostic methods and therapeutical plans, integrating the vaccination with the antiviral and laser therapy to carry out an effective prevention of the cervico-vaginal cancer.

CL3.16
Thyroid fine needle cytology optimization

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Fine needle aspiration cytology is so much specific and sensitive indicator for management of thyroid nodule that it's possible to select patients who need surgery. Neverthless the methods of aspiration are different among ultrasound technicians, as well the percentages of diagnostic specimens obtained. Ultrasound features of lesions correlate with cell density, which affects the number of aspirated cells. We studied 365 fine needle aspiration specimens by thyroid lesions. Four ultrasound technicians performed aspirations. The specimens were prepared both convenzional and thin prep methods. We evaluated aspiration techniques, ultrasound features of lesions, total number of smears and number of diagnostic smears. We also evaluated, by semiquantitative method, the cell density both smears and thin-prep preparations. Results show that total number of smears are significant among ultrasound technicians ($p < .0001$) independently by ultrasound features of nodules, but in evaluation of diagnostic smears are also important ultrasound features of lesions ($p < .005$). Probability to obtain a diagnostic specimen, estimated by logistic regression analysis, is correlated to aspiration methods ($p < .0001$) rather than ultrasound features ($p = .1830$). Results overlapping when thin-prep specimens are considered. Best results are obtained when ultrasound tecnhnician makes aspiration using 22-25 G fine needle, three times each nodule. After aspiration he smears 2 slides, washing needle with Cytolyt solution.

CL3.17
Accuracy of fine needle aspiration cytology diagnosis in breast cancer according to European guidelines and comparison between screening and out of screening women

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Introduction. FNAC is a reliable procedure in breast lesions. This study reports cases collected in a senology centre performing echography and mammography both on screening patients and out of screening women.
Methods. We analyzed FNACs carried out between 2006 and 2008. One single pathologist performed sampling, on site evaluation of sample adequacy and definitive diagnosis, applying the European Guidelines for Quality Assurance in mammography screening (3rd edition; European Commission, 2001).
Results. 822 FNACs was performed on 759 patients: 362 (44,0%) on women involved in screening programs (50-70 years), 374 (45,5%) on women younger than 50 years and 86 (10,5%) on women older than 70 years. The inadequate rate (C1) was 8,0%. Global sensibility and specificity were respectively 95,5% and 67,3%, false negatives 1,1%, false positives 0%. Women younger than 50 and older than 70 showed different sensitibility rates compared to screening patients (93,5% and 95,9% versus 96,0%), and the same was for specificity (69,6% and 53,1% versus 66,0%). For all cases the positive predictive values for C4 and C5 categories were respectively 82,9% and 100%.
Conclusions. FNAC is a safe and reliable procedure in detection of breast cancer, especially when a close collaboration between radiologist and pathologist is ensured; this collaboration, joined with simplicity and less invasiveness, entails that evermore frequently FNAC could replace bioptic procedures in breast cancer diagnosis [1].

[1] Barra Ade A, et al. Diagn Cytopathol 2008;36:26-31.

CL4.1
Clinical and molecular characterization of screening detected MSI-H colorectal cancers

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Introduction. Lynch syndrome (LS) is the most frequent hereditary colorectal cancer (CRC) syndrome and is characterized by microsatellite instability (MSI) due to germline mutations of the DNA mismatch repair (MMR) genes. MSI is also present in 10-15% of sporadic CRCs, due to MLH1 promoter biallelic methylation. Few data are available about detection of LS cases in the context of a population screening program.
Methods. MSI and MMR protein expression were evaluated in CRCs identified in the screening program of the province of Ferrara in the period May 2005 – November 2008. Expression of MMR proteins (MLH1, MSH2, MSH6 and PMS2) was

evaluated by immunohistochemistry and MSI by a fluorescence based PCR method using the Bethesda panel markers. MLH1 promoter methylation was determined by methylation specific PCR in MLH1-negative cases.
Results. MSI was observed in 22 tumors (21 patients: 11 male and 10 female). MSI+ tumors were often localized in the proximal colon (17/22, 77.3%) and were characterized by frequent TNM stage I (n = 9) or stage II (n = 9). All MSI+ tumors showed loss of MMR protein expression. In detail 16 tumors were classified as MLH1-neg, 1 as MSH2-neg, 4 as MSH6-neg and 1 as PMS2-neg.
Based on clinical and immunohistochemical findings, 8 of the MSI+ tumors could be considered as presumed hereditary and 14 (from 13 patients) as probably sporadic (MLH1-neg tumors in patients > 55 years old). MLH1 promoter methylation was observed in 10/16 MLH1-neg tumors and was more frequent in older patients.
Conclusions. Biomolecular analysis was able to identify a significant number of screening detected CRC, most likely due to LS. Further studies are needed to confirm these results and to differentiate sporadic and hereditary MSI+ tumors.

CL4.2
Lymphonode micrometastases in stage I colorectal carcinoma with poor prognosis

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Introduction. Patients with stage I colorectal carcinoma have a good prognosis with a 5 year survival of 90-95%. A small fraction of them die of local or distant recurrence. In a previous study we showed that an infiltrating pattern of growth, absent peritumoral lymphocytic infiltration, presence of tumor budding, vascular invasion and p53 overexpression were related to a poor clinical outcome. It was suggested that the presence of occult metastases on pathologic routine might explain the neoplastic progression in patients with stage II colorectal cancer. In this study we evaluated the presence of micrometastases, using immunohistochemical staining (IHC), in lymphonodes of patients in stage I died for neoplastic disease.
Methods. From the Colorectal Cancer Registry of Modena we selected 43 patients in stage I died for disease progression. The analysis could be executed in 25 cases in whom lymphonodes were examined (6,8 nodes/case). Paraffin embedded nodes underwent step sectioning at 200μm intervals, yielding 4μm thick sections. Alternate sections were evaluated with HE and IHC staining with pancytokeratin antibodies. The micrometastases have been differentiated in glandular structure, clusters and single neoplastic cells.
Results. Micrometastases have been found in 61% of the cases (18 of 25) in a variable number of nodes (from 1 to 3 for case) with a mean of 1,6 nodes. Only in one case we observed glandular structure, in 10 cases clusters of neoplastic cells, and in remaining cases single neoplastic cells.
Conclusion. These preliminary data suggest that lymphonode micrometastases might have a role in the disease progression of patients with stage I colorectal cancer. The study of this "extreme" group of patients might be of relevance for understanding tumor behaviour.

CL4.3
Extrahepatic hilar biliary cystoadenoma

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Background. Biliary cystoadenomas (BCA) are rare neoplasm of liver occurring almost exclusively in middle-aged woman. They represent the 5% of intrahepatic biliary cystic lesions. Less than 10% of BCA occur in extrahepatic biliary tract. Some authors consider BCA premalignant lesions, although malignant transformation is reported in very scantly cases. High rate of local recurrence for extrahepatic-BCA (EBCA) treated with partial resection was reported in a short series of cases. Clinical presentation depend on size and site of origin: obstructive jaundice and abdominal pain are the most common symptoms. Imaging generally demonstrate the presence of multilocular cyst. Preoperative differential diagnosis within benign and malignant lesions could be very difficult.
Materials and methods. We report a case of a middle-age woman admitted to our Hospital for an asymptomatic cyst (diameter: 25mm) at the hepatic ducts confluence. Her past medical history reported a previous diagnosis of common bile duct cystic lesion followed by resection in 1980.
The new lesion was detected during a routinely check-up. She underwent a complete excision followed by biliary reconstruction and biliojejunostomy.
Results. Imaging and gross pathological examination demonstrated a multilocular cyst at hepatic hilum not connected with biliary lumen, microscopically lined by a single layer of columnar mucin-secreting cells, with evidence of mild dysplasia, with focal polipoid projections, surrounded by ovarian-like stRome. The diagnosis of EBCA was performed.
Conclusion. Although very rare, it's important to recognize EBCA because an accurate diagnosis lead to a proper surgical treatment, reducing the rate of loco-regional recurrence and eventually of malignant transformation.

CL4.4
Azathioprine induced acute phlegmonous gastritis in Crohn's disease

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Azathioprine inhibits cell growth interfering with nucleic acid synthesis and is used as immunosuppressant in a variety of autoimmune and severe inflammatory conditions, such as inflammatory bowel diseases. Severe leukopenia, thrombocytopenia, macrocytic anemia, and/or pancytopenia may occur in patients treated with azathioprine [1]. Sweet Syndrome has been described in a patient receiving azathioprine as a ipersensitivity reaction [2]. We report the case of a 25-year old woman affected by Crohn's disease, who was administered azathioprine and came to our hospital for acute addominal pain. The upper tract endoscopy revealed patchy haemorrhages throughout the gastric mucosa with focal erosions in antrum and body, congestion and oedema of the duodenum. Biopsy examination re-

vealed oedema, patchy hemorrages and erosions of superficial lamina propria. In the pits and glands a pronounced number of neutrophils with a non-homogeneous distribution and without a marked chronic infiltrate was present, associated with formation of glandular abscess, like an acute phlegmonous gastritis. Since it is described in literature that Azathioprine causes similar morphological alterations in skin [3], a drug side effect was suspected and azathioprine administration was stopped. Two weeks after she underwent new endoscopical biopsies which demonstrated an almost complete resolution. At once, azathioprine was dismissed with complete resolution of symptoms. The immediate recognition of this association is important to allow the interruption of azathioprine therapy. To our knowledge, no case of gastric abscesses induced by azathioprine therapy have been described before.

[1] *Gisbert JP. Am J Gastroenterol 2008;103:1783.*
[2] *El-Azhary RA. Mayo Clin Proc 2008;83:1026.*
[3] *Watermeyer G. Gut 2009;58:67.*

CL4.5
Histologic risk factors and clinical outcome in malignant colorectal adenomas

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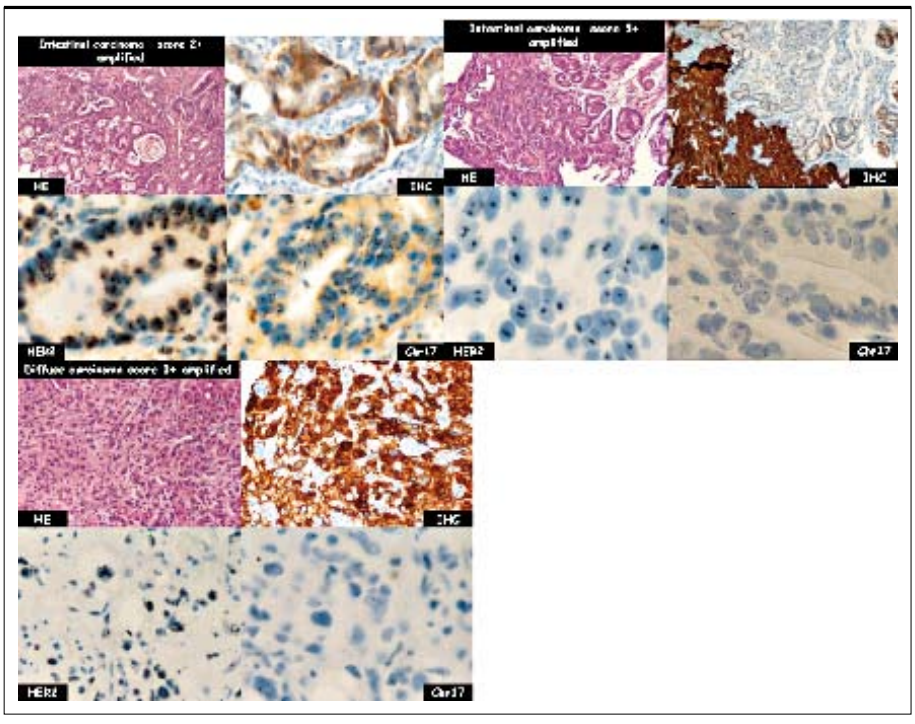
Introduction. Through the Colorectal Cancer Registry we attempted to investigate the clinical and histological features of patients in Stage I, pT1, who were treated with endoscopic polypectomy. Aim of the study was to evaluate the outcome of malignant adenomas on the base of histologic criteria.
Methods. From 1984 to 2004, 105 patients were selected. The presence of one of following histologic criteria (positive resection margin, poor differentiation carcinoma, vascular invasion and tumor budding) defined malignant adenomas and patients at high risk.
Results. Fifty five malignant adenomas were classified as low risk and 50 as high risk. Median polyp size was 19,53 mm. After polipectomy, 10 patients of 55 at low risk were operated on multiple polyps; 15 died of unrelated causes, 40 were alive and in good clinical conditions (median follow-up of 82 months). Of 50 patients at high risk, 23 had surgery; the most frequent cause of inoperability (27 cases) was cardiovascular disease; 7 died of unrelated causes, 3 died for cancer (2 with local recurrence and 1 with metastatic carcinoma), and 40 were alive and in good clinical conditions (median follow-up of 82 months). No patients in the low risk group died for neoplastic disease, while three patients in the high risk group died of cancer; 2 performed only endoscopic polipectomy and showed in one case all histologic criteria of high risk and in the other only vascular invasion. Vascular invasion and tumor budding were present in the remaining case with poor prognosis.
Conclusion. Endoscopic polypectomy seems to be adequate for patients with low risk malignant adenomas. In patients with high risk malignant adenomas the histologic factor more frequently associated with a poor prognosis seems to be the vascular invasion.



CL4.6
HER-2 status by means of SISH technique in gastric carcinoma

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HER-2 status has thoroughly been studied in breast carcinoma. Its over-expression is associated with patients elicited for trastuzumab therapy with reliable benefits. Very few papers, mostly from asiatic countries, have associated HER-2 status with gastric carcinoma. We studied a series of gastric carcinomas evaluating immunohistochemical HER-2 expression and amplification by means of SISH technique in order to detect patients eventually eligible for trastuzumab therapy. One hundred and sixty-one consecutive gastric carcinomas (96 intestinal and 65 diffuse type) were enrolled in our study. HER-2 amplification was performed on all slides according to manufacturer instructions. Statistical analysis was performed in order to reveal associations between immunohistochemical HER-2 expression, HER-2 amplification and gastric carcinoma histotype. Our data reveled only a little rate of diffuse carcinomas with IHC score 2-3 + and amplification. Seventeen out of 96 intestinal type carcinomas (17, 7%) revealed HER-2 over-expression (fifteen score 3+, two score 2+); nine(19,7%)

were amplified (fifteen score 3+, 2 out of 7 score 2+, 2 out of 58 score 0). Six out of 65 diffuse type carcinoma (9,2%) revealed HER-2 score 2-3+ and two (3%) were amplified (one score 2+ and one score 3+). As in breast carcinoma HER-2 over-expression in gastric carcinoma depends on gene amplification. Our novel data revealed HER-2 expression ($p < 0.05$) and amplification ($p < 0.005$) essentially in the subset of intestinal type gastric carcinoma stratifying and addressing patients for target therapy.

CL4.7
Cutaneous metastasis of chordoma: a case report

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Introduction. Chordomas are rare malignant tumors of notochord origin and are locally aggressive with a metastatic potential. The skin is rarely seen as a metastatic site.
Case report. We describe a case of an adult woman with cutaneous metastasis from a primary sacral chordoma excised ten years before, which appeared as a painless cutaneous mass located in the dorsal region. The neoformation (4x2 cm), once removed, was tamponated, formalin fixed and routinely processed for inclusion in paraffin. Sections were stained with haematoxilin-eosin, histochermical and immunohistochemical investigations were performed. Histologically, the neoplasia was characterized by cords or single tumor cells with an abundant myxoid stRome, a conspicuous pale vacuolated cytoplasm (the classic "physaliphorous cells") and mild nuclear atypia. Mitotic activity was scanty. At immunohistochemistry, the tumor cells were diffusely positive for S-100 protein, pan-keratins, EMA and vimentin but negative for EMA and smooth actin muscle. A diagnosis of cutaneous metastasis of chordoma was performed.
Discussion and conclusions. This case illustrates a diagnostic challenge because of the unusual presentation of an already rare tumor. Chordoma can be confused with other pathological conditions but the presence of physaliphorous cells and its immunohistochemical features can be helpful in making the distinction. Immunohistochemically the tumor cells of chordoma label with cytokeratins, vimentin, epithelial membrane antigen (EMA) and S-100 protein. No other mesenchymal tumours histologically similar to chordoma like chondrosarcoma, myxoid liposarcoma and parachordoma, have a similar wide CK positivity.

CL4.8
Primary teratocarcinoma of the third ventricle mimicking adamantinous craniopharingyoma on frozen section

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We report the case of a 20 year old girl who presented with frontal headache, disturbed vision, menstrual cycle disorders and diabetes insipidus of 15 days' duration. MRI of the brain identified a mass in the 3rd ventricle, which was isointense on T1 and T2-weighted images and contrast-enhancing; a T1-hypointense area within the lesion was suggestive of calcification, and supratentorial hydrocephalus was present. The patient underwent surgical removal of the mass, and intraoperative pathologic examination was performed on a small fragment. Two squash slides with modified Giemsa staining were prepared, and the remaining specimen was submitted for frozen section. The intraoperative squash smear showed many groups of large cells, sometimes nucleolated, along with fibrillary material, and frozen section revealed a cystic area lined by squamous keratinizing epithelium with a palisaded basal layer, with surrounding reactive gliosis, returning a diagnosis of probable adamantinous craniopharingyoma; anyway, final diagnosis was deferred pending examination of permanent sections. The excised lesion appeared as a grey-whitish solid mass containing multiple cystic areas. Histological and immunohistochemical examination of formalin-fixed, paraffin-embedded material showed a mixed germinal tumour (immature teratoma, embrional carcinoma, yolk sac tumour and syncytiotrophoblast-type giant cells), with a final diagnosis of teratocarcinoma. We describe herein clinical and pathological features of a primary teratocarcinoma of the brain, which is a very rare tumour, and highlight the limits and inherent difficulties of frozen section due to partial sampling, especially when examining mixed tumours.

CL4.9
"Ependymosarcoma": case report of a rare entity

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Background. Sarcomatous differentiation is a well documented phenomenon in glial tumours (gliosarcomas), where the glial component is usually astrocytic and more rarely oligodendroglial. Rare cases of sarcomatous change in ependymomas have been described.
Case report. White male, 56 years old, with a neoplasia in the region of IV° ventricle.
A first surgical resection showed an ependymoma with evidence of bone metaplasia.
After 4 months the neoplasia relapsed in the same location and the patient underwent a second resection. As in the first surgical specimen, there were areas of an ependymal neoplasia, without features of anaplasia, intermixed with areas made by spindle and pleomorphic cells.
These showed a brisk mitotic index and focal necrosis. The spindle elements were uniformly negative for GFAP, which it was positive in the ependymal component. The final diagnosis was: ependymoma with sarcomatous change. The lesion increased its size rapidly, involving also the soft tissues of the neck and after a few months the patient died.
Discussion. Gliosarcomas are considered subtypes of glioblastomas in the WHO classification of brain tumors. The glial component is usually astrocytic, but cases with oligodendroglial component have been described. The prognosis of such neoplasias is uniformly poor.
Using this definition also the cases of sarcomatous metaplasia in ependymoma should be considered gliosarcomas. The

biological behaviour of these tumors is usually aggressive, although examples of long term surviving are described in the literature. For this reason the term "ependymosarcoma" seems more appropriate.

[1] *Rodriguez, et al. Am J Surg Pathol 2008;32:699-709.*

CL4.10
Quality of and accreditation to Her-2/neu FISH testing in breast carcinoma

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Introduction. There is an increasing requests from oncologists to pathologists of molecular analysis related to targeted-therapy, being the Her-2/neu gene status in breast carcinoma by fluorescence in situ hybridization (FISH) method, the most frequent evaluation in a surgical pathology laboratory. Our staff (Verona University) takes into account the recommendations that grant accreditation to Her-2/neu FISH testing. We sought to evaluate the procedures of this molecular analysis in order to assess its accuracy.
Methods. We analyzed steps involving biological material and personnel along timing of Her-2/neu FISH analysis in 100 breast carcinoma consecutive cases.
Results. Her-2/neu FISH test is routinely requested by the pathologist after referring the histological diagnosis of breast carcinoma. He identifies the biological sample on which Her-2/neu testing has to be performed (1th day). The technical personnels prepare tissue sections and a molecular code number (2009-FLA-1000) is given onto and linked to the matched histological code number (2th day). A dedicated technician performs the FISH analysis by using commercial kits (3-4th day). The evaluation of the test is screened by the technician, then verified by pathologist. Overall Her-2/neu assessment by FISH is reported onto the Hospital Internal Database, printed as official diagnosis and sent to oncologists (5th day). The print form of the molecular diagnosis requires patient's name, FLA (fluorescence analysis) and histological code numbers, and assessment of Her-2/neu amplification.
Conclusion. The identification of standardized-steps assesses the quality of and accreditation to the Her-2/neu FISH molecular testing in breast carcinoma.

CL4.11
Medical bioengineering in the Middle Age. The upper-limb and lower-limb prostheses. Description and study of limb prostheses belonging to Stibbert Museum of Florence

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The use of prostheses dates back to many centuries b.C.. In 1971, Russians disclosed the skeleton of a woman (2300 b.C.) with a leg of a goat instead of the amputated limb. An Egyptian mummy has a woody great toe. Erodoto and Plinio the elder report men with artificial limbs. Medioeval prostheses were produced by armourers: the artificial hand of "Alt-Ruppin" and G. Von Berlihingen allowed to brandish the sword. Here we describe the characteristics of five medioeval prostheses belonging to the Stibbert Museum of Florence. An iron made hand shows a fixed thumb and four movable fingers; thanks to a rack, the fingers could be flexed and fixed in a desired position; a small lever allowed to remove the blockade of fingers and to bring them back to the initial position. A second iron made hand has five movable fingers and some holes with geometrical or floral shape, decreasing the weight of the prosthesis. An iron made lower-limb, including a knee, a leg and a foot, allows to flex and extend the leg thanks to the structure of the knee, composed of sliding sheets of iron; the distal part of the foot is also movable. This prosthesis was devised to allow a less lame deambulation. Two further very light iron made lower-limbs prostheses, made it possible to correct or hide a leg's deformity in childhood. Conclusion: in the Middle Age, the limb prostheses became more functional thanks to new knowledge in human anatomy, surgery and anaesthesiology. The analysis of medioeval prostheses of Stibbert Museum allow to evaluate the mechanisms that move these artificial limbs. These ingenious mechanisms were devised by armourers that we can consider the pioneers of medical bioengineering.

Viladot Pericè R, et al. Ortesi e Protesi dell'Apparato Locomotore, Verduci Editore, 1991.

CL4.12
Her-2/neu gene amplification and chromosome 17 status in common renal cell neoplasms

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Background. Significant progress has been made in understanding the biology of kidney cancers and the treatment of different renal cell carcinomas has undergone a paradigm shift from biologic response modifiers to new targeted therapies. Cytogenetic abnormalities of chromosome 17, which harbors Her-2/neu gene, are implicated in cancerogenesis of some renal cell carcinomas, such as papillary RCCs characterized by gains of chromosome 17, chromophobe RCCs by chromosome 17 losses and a subset of advanced stage clear cell RCCs by chromosome 17 polysomies. Because herceptin therapy targets neoplastic cells which harbour Her-2/neu gene abnormalities on chromosome 17, we sought to determine the status of Her-2/neu located among different subtypes of RCCs and assess their suitability for Herceptin immunotherapy.
Methods. We evaluated 10 clear cell RCCs with matched distant metastases in 5 cases, 5 papillary RCCs, 5 chromophobe RCCs and 5 oncocytomas. Fluorescence in situ hybridization was performed using the PathVysion kit. Her-2/neu amplification and polysomy of chromosome 17 were assessed.
Results. No Her-2/neu gene amplification has been observed. Polysomy of chromosome 17 was present in 4/5 papillary RCCs, in 1/10 primary clear cell RCCs and in 2/5 metastases

of clear cell RCCs. Loss of chromosome 17 was observed in the 5 chromophobe RCCs.
Conclusion. Her-2/neu gene amplification is a cytogenetic abnormality absent in common subtypes of renal cell neoplasms. These observations weigh against the potential suitability of the target therapy Herceptin in the treatment of RCCs.

CL4.13
Diagnostic accuracy of renal tumors from core biopsies

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Background. New treatment options and integration of clinico-pathological factors into prognostic and therapeutic algorithms have stimulated renewed interest in renal tumors biopsy. We evaluated the diagnostic accuracy of renal tumors on core biopsies.
Methods. Renal tumor biopsies were performed using 18G needles on 50 consecutive cases from Department of Pathology, University of Verona. At least two cores per tumor were obtained. All biopsies were performed ex situ on back table visually guided and they were evaluated on hematoxylin-eosin stained slides by 4 pathologists with variable expertise in uropathology. Clinical data and diagnosis on whole sections were blinded.
Results. Biopsy material was considered insufficient for evaluation in 5-9 (mean 6; 12%) cases due to the absence of tumor or the presence of only few neoplastic cells. Pathologists assessed a confident diagnosis from biopsies in respectively 80% (35/44), 84% (38/45), 82% (37/45), 76% (31/41) of cases. According to 4 pathologists, the diagnosis was correctly assessed in respectively 86%, 90%, 95%, 95% (mean 90%) of clear cell renal cell carcinomas (RCCs); 44%, 67%, 77%, 77% (mean 66%) of papillary RCCs and 77%, 77%, 83%, 100% (mean 84%) of chromophobe RCCs. Oncocytoma was correctly assessed in respectively 80%, 80%, 80%, 100% (mean 85%). Overall, from 66% to 95% of available tumorous material a correct diagnosis may be referred from biopsies. In 8 cases (16%), although the presence of tumor was recognized, the correct subtyping was not reached.
Conclusions. Core biopsies from renal tumours provide diagnostic material in most of cases (81%). We found that needle core biopsy is a safe and accurate technique for distinguishing between renal epithelial tumors.

CL4.14
Primary prostatic large B-cell lymphoma. Histological and immunohistochemical study of a case and review of the literature

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Introduction. Primary prostatic lymphoma is rare. Symptoms are: urinary obstruction, frequency, infections, hematuria, fever, night sweats and weight loss. A case of primary prostatic large B-cell lymphoma, is described.
History. A 71-year old man (PSA: 0,31 ng/ml) with urinary obstruction, hematuria, abdominal pain and an echographic diffusely enlarged prostate was submitted to a TURP. Histologically, the prostatic fragments (cm 3x2 in all) showed a diffuse neoplastic large cell population, with single centrally located nucleolus and basophilic cytoplasm.
Immunohistochemical staining results: CD20+, lambda+, CD10–, kappa–, CD3–, CD30-, PSA–, MNF116–.
Discussion. Primary prostatic lymphoma is a rare neoplasia (less than 200 cases reported in the literature) [1] of older men (mean, 62 years). Diagnostic criteria are: symptoms of prostatic enlargement, lymphoma chiefly involving the prostate but lacking liver, spleen, lymph nodes and marrow involvement within 1 month diagnosis. The reported cases were B-cell lymphoma of the small lymphocytic, marginal zone and large cell types. The patient in the reported case had an enlarged prostate without any haematologic disease. TURP fragments showed a diffuse large B-cell lymphoma, immunoblastic variant. Poorly differentiated prostatic carcinomas can also mimic large cell lymphoma: antibodies to PSA, PSAP and lymphoid markers are useful tools to make a correct diagnosis. Prognosis of lymphoma is poor: the patient was subjected to a chemo-radiotherapy and died after 18 months.

[1] Epstein JI, et al, Biopsy Interpretation of the Prostate, 4th edition, Lippincott William & Wilkins 2008, pp. 309-311.

Posters

PD1
Liver fine needle aspiration using liquid based cytology

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Introduction. Only few papers have been published on the issue of LBC in Fine Needle Cytology (FNC), because of its limited use. The aim of this study is to verify the use of LBC-FNC as compared with Cell Block (CB) technique, according to cellular and architectural features, in liver FNC.
Material and methods. We analyzed 114 patients who underwent hepatic FNA under US guidance using a 21 G-CIBA needle. We focused on 92 (43 females and 49 males; mean age 57.2 ys,range 23-87) out of 114 cases, in which malignant cells were evidenced: 21 FNCs resulted as primary of liver whereas 71 were metastatic. The mean size of 92 nodules was 24 mm (range 3-90); 41% of the nodules were single. The mean age was 73.4 and 52 in primary tumors (13 m and 8 f) and in metastatic patients, respectively.
Results. A similar amount of cells was founded in 72.5% of samples. The nodules were single in 62.5% of primary tumors and in 33.3% of metastatic patients ($p = 0.022$). Primary tumors were ipoechogen in 38.1% whereas metastatic nodules in 52% of the cases ($p = 0.015$). Two cases showed to be inadequate for one method; the diagnoses of the remaining cases performed on LBC, were confirmed on the corresponding CB. Immunocytochemical assay (ICA) was performed on 92 cases: 34 (13.4%) on the monolayered smears, 225 (83.3%) on the CBs and 9 cases by both the two methods according to availability of the material.3 CB and 1 LBC samples were unables for ICA.
Conclusion. Morphologically a better nuclear detail was evident in LBC whereas structural features were better appreciated on CB. In malignant primary or metastatic hepatic nodules, FNC on LBC evidenced a similar diagnostic accuracy as compared with CB technique even though a smaller amount of cells useful for immunochemistry was available.

PD2
Circulating tumor cells in blood of breast cancer patients: cytological detection and technical characterization

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Purpose. To assess the feasibility of detecting CTCs in blood samples using the Thin Prep® cytological preparation after a concurrent analysis with the CellSearch system (Veridex LLC, Warren, NJ) and to further characterize these cells.
Materials and methods. 7.5 ml of whole blood were drawn from breast cancer patients into the CellSave Preservative Tube containing a cellular preservative and processed within 72 hours. The CellSearch Profile kit was then utilized to separate the CTCs by treatment with ferrofluid particles coated with specific anti-EpCAM antibodies against enrich epithelial cells; thus CTCs were magnetically separated out and concentrated into a remaining aliquot of 1ml. This aliquot was centrifuged at 1700RPM for 7 minutes and the supernatant discarded. The pellet was resuspended and then added directly into the Preservcyt® vial for subsequent processing of ThinPrep slides. The samples were colored with H&E and evaluated microscopically.
Results. A total of 106 blood samples where the CellSearch System obtained CTCs were further evaluated cytologically. Of these, 60 were negative and 46 were positive for malignant cells (range: 1-615). Immunocytochemistry for estrogen receptor was performed in 7 samples and only one case showed ER-positivity. 10 cases were analyzed by fluorescent in situ hybridization (FISH): 7 cases had no amplification while in 3 cases no more cells were detected.
Conclusions. Cytological detection of CTC in blood specimens from breast cancer patients can be useful in providing samples for testing predictive indicator of prognosis and clinical response during therapy.

PD3
Cytomorphologic analysis of adenoid cystic carcinoma of the breast: a case presentation

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Objective. Adenoid cystic carcinoma (ACC) of the breast is a rare malignant tumor with a favourable prognosis, accounting for < 1% of all breast tumors. We present a case of ACC of the breast, its diagnostic features and the problems of differential diagnosis.
Materials and methods. Material from fine needle aspiration (FNA) was smeared on glass slides: 2 slides were air-dried and stained with MGG and 3 slides were fixed in 95% alcohol and stained with H&E and/or PAP. An aliquot of material was placed in 2.5 ml of Cytolyt solution (CYTYC, Italia s.r.l.), processed with the ThinPrep 2000 and slide obtained colored with PAP. Additional ThinPrep slides were made for immunocytochemistry (ICC).
Case. A 60-year-old woman presented with a painful 1.5 cm lesion in the left breast. A FNA was performed: the specimen was represented by numerous small, round to oval, epithelial-like cells, isolated or in masses with marked chromatin streaking. In MGG stained slides there were rare globules of amorphous metachromatic material.
ICC was performed to further classify this tumor; results supported an epithelial origin, suggesting a lesion with an epithelial and myoepithelial component.
Conclusions. ACC of the breast is a rare neoplasm that accounts for 0.1-1% of breast malignancies. In our case, the predominance of small fragile cells and cellular molding led to difficulty in differentiating this tumor from other rare mammary neoplasms, such as lymphoma and small cell carcinoma (respectively 0.05-0.5% and 2-5%). The presence of small, epithelial-like cells allowed us to suspect an epithelial-myoepithelial neoplasm whose definitive classification was made on histology.

PD4
Dendritic cells and mast cells: their role in the progression of the Mycosis Fungoides

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Background. Mycosis fungoides (MF) is a special type of tumor, in which the neoplastic cells and those responsive to them are represented by the same cell: lymphocytes. The prolonged course of disease and bystander intense infiltrate suggest a strong antineoplastic immune response. We studied the role of epidermal Langherans cells (LCs), dermal dendritic cells (DDCs) and mast cells (MCs) in the progression of MF.
Materials and methods. The study included 32 patients. The immunoreactivity was investigated quantitatively and topographically using monoclonal antibodies CD3, CD4, CD8, CD1a, Lag, langerina, S100, tryptase, cKit, CD31, CD34, FactorVIII, VEGF. The average number of positive cells was calculated for 5 fields with higher cell density at 400X. The intensity of cell immunostaining was scored as 0 (0-5 cells), 1 (5-10 cells) and 2 (+10cells). The neovascularisation was studied using MAGS [2] and MD [3].
Results. LCs were significantly increased in plaque stage; conversely during tumor stage they decreased to the point that microabscesses were almost free. To compensate for this reduction, however, during the nodular period, we noted an increase in the population of DDCs. MCs and neoangiogenesis was accentuated in tandem with progression of MF.
Conclusions. This study support dual role of LCs and DDCs in the MF: in the early stages they are involved in the host defense against malignant T lymphocytes, while in advanced stages they are involved in a immune fall of the host to which they themselves would be involved. Besides the neovascularization closely is correlated with tumor progression and amount of perivascular tryptase+ MCs.

[1] *Kim EJ, et al. J Clin Inv 2005:798*
[2] *Marasà L, et al. Pathologica 1983:353.*
[3] *Weidner N, et al. N Engl J Med 1991:1.*

PD5
Primary cutaneous marginal zone B-cell lymphoma (MALT-type) of the scalp: a case report

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Introduction. Primary cutaneous marginal B-cell lymphoma (PCMZL) is a low grade lymphoma of the skin-associated lymphoid tissue (SALT), that occur as solitary or multiple red to violaceous papules, nodules or plaques, accounting for approximately 10% of all cutaneous lymphomas; the prognosis is excellent, with a 5-year survival rate of greater than 95%, although there is high risk of recurrence (30%). PCMZL are usually located on the trunk or extremities, especially the arms, while are uncommon on the head-neck and fairly rare on the scalp: there is only 1/50 cases of PCMZL of scalp in the large series of Dutch Cutaneous Lymphoma Working Group (2005).
Materials and methods. A 67 years old men presented with a cutaneous reddish brown papule of the scalp, measuring 1.3 cm at its longest diameter. For immunohistochemistry, the avidin-biotin peroxidase complex method was used.
Results. Histologically, the skin biopsy specimen revealed a lymphoid neoplasm CD20/bcl–2+ (CD5/CD10/CiclinaD1–) occupyng the whole dermis, with a subepidermic grenz zone; there was a diffuse proliferation of small centrocyte-like and monocytoid B-cells outside reactive follicles, with sometimes follicular colonization and with lymphoplasmacytoid cells and aggregations of plasma cells, predominantly located at the edge of the infiltrate. The reactive cellular population in the interfollicular areas is represented by T-lymphocytes CD3+, a few activated lymphocytes CD30+, histiocytes CD68+ and eosinophils; there was a large number of cells S100+ intermingled with the neoplastic infiltrate, as well as along the basement membrane of the epidermis and adnexal structures (Langerhans and interdigitating dendritic cells).
Conclusion. The differential diagnoses are PCFCL and B-PSL: immunophenotype is diriment

PD6
Intranodal palisaded myofibroblastoma (IPM): report of a case in an inguinal lymph node

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IPM is a rare benign mesenchimal neoplasm usually arising from the inguinal lymph nodes but cases in mediastinum and submandibular lymph nodes have been reported. It appears as a painless, slow-growing inguinal mass; two thirds occur between the ages of 45 and 55 years and the male: female ratio is 2:1. In 1989, 3 different groups (Weiss's, Suster and Rosai's and Lee's) published their findings of IPM, establishing the characterization of this lesion. We report the case of a 75 years old woman with a past diagnosis of "presence of myoid cells" in an inguinal lymph node, followed by hysterectomy for uterine leiomioma. 35 years later she presented an inguinal indolent mass is excised to rule out a lymphoma or bacterial infection. Grossly, the tumor was well circumscribed, firm and had a gray-white cut surface with hemorragic areas. The microscopic features were: compressed remnants of lymphoid tissue at the periphery; at this interface, a collagenous pseudocapsule surrounding the lesion; spindle cells arranged in short intersecting fascicles with nuclear palisading; little nuclear atypia and rare mitotic figures; intraparenchymal hemorrage and erythrocyte extravasation; intracellular and extracellular fuchsinophilic bodies that stained positive for smooth muscle actin; giant collections of collagen fibers so-called amianthoid fibers. The spindle cells were positive for vimentine, muscle specific and smooth muscle actin; negative for desmin, neural and endothelial markers. SEM demonstrated features of myofibroblasts and smooth muscle cells. These cells are usually numerous in inguinal lymph nodes that are subject to striking degree of drainage function. Prognosis is excellent with a 6% recurrence rate and no malignant transformation.

PD7
p16INK4A expression and HPV-L1 detection in the uterine cervix: cyto-histological and immunoistochemical study

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Object. The most important event in SCC carcinogenesis is the infection by HPV and the viral integration in cellular DNA. The "progression timing" between the development of the lesion in SCC after viral integration is unknown. p16INK4A protein is tightly correlated to expression of HPV-L1 protein.
Methods. 137 women underwent Pap Test using LBC. In 78 cases the corresponding bioptic sample was also available. We observe the distribution of the lesions, the cyto-histological correlation, the presence of HPV and the incidence of p16. In 40 cases we have detectet HPV-L1 protein expression in the same samples.
Results. In none of the 48 evaluated cases with Negative cytology, including 6 HPV+, p16 immunoreactivity (IR) has been evidenced. IR has been found in 1/22 cases ASCUS/AGUS, in 4/20 L-SIL, in 6/13 H-SIL and in 2/4 SCC. In 51/78 cases, of which the histological sample was available, the p16 IR has been determined: a strong IR was present in 1/28 negative cases, 2/8 lesions CIN1, 9/12 CIN2-3, 3/3 SCC and in 2 lesions condylomatous. In the evaluable cases (excluding ASCUS, AGUS and L-SIL) the Overall Agreement was 91% (83. 3% with LSIL). In 18 cases, p16 has been determined both on the cytological and histological samples: in 2 cases IR was present only on the cytological sample, while in 1 case the opposite event was observed. In 75 cases the presence of HPV has been determined: 15 case p16 IR positive also presented infection by LR-HR HPV; in 21/44 cases that didn't express p16 IR, LR-HR HPV infection was detected. In 40 cases which expressed p16, HPV and/or cyto-histological lesions we have observed 1 case L1+/p16+ in HSIL.
Conclusions. Our study shows that the presence of p16 and HPV-L1 protein in cyto-histological samples could be useful in giving information on viral integration and lesions progression.

PD8
Primary CD30+ lymphoproliferative disorder of the vulva

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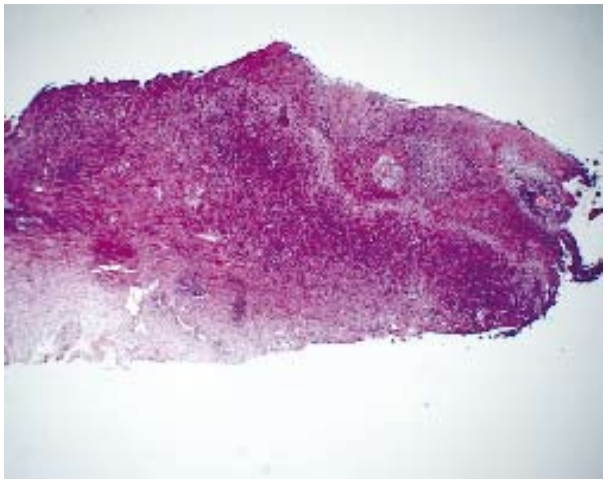
Primary non-Hodgkin lymphomas (NHL) of the female genital tract are rare and those in the vulva are extremely uncommon (117 cases reported in a survey of the literature up to 2005), mostly featuring the B immunophenotype (particularly, diffuse large cell and follicular NHL). CD 30+ lymphoproliferative disorders of the skin constitute a heterogeneous group of T-cell NHL, sharing the expression of CD30, a cytokine receptor of the tumour necrosis factor receptor superfamily, and showing variable morphology, immunophenotype and clinical behaviour.
Case report. A 17-year-old female was submitted in July 2008 to an excisional biopsy of a solitary ulcer of the labius major of the vulva of 0,5 cm. in its greatest diameter, of a few months duration and non-responsive to local anti-inflammatory therapy. Histologically (Fig. 1) the lesion showed a skin ulcer with the epidermis replaced by a thick layer of necrotic material overlying a recently-formed granulation tissue extensively involving the dermis. In the necrotic material, there were multiple foci of a surviving cell popolation arranged in a perivascular pattern and consisting of medium- to large-sized lymphoid cells featuring the following immunophenotype: CD3+, CD45R0+, CD30+ (Fig. 1), CD1a-, CD20-, CD43-, CD56-, Alk- and EMA-. In situ RNA hybridisation for EBV (EBER ISH) was negative and PCR analysis revealed no clonal rearrangement of either TCR or heavy chain Ig. The final histopathological diagnosis was CD30 + T-cell lymphoproliferative disorders with unpredictable biological behaviour. A close follow-up was recommended, which in the following 6 months revealed no further lesions, either local or at distance.



PD9
Two cases of low grade endometriod carcinoma associated with undifferentiated carcinoma of the uterus (dedifferentiated carcinoma): a molecular study

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Introduction. Dedifferentiated carcinoma (DC) is a uterine neoplasm containing both low-grade endometrioid carcinoma (LGEC) and undifferentiated carcinoma (UC). DC is an aggressive tumour even when the UC component represents only 20% of the entire neoplasm [1].
Materials and methods. Two uterine DCs at different stages of developmement were investigated for microsatellite instability (MSI) and loss of heterozygosity (LOH). Case #1 presented metastases at diagnosis, while case #2 was at a lower stage. LGEC component was invasive in case #1 and intramucous in case #2. Nine microsatellite markers, including those recommended in the original or in the revised National Cancer Institute panel for MSI testing in colorectal cancer, were PCR amplified from tumor and normal DNA using fluorescently labeled primers. MSI was characterized by novel allele appear-

ance or by allele mobility shift. Disappearance or significant reduction of one allele in the tumor DNA, as compared to the ratio observed in normal DNA, was described as LOH.
Results. In both cases, UC components were characterized by a high degree of instability, with MSI at four loci in case #1 and at five loci in case #2. Moreover, LOH and MSI occurred simultaneously at the same marker at three loci in UC component of case #1 and at two loci in case #2. Invasive LGEC component of case #1 showed high level of instability (MSI at 7/9 loci investigated), while intramucous LGEC of case #2 showed only a single alteration (LOH).
Conclusions. Molecular damages in uterine DC are frequent, in accordance with its aggressive behaviour. Genetic alterations in the two components increase with the stage of development.

[1] *Silva EG, et al. Int J Gynecol Pathol 2006;25:52-8.*

PD10
Uterine mesenchymal neoplasia and biological markers

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Uterine sarcomas represent approximately 3% of malignant tumors of female apparatus that originate from primitive mullerian epithelium. They are neoplasm with high aggressiveness and unfavourable prognosis. The most common histological variants are endometrial stromal tumors, leiomyosarcoma and mixed mullerian tumor of omologous and etherologous type. The pattern of diffusion is through the myometrium, blood and linfatic vases, to adjacent pelvic structures and lungs. We examined 20 cases that including 7 leiomyosarcoma, 11 mixed mullerian tumor, 1 endometrial stromal sarcoma and 1 sarcoma botryoide. Immunoistochemically we evaluated c-Kit (CD117) and Metalloproteinasi (MMP2 and MMP9). The pro-oncogene c-Kit encodes for a receptor factor, identified by CD117 antigen, whose expression has been found for the first time in GIST. The Metalloproteinasi are endopeptidases zinc-dependent and they are responsible for extra-cellular matrix degrade. At the end of our study, we can hypothesize that the c-Kit expression could be an independent prognostic factor, even if the number of cases is limited and the expression of metalloproteinase is mainly present in the advanced stages and/or in the high grades with unfavourable prognosis. Currently, it is not possible to consider and/or cure uterine sarcomas as GIST (gastrointestinal stromal tumors). However, by inclusion of genetic sequencing (sequenziamento genico) of c-Kit gene and the valuation of new bio-molecular parameters, it could be possible to establish their biological behaviour and subsequently new choices of therapeutic strategies.

PD11
Squamous cell carcinoma arising in a dermoid cyst of the ovary: a case report

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Introduction. Mature cystic teratomas make up almost 20% of all ovarian neoplasm. Squamous cell carcinoma (SCC) arising in mature cystic teratoma is rare, approximately 1-3%, and affects elderly persons. Approximately 50% of the patients present with FIGO stage I, while 35-38% present with stage III disease.
Methods. We report a case of SCC originating from a dermoid cyst of left ovary in a 78 year-old woman. The patient underwent unilateral salpingo-oophorectomy. The specimens were fixed in 4% formaldehyde and embedded in paraplast. Sections 4 micron thick were stained with H&E and PAS stain. Immunohistochemistry was performed.
Results. The left ovary was cystic, unilocular, mass measuring 10 x 7 x 5 cm, with external and internal surfaces pink and smooth and showed abundant hair and yellow sebaceous material. Microscopically, the cystic wall was lined by mature and immature squamous epithelium, involved by extensive SCC in situ, distinguished by a diffuse expansion of epithelium by large cells with a variety of atypical changes, such as cytoplasmic vacuolization, nuclear hypercromasia, marked anaplasia. The tumor cells were positive for CKAE3, EMA, CEA, ulex europaeus, negative for S100, vimentin. Microscopic invasion of the wall by single malignant cells and small clusters was observed. No malignant cells were observed in peritoneal washing. The patient was categorized as FIGO stage IA. She is now doing well without recurrence of disease six months after the surgery.
Conclusions. Every case of SCC originating from dermoid cysts may not have the same clinico-pathologic characteristics and management should be individualized. To our knowledge, it is the first report of an extensive SCC in situ with microinvasion arising in a mature cystic ovarian teratoma.

PD12
Mullerian adenosarcoma of the ovary. Case report

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Mullerian adenosarcoma is a rare biphasic neoplasm whit a benign epithelial and a malignant mesenchymal component. In rare cases metastasis can occur, and they usually have mesenchymal component. They are typically originated in the endometrium, but they can also originate in other mullerian areas. Here we report a clinical case, characterized by a multifocal start and sarcomatous metastasis. A 47 years old woman, with hyperpyrexia, pelvic mass assessed through ecography was submitted to an hysteroannessiectomy operation. At histology, a mixed neoplasm with malignant mesenchymal component in the ovary area was observed and similar superficial lesions of small dimension in the endometrium, endocervix and abdominal peritoneum area. After 2 years the tumor reappeared in the colon with only the malignant sarcomatous component. The multifocal presentation represented in this case a discriminant element and made difficult the attribution of the tumor to one organ only. The relapse morphologically presented a remarkable growth of anaplastic and cells proliferation.

PD13
Expression of WT1 and claudin-5 in angiogenic tumors

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Background. Identification of endothelial cells is based on the expression of angioblastic linage-specific genes like CD31, CD34. WT1 protein is a zinc finger transcription factor expressed in endothelial progenitor cells that has been reported to be maintained during the differentiation. Claudin-5 is a key component of tight endothelial junctions. Considering the presumed specificity of these two proteins for the endothelial cells we sought to analyse their expression in different benign and malignant tumors originating from endothelial cells.
Methods. We collected 15 angiogenic tumors including 6 angiomas, 5 Kaposi's sarcomas and 4 angiosarcomas. All the Kaposi's sarcomas and the angiosarcomas have been confirmed by the immunoexpression of CD31 and CD34. We tested the expression of WT1 and claudin-5 by immunohistochemistry using respectively the antibodies anti-WT1, clone 6F-H2 from Dako and anti-claudin-5, clone 4C3C2 from Zymed; both diluted 1:100.
Results. The 6 angiomas showed a consistent cytoplasmic and membranous immunoexpression of WT1 whereas claudin-5 was expressed especially on the luminal membrane with a weak cytoplasmic labelling. All the Kaposi's sarcomas showed a strong cytoplasmic and membranous expression of both the markers. All the angiosarcomas expressed claudin-5 in the cytoplasm and on the membrane whereas only 2 of 4 (50%) showed a strong immunoexpression of WT1.
Conclusions. WT1 and claudin-5 are specific endothelial markers and are maintained in angiomas and Kaposi's sarcomas. Angiosarcomas maintain the expression of claudin-5 whereas WT1, that has been proven to behave as a tumor suppressor protein, tends to be lost in the development of the neoplasm.

PD14
Critical values for professional staffing in anatomic pathology

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Professional staffing requirements in Histopathology is a controversial, ill-documented but badly needed standard. Furthermore, strain on the service caused by temporary or long lasting reduction of the staff is scarcely reported and almost never methodically analyzed, also because there are no standards or methods to measure such strain. We analyze the workflow in our Department of Histopathology over 2 years (2007-2008), by data mining from the histopathology laboratory information system (LIS) database and paper logs, including repeated occasions in which we experienced staff reduction. Our data show that 13 full time pathology technicians or more are required to handle not more than 1700 blocks per week for a total of approximately 25,000 specimens/year; a minimum of 5 technicians per day must do block sectioning. Unexpected reduction of professional technical staff, resulting in diagnostic delays due to overload of the productive capacity, must be matched upfront by enough technical staffing to resume regular case output in the shortest time possible. The workload for pathologists was analyzed by tallying the daily workload in trays, the SIAPEC Unit weight of the cases assigned and the total time on duty, including overtime. 10 minutes for each specimen Unit is a minimum load for experienced pathologists in a community Hospital, barely if adequate for a Teaching Hospital. Overwork for understaffed pathologists, a health care hazard, was addressed by setting limits to the daily and weekly case load (trays), a task managed by the technicians. Active management of the workflow and workload of Histopathology professionals is key for optimal service for the patient and the Hospital.

PD15
Autopsies: still a gold standard?

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Background. Pathology in Switzerland is confronted with a declining number of medical autopsies. This can be explained by progressing in medical imaging which offers clinicians a high level of certainty in diagnosis. For this reason, clinicians may regard autopsies as useless. In this study, we compared clinical with autopsy pathological diagnosis in order to evaluate the value of autopsies in the quality control of local health care.
Methods. Between January 2004 and December 2008, we reviewed all adult autopsies cases in our Institute. We excluded autopsies cases limited to isolated organs such as the central nervous system and also pediatric autopsies (< 16 years old). Sudden death was defined as death occurring less than 12 hours after the first symptoms.
Results. Among 718 autopsy cases, 226 (31.5%) were qualified as sudden death. The cause of death was established in 63.3% of the cases. Of the non sudden death population, the cause of death was established in 85.2% of cases. At autopsy clinically important diagnoses 94 (13.1%) were established that were missed by the attending clinicians and 83 (11.6%) missed diagnosis without important clinical implications.
Conclusions. In a high % of cases the autopsy allowed to establish cause of death. Our case series shows that missed diagnoses with important clinical implications remains an important issue spite of progress in medical imaging. In our era with strong emphasis on quality control, autopsies should be reconsidered as essential approach forwards achieving this goal.

PD16
Epileptogenic neoplasm with features of astrocytoma and ependymoma

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Ultrastructural studies play a role in identifying many cerebral neoplasia especially in rare tumors.

Case report. A 62 years-old man presented with partial epilepsy and aphasia. MRI showed somewhat high-signal lesions on T2-weighted of precentral gyrus. The tumor had infiltrated the cortex and the subcortical white matter. Histologically the constituent cells were predominantly composed of elongated, monomorphous and astrocityc cells, in addition to oligodendroglioma-like cells. The perivascular arrangements with tumor cells arranged longitudinally and circumferentially in relation to vessels was observed in some regions. The tumor cells exhibited marked vascular polarity or orientation. Immunohistochemically, tumor cells stained with GFAP(+++), EMA (+ dot-like), neurofilament protein, synaptophysin (++), and Ki67 (4%). Ultrastructural analysis showed a mixture of tumor cells: microtubuli, intraluminal microvilli and cilia, and elongated,"zipper-like" intercellular junctions (ependymal features), cells with rich cytoplasm in thin fibrills, then aggregated in electrondense crystalline-like structure (astrocytic features). We feel that these tumors are difficult to categorise due to their broad morphologic spectrum therefore the diagnosis can be included in poorly differentiated tumors which includes features of ependymoma and astrocytoma. The diagnosis may not become apparent until electron microscopy is performed. In these difficult cases, electron microscopy is useful for identifying the tumor cell lineage.

PD17
Extramedullary hematopoiesis as a potential pitfall for meningeal mass in patients with myelofibrosis

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Introduction. Myelofibrosis is a disorder that leads to ineffective erythropoiesis accompanied by bone marrow fibrosis. As a consequence, extramedullary hematopoiesis (EH) characteristically develops predominantly in the spleen and liver but serous membranes, lungs, gastrointestinal tract, or urogenital system can be involved too. The central nervous system (CNS) is rarely affected and, when this occurs, the spinal canal and the cranial meninges are generally the preferred locations.
Methods. A 63-year-old woman presented with a history of worsening headaches. The patient was known to have histologically documented bone marrow fibrosis. MR imaging revealed a bilateral, frontal-solid-lesion, attached to the dura, that was hyperintense to contrast. The patient underwent a frontal craniotomy for suspected meningioma and the lesion was removed with its dural implant. Grossly the tumor resection measured in aggregate cm 4,5x3,5x3.
Results. Histological examination revealed multiple foci of hematopoietic cells of erythroid, myeloid and megakaryocytic lineages confirmed with stains respectively for glycophorin, myeloperoxidase and Factor VIII. No neoplastic tissue was found. The hematopoietic cells were of course immunonegative for pankeratin and glial fibrillary acidic protein. The diagnosis was meningeal EH. Follow up MR imaging 2 years later revealed no recurrence.
Conclusion. The presence of EH loci in primary brain tumors is fairly rare. Nevertheless, because of the specific meningeal tropism of extramedullary myeloid metaplasia and its likely growth influence to meningeal cells, we suggest that intracranial myeloid metaplasia should be ruled out in patients experiencing idiopathic myelofibrosis and considered as a potential differential diagnosis in brain lesions.

PD18
Pulmonary metastasis from submandibular benign pleomorphic adenoma: report of a case

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Introduction. Metastasizing pleomorphic adenoma (MPA) is an extremely rare metastasis of histologically benign tumour. Nevertheless, the aggressive outcome of some cases indicates that the adjective benign is inappropriate. Typically, both metastatic and primary salivary gland tumours are histologically identical to the benign tumour. We describe a case of pulmonary metastasis from submandibular pleomorphic adenoma.
Case history. A 51-year-old woman with past medical history of pleomorphic adenoma of the submandibular gland and a local recurrence 7 years after primary excision, showed a lung nodule and two pancreas lesions. A computed tomography-guided needle biopsy of the pulmonary nodule was performed and the histologic exam of formalin-fixed and paraffin embedded tissue evidenced a neoplasm made up of ductal and myoepithelial cells with no atipia in a variable amount of fibromyxoid tissue. The cells were immunoreactive for cytokeratin pool, smooth muscle actin and S-100 protein, while they were negative for chromogranin, synaptophysin and TTF-1. The proliferative index Ki-67 (MIB-1) was 3%. The histologic and immunohistochemical features were consistent with metastasizing pleomorphic adenoma. Discussion: MPA was first described in 1942; the mechanism underlying its metastatic behaviour is still not certain. The majority of the tumours origins from the parotid gland, while there are a few reports of metastasis from the submandibular gland. Bone, lung and lymph nodes are the most common sites of metastatic disease, but kidney, liver, CNS, retroperitoneum and skin can also be involved[1]. As in our case, there is often a long interval between diagnosis of the primary tumour and metastases.

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PD19
Intercellular adhesion molecules in spontaneous abortions: an immunohistochemical study

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Background. Early placental development is associated with complex regulatory mechanisms, and molecular path-

ways involved during placental development are critical for pregnancy progression.
Aim. We examined the role of adhesion molecules in the mechanism of spontaneous abortions.
Study design. Curettage materials from abortions were examined retrospectively by hystochemical and immunohistochemical methods through CD31, CD44, E-cadherin expression. The results of spontaneous abortions in the first trimester were compared to those of abortions in the 2nd and the 3rd trimesters.
Results. Microvascular density (MVD) through CD31, CD44, and E-cadherin expression decreased in cases of spontaneous abortion. Statistically significant differences have been detected between spontaneous abortion materials in the first trimester compared to those of abortions in the 2nd and the 3th trimesters with regard to cytotrophoblasts (CTs), syncytiotrophoblasts (STs), and extravillous trophoblasts (EVTs) with the anti-CD31 antibody ($p < 0.05$). Moreover E-cadherin expression in CTs and STs ($p < 0.05$ and $p < 0.05$), respectively, was significantly different. A down-regulation of CD44 was observed in decidual(D) cells.
Conclusions. Decreased MVD in CTs in spontaneous abortion cases can suggest that MVD is critical in uteroplacental adequacy. Moreover,a down-regulation of E-cadherin could be responsible for impaired CTs differentiation and loss of the pregnancy. Furthermore, decreased CD44 expression in D cells may be cause of impairing stroma-villous connections. Many adhesion molecules are known to be effective in the normal development of pregnancy and thus their analysis in spontaneous abortions will provide useful information for clarifying the physiopathology of spontaneous abortions.

PD20
Correlation of morphologic findings and genetic anomalies by CGH-array in a intrauterine fetal death at 20 weeks

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Genetic anomalies are frequent in spontaneous abortions. Conventional cytogenetic analysis is limited by low sensitivity; the array-CGH technology allows genome-wide analysis. We report the case of an intrauterine fetal death at 20 weeks. Autopsy of fetus and examination of placenta were performed. Genetic analysis included conventional karyotype and array-CGH analysis by genome-wide microarray platform (44k Agilent). At autopsy, the male fetus revealed morphologic changes due to advanced maceration. Major abnormalities were:microcephalia, low plant ear, flat nose, triangular faces, large mouth, micrognatia and intrauterine growth retardation (weight g 162; crown-rump length 14 cm). Situs and morphology of internal organs were normal. The encephalus was modified by autolysis. At histology, cells with nucleomegalia and eosinophilic incluses were identified in lungs, heart, kidneys and adrenal glands; these cells were immunoreactive with antibodies for Human Cytomegalovirus (HCMV) IE antigen. Examination of placenta revealed chronic villitis with similar cytopathic changes. Chromosome analysis on chorion villi cells from long term culture preparations was performed but few partial metaphases were analyzed, showing an apparently balanced translocation between the short arm of chromosome 4 and the long arm of chromosome 9, that resulted to be "de novo" after extension of cytogenetic investigations to parents. Array-CGH on fetal DNA showed deletion of the chromosome 4p of about 7.1 Mb [del(4)(pter-p16.1)], with the deletion proximal breakpoint falling between 7,183 Mb and 7,185 Mb.
The phenotype of the fetus was compatible with deletion of chromosome 4p. In this abortion, viral sepsis by HCMV was associated with morphologic anomalies due to deletion of the chromosome 4p.

PD21
An efficient and rapid method for DNA extraction from formalin fixed and paraffin embedded tissue suitable for analysis by polymerase chain reaction (PCR)

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Background. There are several methods for DNA extraction from paraffin embedded tissue reported in the literature and most described methods report an amplification success rate of 60-80%.
Objective. In a prospective study, we compared two methods to identify an optimal and rapid protocol for DNA extraction from paraffin-embedded samples.
Material and method. Nuclear DNA was extracted from forthy-five cases of surgically resected colorectal adenocarcinomas which had been fixed in buffered formalin and embedded in paraffin. DNA extraction was performed using two different methods: a) a commercial kit (DNA extraction kit, *BioDiagene*) with a simple and rapid protocol consisting of xylene/ethanol dewaxing, followed by a kit base extraction without proteinase K digestion and b) a more conventional method consisting of xylene/ethanol dewaxing followed by overnight proteinase K digestion in lysis buffer. We checked the quality of samples by PCR for the housekeeping gene, β-globin (fragment of 268 bp).
Results. Specimens amplification of the β-globin gene sequence was successful in 42 of 45 (93%) paraffin-embedded samples using DNA extracted by commercial Kit and in 38 of 45 (84,4%) by conventional methods.
Conclusion. Both methods used to obtain genomic DNA from formalin fixed and paraffin-embedded tissues produced DNA suitable for amplification, but the rapid and non-laborious commercial Kit *BioDiagene* should facilitate the molecular analysis of a large number of archivial specimens in retrospective studies. Infact, in only thirty minutes genomic DNA is extracted and made to use for PCR amplification.

PD22
Evaluation of an alternative method for DNA extraction from paraffin-embedded tissues for PCR amplification

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Background. Formalin-fixed paraffin-embedded tissues are a valuable source of DNA for molecular studies.
Object. Two efficient and rapid procedures for DNA extraction from formalin-fixed and paraffin embedded tissues were tested.
Material and methods. DNA was extracted using two methods: 1) overnight proteinase K digestion followed by 5% Chelex-100® (BioRad) purification and 2) a commercial Kit (*BioDiagene,* DNA extraction kit).
Results. Using *BioDiagene* DNA extraction kit, we were able to extract amplifiable human DNA from formalin-fixed and paraffin-embedded tissue sample obtained from thirty blocks of various organs (lung, thyroid, lymph node and pancreas). Successful extraction was determined by the ability to amplify a 268-bp fragment of the house-keeping gene beta-globin.
Conclusion. Both methods produced DNA suitable for amplification in 28 of 30 samples (93,3%), but while Chelex-100®method requires an overnight incubation, the *BioDiagene* method is faster.Infact, in only 30 minutes the DNA is extracted and ready to use for PCR amplification.

PD23
Changes in mitochondrial shape and distribution in gastric carcinoma: a morphologic, immunohistochemical and ultrastructural study

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We evaluated the antimitochondrial antibody 113-1 and electron microscopy in an attempt to ascertain differences in mitochondrial morphology and distribution in intraepithelial neoplasia (3 cases) and in invasive carcinomas of the stomach, including 16 intestinal and 8 of diffuse-type. Immunohistochemistry revealed a prominent supranuclear mitochondrial reactivity in all cases of intraepithelial neoplasms and in 4 cases of intestinal type adenocarcinomas. A diffuse cytoplasmic reactivity was found in 3 cases of intestinal type adenocarcinomas, indicative of an oncocytic differentiation. Irregular cytoplasmic mitochondrial immunoreactivity was found in the other cases. The same pattern of mitochondrial staining was confirmed in the ultrastructural study. Mitochondria both in intraepithelial neoplasia and in invasive carcinomas exhibited heterogeneous morphology. They were predominantly seen with lucent-swelling matrix associated with disarrangement and distortion of cristae and partial or total cristolysis. Functionally, the structural alterations suppose the presence of hypoxia-sensitive cancer cells. Hypoxia-sensitive cells are linked with lucent-swelling and cristolysis mitochondria profile and have an inefficient or null oxidative phosphorylation, which consequently use the glycolytic pathway to generate energy. In conclusion, our data confirm the presence of hypoxia-sensitive mitochondrial changes in invasive carcinomas and extend these observations to the intraepithelial stage of gastric carcinomas. Mitochondrial immunohistochemistry and electron microscopy can be used as complimetary techniques to reveal oncocytic differentiation in gastric carcinomas.

PD24
A case of a solitary primary mesenteric paraganglioma

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A mass was accidentally discovered during routine sonographic evaluation in a 65-years-old woman; no other masses were found by computed tomographic scan. The resected specimen consisted of a segment of normal-appearing small bowel with an attached wedge of mesentery, which contained a well-circumscribed mass 6 cm. in diameter showing a peripherally solid white area with predominantly central hemorrhage. The mass was distinct from the small bowel. Histologically, the tumor showed trabecular and predominantly "zellballen" pattern with epithelioid cells, CK-, EMA-, chromogranin+, synaptophysin+, CD56+, bordered by sustentacular cells S100+.The stroma was highly vascular. In some areas, the pattern was more diffuse; the cells showed clear cytoplasm and were less monomorphic or even more elongated with sparse S100 positive cells. Follow-up was negative after 6 months. Approximately 5% to 10% of paragangliomas occur in extra-adrenal sites, which can extend from the upper cervical region to the pelvis. Extra-adrenal paragangliomas are predominantly intra-abdominal. Extremely rare are those primary in the mesentery (< 10 cases reported in the literature), mostly arising in the mesentery of small bowel. They all pursued a benign course, except one which presented with liver and lymph nodes metastases. Normal paraganglionic tissue has been described at the roots of the superior and inferior mesenteric arteries, theoretically explaining the origin of the posterior mesenteric paragangliomas. The anterior mesenteric paragangliomas can best be explained by vertebral migration of

paraganglionic cells through these vessels to reach the anterior small bowel mesentery, where they could potentially give rise to paragangliomas in this site.

PD25
Autophagy in colorectal cancer: preliminary observations

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Background. Autophagy is a catabolic process involving the degradation of cellular components through the autophagosome pathway. This highly-regulated process plays a pivotal role in tissutal differentiation and homeostasis [1]. Recent studies suggest that autophagy may be important in cancer development and progression as well as in determining the response of tumor cells to anticancer therapy [2]. However, the role of autophagy in these processes may depend on a number of circumstances.
Methods. In the present work we are studying some molecules involved in the regulation of autophagic process in a series of colorectal cancers. Particularly, we are performing immunohistochemistry for Beclin-1, UVRAG, mTOR, phospho-mTOR, AKT and phospho-AKT.
Results. Preliminary results indicate that both Beclin-1 (ABGENT, cat. No. AP1818a, 1:50) and UVRAG (ABGENT, cat. No. AP1850b, 1:50) are downregulated in cytoplasm of tumoral cells, compared to normal epithelial ones. Experiments for the other molecules are currently under running.
Conclusions. UVRAG and Beclin-1 interdependently induce autophagy. Our results for Beclin-1 are in contrast with what showed by other groups [3 4], but in agreement with other studies conducted in other types of neoplasms [5]. By contrast, no data are available concerning UVRAG expression in colorectal cancers. This study will add important information concerning the role of this molecules in colon cancer development and progression.

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PD26
Multiple adenocarcinomas and synchronous leiomyosarcoma of the colon: a case report

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Methods. The formalin-fixed surgical specimen was stained with hemathoxylin-eosin. Immunohistochemistry performed: ASMA, Desmin, Vimentin, CAM 5.2, AE1-AE3, CEA-p, Mib-1, CD34, CD117.
Results. A 73 year old man was admitted to surgery for a colic stenotic neoplasm. The surgical specimen consisted of 76 cm colon showing 8 polypoid neoplasms ranging from 0,3 cm to 5 cm. At gross examination, the largest neoplasm was composed of a polypoid component and a hard infiltrating one. The remaining lesions were polypoid only. At microscopic examination, the 6 smaller polyps were tubulo-villous adenomas, the second largest neoplasm was an adenocarcinoma (G2, pT3 N0 Mx). Interestingly, the largest lesion turned to be a collision between two different and indipendent tumours: a typical adenocarcinoma G2 of the colon and a low grade leiomyosarcoma. The identification of discrete sarcomatous and glandular components with no morphological "transition" ruled out a possible diagnosis of carcinosarcoma. The adenocarcinomatous cells were positive for cytokeratins and CEA-P and negative for muscolar markers while sarcomatous cells stained diffusely and strongly with Vimentin, Actin and Desmin and were negative for cytokeratins and CEA-p. This immunohistochemical profile supports the hypothesis of two different neoplastic clones.
Conclusion. To our knowledge, only few cases of collision tumour of the intestine are reported. Moreover, we find interesting the synchronous presence of a low grade leiomyosarcoma with multiple adenomas and infiltrating adenocarcinomas in a patient with no known cancer predisposition syndrome.

PD27
Role of CXCR4 and VEGF expression and metastatic loco regional limphnodes in rectal carcinoma

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Introduction. Chemokine receptors are known to be involved in the lymphatic spread of many solid tumors, while VEGF (Vascular Endothelial Growth Factor) is implicated in metastatic process. Their role in the prediction of lymphnode spread and possible occurence of distant metastasis in rectal carcinoma could be of great importance for the selection of rectal carcinoma patients with worst prognosis. The aim of this work is to evaluate the expression of both CXCR4(chemokine receptor) and VEGF on stage II-III rectal carcinoma specimens, relating it to the expression of other biological markers as p53 and TS106, and to "traditional" pathological parameters predictive of disease progression, sugh as pT category, tumor grading and presence of lymphovascular invasion.
Materials and methods. A total of seventy cases of stage II-III rectal carcinoma, operated in our institution from 2003 to 2007, were examined using routine staining methods and immunohistochemical staining for CXCR4, VEGF, p53 and TS106 on paraffin embedded specimens. All 70 patients were clinically and pathologically R0 after surgery. Receptor expression (absent vs. positive)from the overall tumor and from the invasion front of tumor was related with lymphnode status and other prognostic pathological parameters(pT stage, grading, lymphovascular invasion). These data were also related with presence of distant metastases and overall survival.
Results. We obtained these preliminar data about histopathological and immunohistochemical evaluation: Lymphnode status is significantly related with lymphovascular invasion and with CXCR4, VEGF, TS106 and p53 expression. Notably, high expression of CXCR4 and VEGF was significantly associated with lymphnodal status and the presence of distant metastasis.

PD28
Phagocytosis of apoptotic neutrophils by adenocarcinoma cells and foveolar cells of the gastric mucosa with Helicobacter pylori infection: A histologic, immunohistochemical and ultrastructural study of three cases

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We report 3 cases of an unusual type of gastric carcinoma that occurred in elderly patients 62-80 years of age. Histologically, the tumours were well to moderately differentiated intestinal-type adenocarcinomas with a focal micropapillary component. There was an abundant neutrophilic infiltration around the tumour cells both intraepithelial ("cannibalism") and stromal. At the electron microscopy, apoptotic neutrophils were found within vacuoles of adenocarcinoma cells. Immunohistochemical staining for caspase-3 confirmed the presence of apoptotic neutrophils within the cytoplasm of the tumour cells. Antral and corporal non-neoplastic mucosa showed a Helicobacter pylori–positive gastritis with high degree of neutrophil activity. Neutrophil phagocytosis by foveolar cells was also documented by light and electron microscopy. This is the first study that provide morphological evidence of apoptotic neutrophils phagocytosed both by gastric adenocarcinoma cells and foveolar cells of gastric mucosa with Helicobacter pylori infection.

PD29
Adenocarcinoma intestinal-type of the nasal cavity and paranasal sinuses: report of two cases

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Intestinal-type adenocarcinoma (ITAC) is a primary malignant glandular tumour of the nasal cavity and paranasal sinuses histologically resembling adenocarcinoma of the intestines (Achenson et al. in 1968). The frequency of ITAC is 4% of the malignant tumors of nasal and paranasal sinuses, with pronounced male predominance, possibly because of occupational exposure, in particular in wood workers. ITAC involve the ethmoid sinus, nasal cavities and maxillary sinus. Advanced tumors tend to invade the orbit, the pterygopalatine and infratemporal fossae, and the cranial cavity. We have observed two cases of ITAC in wood workers patients, with evidence in the first case of the natural evolution of tumor with extension in anterior cranial cavity miming meningioma, because the patient refuses surgical excision, with neurologic and visual disturbances. Macroscopically ITAC appears as irregular exophytic pink or white mass bulging in the nasal cavity or paranasal sinus, with a necrotic friable area. Histopathologically have been proposed two classification of ITAC. Barnes divided these tumors into 5 types: papillary, colonic, solid, mucinous and mixed. Kleinsasser and Schroder divided ITAC into 4 types: papillary tubular cylinder cell I-III, alveolar goblet, signet-ring and transitional type. The differential diagnosis must be made among ITAC, sinonasal adenocarcinoma (non-ITAC) and metastatic adenocarcinoma of intestinal origin. Sinonasal ITAC have a distincitive phenotype, with most of all cases expressing CK20, CDX2. Most ITAC also express CK7, although a proportion of tumors are CK7 negative. ITAC seems to be preceded by intestinal metaplasia of the respiratory mucosa, which is accompanied by a switch to an intestinal phenotype.

PD30
Immunocytochemical and molecular help in thyroid liquid based cytology

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Introduction. Fine needle cytology (FNC) is the most important tool in the diagnosis of thyroid nodules. It has been demonstrated that Liquid Based Cytology (LBC) improves the quality of the smears and the diagnostic accuracy (D.A.) because of well preserved cellularity useful to immunocytochemical (ICA) or molecular assays.
Material and method. 106 FNCs were observed: the nodules occurred in 88 females and 12 males (mean age: 46.8; Fem 46.4; Males 43), 69 single and 25 in multi-nodular thyroids. The material was processed in Thin Prep 2000 TM. ICA for HBME-1 were carried out on 48 LBC slides. The presence of BRAF gene mutation (V600E) was investigated appling MASA tecnique on remaining 48 patients; both HBME-1 and BRAF were available in 5 patients. FNCs were classified according to SIAPEC classification.
Results. We analyzed cyto-histological correlation and D.A. On 97 evaluable cases, D.A. was 88.6% (we considered as false negative 10 Thy3 that resulted PC at histology). Considering HBME-1 expression and BRAF mutation as detected on LBC, the prevalence of HBME-1 and BRAF mutation was 44% and 27%, respectively; D.A. resulted 90% and 86.5%, respectively. The Positive Predictive Value was 96%, 100% and 100% for cytology, immunocytochemistry and molecular assay, respectively. The relation between histological diagnoses and cytological, molecular and ICA findings, were s.s. ($p < 0.001$). On the 5 cases, where both HBME1 and BRAF mutation were investigated, 2 cases were HMBE-1 positive and BRAF mutated whereas, the remaining 3 cases were negative for both the markers.
Conclusions. Our study showed that ICA and molecular assays can be successfully applied on monolayered smears, on well preserved cells obtained by LBC and could increase diagnostic accuracy in thyroid FNC.

PD31
Dentinoma: a case report

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Introduction. "Dentinoma" has been defined in 1971 by WHO as "a very rare neoplasm composed of odontogenic epithelium, immature connective tissue and dysplastic den-

tin". It generally occurs in young adults, without sex predilection. The mandible represents the most common site of onset. Clinically, it generally occurs an asymptomatic mass, even though sometimes associated with unerupted or carious teeth. Surgical excision represents the gold standard therapy.
Case report. We report on a case of a 41-year-old woman presented to our hospital for an asymptomatic, 1,5 cm in-diameter, lesion of the mascellar.
Results. After radical excision, the lesion presented as a white, hard consistent mass. Microscopically, the lesion consisted of dysplastic dentin without clear tubules, scanty epithelium and rare fibrous immature elements, stained positive for vimentin, calretinina and cytocheratine. A diagnosis of dentinoma was suggested.
Conclusions. First described by Straith in 1935, "dentinoma" is an entity that has not been fully accepted. Some pathologists assume that this lesion must be regarded as ameloblastic fibroma or ameloblastic fibro-odontoma. In our case, the presence of dysplastic dentin seems to preclude the diagnosis of ameloblastic fibroma, whereas the absence of enamel excludes the diagnosis of ameloblastic fibroodontoma. Thus, we hypothesize the diagnosis of dentinoma.
Up to now, only 20 cases diagnosed as dentinoma have been reported in literature. The rarity of this entity and the difficulties in the differential diagnosis lead us to report on this case.

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PD32
Granular cell tumor of the larinx and of the esophagus: report of two cases

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Introduction. Granular cell tumor (GCT) is an uncommon and nearly always benign neoplasm of presumably Schwann cell origin, with predilection for the skin, the soft tissue and the tongue, while laringeal and esophageal involvement is fairly rare and their diagnosis is mainly based of histopathologic examination of endoscopic biopsies.
Clinico-pathologic cases. Case 1. A 40-year-old white women presented with a recurrent smooth sessile tumor of the right vocal cord-subglottic area causing hoarseness (the first lesion revealed five years ago). Case 2. A 42-year-old white men with a flat irregular lesion detected incidentally near the esophago-gastric junction during esophagoscopy: the endoscopic diagnosis was Barrett's Esophagus.
Methods. Tissues were fixed in buffered formalin, paraffin embedded; for immunoistochemistry, the avidin-biotin peroxidase complex method was used.
Results. Histologically, the tumor was located in the subepithelial area and consisted of sheets of large polygonal and round-oval tumor cells with ample granular eosinophilic cytoplasm and with small round nuclei centrally or eccentrically located, with -in the recurrent tumor of case 1- nuclear pleomorphism and a few prominent nucleoli; the overlying epithelium was thin and unremarkable in the case 1, while pseudoepitheliomatous hyperplasia is seen in the case 2.
The neoplastic cells showed immunoreactivity for S-100 protein and CD68 (KP-1), while ki67-MIB1 was unremarcable.
Conclusion. Pseudoepitheliomatous hyperplasia may lead to misdiagnosis as squamous cell carcinoma when a shallow biopsy is performed. Ki67 proliferative index did not distinguish reliably between typical and recurrent atypical GCT. Lastly the endoscopic differential diagnosis of Barrett's Esophagus should include GCT.

PD33
Intraneural schwannoma. An incidental observation

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Introduction. Peripheral nerve tumors are neoplasms that includes benign (schwannomas, neurofibromas and perineuriomas) and malignant forms, collectively designated as malignant peripheral nerve sheath tumors (MPNST). Most schwannomas are uninodular, truly encapsulated, eccentric masses, dislocating the nerve fibers that can be demonstrated in the periphery, flattened along the capsule, but not penetrating the substance of the tumors.
Case report. A 64-year-old man was referred to Hospital of Vallecamonica for excision of a 2 cm wide, nodular, formation on the left auricle. The lesion recurred 15 months later in the adjacent soft parts and a 3,6x1,5 cm. mass was removed with homolateral cervical lymph node clearance.
Results. Histopathology revealed a moderately-differentiated squamous cell carcinoma (G2) in the auricle and recurrent nodule without tumor invasion in 33 cervical lymph nodes. One of the cervical nerve showed a 6 mm. fusiform expansion of its structure. Microscopically the lesion was an encapsulated neoplasm growing inside the nerve. It was composed of short fascicles of bland spindle cells with focal nuclear palisading and Verocay bodies. The tumor cells expressed S-100 protein and were negative for EMA and GLUT-1 which stained instead the peripheral perineurial cells. These findings were consistent with the diagnosis of intraneural schwannoma.
Conclusions. The differential diagnosis includes traumatic and Morton's neuromas, palisaded encapsulated neuroma, nerve sheath ganglion and intraneural perineurioma.
In the present case, schwannoma was centrally located inside the nerve, a position that, to the best of our knowledge, has been reported in the literature only once [1].

[1] *Fellegara G. Int J Surg Pathol 2008;16:57-8.*

PD34
Nuchal-type fibroma: a case report

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Introduction. Nuchal-type fibroma (NTF) is a rare, tumorlike proliferation typically located in the subcutaneous tissues of the posterior neck and interscapular regions of patients between ages 25 and 60 years. The lesion has a low recurrence following excision, not metastatize and has a strong association with diabetes and Gardner's syndrome.
Methods. A 72-year old man presented with a mass in the posterior neck region. It was locally excised. Gardner's syn-

drome and diabetes mellitus has not been documented. Specimens had been fixed in 4% formaldehyde and embedded in paraplast. Sections 4 micron thick were stained with H&E and Van Gieson stain. Immunohistochemistry was performed.
Results. The poorly circumscribed, withe, subcutaneous, 2 cm in the greatest diameter excised mass, microscopically was paucicellular and composed of thick, irregularly arranged bundles of collagen, and scattered fibroblast, with island of trapped adipose tissues, and a localized proliferation of nerve twigs, similar to that seen in traumatic neuroma. A delicate network of elastic fibres is observed between the collagen fibres. The fibroblastlike cells were positive for vimentin and CD34, negative for actin, desmin, S100, GFAP.
Conclusions. NTFs, typically present in the posterior neck region, appear to represent a localized accentuation of the collagenous connective tissue that normally resides in these sites. NTF represents a peculiar, non neoplastic tumorlike reactive process and should be distinguished from other fibrous tumors and tumor like conditions, especially the desmoids-type fibromatosis, circumscribed storiform collagenoma, collagenous fibroma, lipoma. Local recurrence probably reflects the persistence of local or systemic factor related to its pathogenesis.

PD35
CpG island hypermethylation in lung neuroendocrine tumors

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Background. Hypermethylation of promoters' CpG islands, associated with epigenetic silencing of tumor suppressor genes, has not been sistematically investigated in the spectrum of neuroendocrine tumors (NETs) of the lung.
Methods. DNA from formalin-fixed, paraffin-embedded NETs, 21 typical (TC) and 20 atypical carcinoids (AC), 22 large cell neuroendocrine (LCNEC) and 26 small cell lung carcinomas (SCLC), was modified with sodium bisulfite. Methylation-specific PCR was performed for the following genes: APC, BRCA1, CST6, DAPK, FHIT, MGMT, p16, RARb, RASSF1A, Rb, RIZ1, RUNX3.
Results. Median methylation index (fraction of methylated genes/tumor) was 25% for TC, 17% for AC, 33% for LCNEC and 42% for SCLC, all comparisons between classes being statistically significant, except for TC vs AC. Methylation frequencies above 40% were observed for BRCA1, FHIT, RASSF1A and RUNX3 in all NET types. Higher methylation levels were observed in high grade tumors (LCNEC and SCLC) as compared to carcinoids, with statistical significance reached for APC, DAPK, RARb, RASSF1A and RUNX3. Among carcinoids, TC showed slightly higher degrees of methylation than AC. Among high grade neoplasms, SCLC showed higher methylation rate than LCNEC for all genes except APC, a difference that reached statistical significance for p16 and RARb, the latter being hypermethylated only in SCLC.
Conclusions. Hypermethylation is frequently observed in lung NETs. Hypermethylation of BRCA1, FHIT, RASSF1A and RUNX3 in both lower and higher grade NETs suggests their involvement in early stages of tumorigenesis. Higher degree of methylation associates with high grade NETs, suggesting progressive accumulation of epigenetic defects.

PD36
Chromosome 17 polisomy (Ch17polisomy) on HER/Neu status in breast cancer

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Introduction. Ch17polisomy is often found in breast cancer and may complicate the interpretation of HER2 testing results. The aim of the study was to analyze: a) HER2 amplification in tumors where HER2 status scored as 2+ (Dako/FDA) on IHC; b) the prevalence of polisomy17,defined as ≥ 3 copies of the chromosome and its correlation with HER2 protein expression and gene amplification; c) the correlation of polisomy with biologyc and pathologic features as compared with tumors HER2 amplified.
Methods. 428 cases of invasive breast cancer were collected. 327 cases with weakly or moderate positive staining for HER2 on IHC (0/1+/2+) were selected. Immunohystochemical staining, for detection of Her2 protein, was carried out using polyclonal antibody A0485.FISH analysis (Vysis) was carried out with the protocol recommended. The HER2 gene was considered amplified in tumors with average ratios ≥ 2.2.
Results. Ch17 polisomy was observed in 114/428 cases (26.7%), in 106 of those HercepTest was evaluated: 72 (67.9%) were scored HER2 2+, 18 (16.9%) 3+, 16 as 0/1+ ($p = 0.007$). On 114 polisomic cases, HER2 gene amplification was detected in 16 cases (14%) enclosing 12 overespressed (3+) and 4 scored as 2+. One case showed an histological low grade (G1). With regard to nodal status, 158 out of 328 evaluable cases were N0 and 170 N+: polisomy was detected in 39 (24%) and 53 (31.3%) cases, respectively. Hormone receptor and kinetic activity were evaluable in 110 cases. ER and PgR: 78 tumors (71%) resulted ER+/PgR+ and 20ER-/PgR-(18%); moreover 76 cases (69%) showed high MIB1 (Cut off: 20%).
Conclusions. Ch17Polisomy had high incidence in HER2 not amplified cases and confirmed that HER2 gene amplification is indipendent from polisomy17. High incidence of polisomic cases in tumor 2+ suggest that this subgroup could benefit by target therapy.

PD37
NHERF1 and angiogenesis in familial breast cancer

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Background. Na+/H+ exchanger regulatory factor 1 (NHERF1) is a scaffolding protein that recruits membrane and cytoplasmic proteins into functional complexes. Our recent evidences demonstrate that in breast cancer NHERF1 overexpression is associated with increased tumor hypoxia and poor prognosis. Our aim was to determine NHERF1 expression on

a series of familial and sporadic breast cancer patients and examine the relationship with other progression markers.
Methods. NHERF1, VEGFR1, HIF1α and HER2/neu proteins expression were analysed by immunohistochemistry on a tissue microarray, including 94 familial and 93 sporadic breast tumors. Cytoplasmic, membrane and nuclear NHERF1 reactivity was analysed.
Results. Membrane NHERF1 expression was significantly higher in sporadic than familial patients (p = 0.000). Familial cancers showed high levels not statistically significant of cytoplasmic NHERF1 expression compared with sporadic cancers. In familial breast patients, cytoplasmic NHERF1 overexpression was related with VEGFR1 positivity, in 48.3% of cases (p = 0.009). Furthermore, high levels of nuclear NHERF1 in familial cancers were associated with positive HIF1α tumors (p = 0.003). No significant correlation was found between NHERF1 and HER2/neu. In contrast, 48% of overexpressing HER2/neu sporadic tumors, showed a significant association with high cytoplasmic NHERF1 levels (p = 0.007). Moreover, in these tumors, nuclear NHERF1 protein is significantly correlated with HIF1α expression (p = 0.019). Any NHERF1 significant association between both VEGFR1 and HIF1α was found.
Conclusion. In familial breast cancer, NHERF1 resulted strongly related with VEGFR1 and HIF1α proteins with respect to sporadic tumors. In this context, we suggest an emerging role of NHERF1 in angiogenesis.

PD38
Risk evaluation in breast cancer: a preliminary study of pre-metastatic niche in 252 negative lymphnodes

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Normal and tumorigenic stem cells have extensive proliferative potential and ability to give rise to new tissues. The identification of bone marrow-derived hematopoietic progenitor cells (HPCs) emerged as key event, creating microenvironment for distant tumor growth and establishing the "Pre-Metastatic Niche". In order to identify HPCs of the premetastatic niche in breast cancer, we have analyzed 252 (17 of which pN1a) lymphnodes, obtained from 25 patients (mean age 58.6; age range 47-79 yrs), surgically treated in the period 1998-2000 for ductal invasive carcinomas. The mean follow-up was 84.2 mo. (range 36–136). Samples have been fixed in 10% μneutral buffered formalin for 12-72 hrs and included in paraffin at 56°C; 4 m thick sections were pre-treated in microwave owen in 10 mM citric acid, ph 6.0 and incubated overnight with the folllowing poly/mono-clonal antisera: VEGF-R1 (Santa Cruz Biothechnology 1:400), CD 133 (Abgent 1:80), CD 117 (DAKO 1:500), CD-34 (DAKO 1:50). In lymphnodes, clusters of immunoreactive cells were always evident with CD 117 and CD 34. A different degree of immunopositivity was found with VEGR-R1 and CD 133 antibodies, based on different sites of reported metastases; in particular, cases with spread in lungs, liver and central nervous system were more immunoreactive in comparison to the bone localization. Seventeen lymphnodes classified as pN1a showed no immunoreactivity. The clinical significance of "node negative" breast carcinomas needs to be further investigated, since the identification of HPCs able to predict possible, imminent or future, spread to other sites become mandatory; this immunomorphologic aspect of the lymphnode microenvironment could have significant clinical implications for the breast cancer treatment.

PD39
Cancer-stroma interactions: role of cancer-associated fibroblasts and mast cells in breast carcinogenesis

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Background. Carcinogenesis is influenced and controlled by cellular interactions derived from a complex relationship between epithelial, stromal, and extracellular matrix components. Recent evidence shows that fibroblasts and inflammatory elements may not be passive bystanders but might have an important role in modifying tumor growth and cancer progression.
We investigated the distribution of a subpopulation of activated fibroblasts called carcinoma-associated fibroblasts (CAFs) and tumor infiltrating mast cells (MCs), and their simultaneous interaction in invasive human breast cancers.
Methods. Expression of α smooth muscle actin (αSMA), CD34 stromal fibroblasts by immunohistochemistry, and accumulation of intact MCs with toluidine blue staining, was examined in 30 breast cancers. Tumor (T), peritumor (PT) and non malignant (PM) tissues from the same patient have been investigated in order to identify and quantify CAFs and intact MCs. Ten cases were studied with electron microscopy.
Results. αSMA+ fibroblasts were predominantly observed in T and in some PT specimens within the fibrous stroma. Ultrastructural study confirmed the presence of cells with myofibroblast features in the tumor tissues. CD34+ fibroblasts were found prevalently in PM and PT tissues. Compared with PM, the number of intact MCs was significantly increased in T, distributed in the stroma near blood vessels, but not interacting with α-SMA+ fibroblasts. Notably, the CAFs increased in parallel with the MCs infiltration and their values were substantially greater in the T than in the surrounding PM.
Conclusions. Preliminary findings suggest that MCs may contribute to breast cancer stromal remodelling, characterized by a loss of CD34+ fibroblasts and subsequent progressive fibroblast activation.

PD40
Myoid hamartoma of the breast

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Introduction. Mammary hamartoma (MH) is an unusual benign lesion first described by Arrigoni in 1971. Hamartomas accounted for 1% of benign tumors and 5% of benign breast tumors. Myoid hamartoma is a very rare subtype of breast hamartoma. It is composed of varying amounts of mammary ducts, lobules, stroma and bands of smooth muscle cells. Clinically suggests a fibroadenoma whereas the heterogeneous echogenicity with the radiolucent halo on mammography represent a characteristic feature.
Case report. A 42-year-old woman presented with a 4,5 cm lesion in the right breast. Her medical history was unremarkable as well as her family history. Mammography revealed a sharply, circumscribed lesion with a radiolucent halo. Ultrasound echogenicity was heterogeneous without calcifications. Treatment was tumorectomy. The lesion was rubbery and yellow-white on cut sections. Histologically the lesion exhibited pushing borders and contained lobular and ductal mammary tissue without atypia and interdispersed bands of fibrous and smooth muscle cells. Many aspects of fibrocystic changes were present. Immunochemistry showed positivity of smooth muscle cells for desmin and smooth muscle actin. The diagnosis was myoid hamartoma of the breast.
Discussion. Mammary hamartoma is an uncommon tumour. In contrast to many other benign or malignant breast lesions, the diagnosis of hamartoma can easily be missed. Necessary is to correlate pathological, clinical and radiological features for the correct identification of this entity because there are related problems of coincidental epithelial malignancy. Hamartomas result more from breast dysgenesis than from tumoral process. The treatment is excisional biopsy.

PD41
Pure type mucinous carcinoma of the breast with neuroendocrine differentiation: a case report and short review of literature

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Introduction. Mucinous carcinoma (MC) is a special type of invasive breast neoplasm grouped into pure and mixed types. Some authors subdivided mucinous carcinoma components on the basis of mucin content, epithelial growth pattern and associated figures in type A (tumours contain 60-90% mucin) and type B (tumours contain 33-75% mucin). Neuroendocrine differentiation in mucinous carcinoma has long be observed, either immunohistochemically or by electron microscopy. The significance of neuroendocrine differentiation is still unclear.
Case report. An 81-year old woman showed a right breast nodule in the retro-areolar region. The neoformation (4x4x2 cm), once removed, was fixed in buffered formalin and paraffin embedded. Sections were stained with haematoxilin-eosin, histochemically and immunohistochemically investigated. Microscopically we observed small clusters of tumor cells with abundant extracellular mucin accumulation (65%). The axillary lymph nodes were found negative for metastasis. Immunohistochemical stains for Neuron Specific Enolase (NSE), chromogranin (CGA), synaptophysin (SYN) were strongly positive with a marked histochemical expression of Grimelius. The tumor was positive for estrogen (90%) and progesteron (80%) receptors, incompletely positive for c-erbB2 (15%) with a low Ki-67 expression (10%). A diagnosis of a pure mucinous carcinoma of the breast (hypercellular variant) with neuroendocrine diffrentiation was performed.
Discussion and conclusions. As reported in literature the diagnosis is based on the expression of CGA, SYN and NSE. The tumor occurs in older patient age and is associated with favorable parameters like lower Ki-67 and cerbB2 expressions and lower incidence of axillary lymph nodes metastasis, as we observed in our study.

PD42
The 70 gene Mammaprint ™ signature: a comparison with traditional clinco-pathological parameters

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Background. The pathologist is increasingly engaged in testing biomarkers that provide prognostic and predictive information to direct treatment in breast cancer patients. The 70 gene signature (MammaPrint™) is validated as an independent prognostic indicator for patients pN0 and pN+; moreover may be clinically more useful for prediction in endocrine responsive cancer to assign adjuvant-therapy.
Methods. Our study involves 57 patients (aged 38-88) with invasive breast cancer and submitted to surgical procedure, tested on a biopsy punch of fresh tissue by Mammaprint™ (Agendia).
Results. 38 cases were ductal, 7 lobular, 8 special, 4 mixed type. 4 were G1, 37 G2, 15 G3. 41 cancers were pT1, and 15 were pT2. 24 were pN0, 25 pN1 and 8 were pN2. In all cases. NPI, ER, PR, Mib1 and HER2 were evaluated. Applying St.Gallen criteria 5,5% of patients were low risk, 74,5% intermediate, and 20% high risk. Among the 57 patients, 24 had a good prognosis-signature, whereas 33 had a poor prognosis-signature. At univariate analysis we detect a direct correlation between Mammaprint™ vs G, vs NPI, vs Mib1 expression and vs St. Gallen Categories. Changing categories by Mammaprint: 7 good prognosis NPI: High Risk Mammaprint; 9 intermediate NPI: Low Risk Mammaprint and 8 intermediate NPI: High Risk Mammaprint; 2 High Risk St.Gallen: Low Risk Mammaprint.
Conclusions. The gene signatures integrated in clinical practice could help oncologists to optimize clinical management decisions. Today's pathologist plays a traditional but also emerging roles in risk assessment, diagnosis, staging and management of patients.

PD43
Ewing sarcoma of the kidney

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Introduction. Ewing sarcoma (ES) is a rare malignant tumor usually occurring in patients under the age of 19 and rarely found in adults. ES typically involves the bones and can also arise in almost any soft tissue. This tumor is characterized by translocations like the most common t(11;22)(q24;12) involving the EWS gene at 22q12. Prognosis is generally poor and pathologic stage is the major determinant factor.
Methods. A 39-year-old woman presented with flank left pain and hematuria. CT scan revealed a 9-cm mass in the left kidney and radical nephrectomy was performed.
Results. Macroscopically the lesion showed greyish-tan color. Microscopically the cells were monotonous, arranged in nodules and sheets with formation of rosettes and with hyperchromatic rounded nucleus with finely dispersed chromatin and micronucleolus. Scattered apoptotic elements were observed, mitotic figures were numerous and necrosis present. Immunohistochemically, the neoplastic cells showed strong expression for CD99, FLi-1, vimentin, focally for cytokeratin, synaptophysin and were negative for WT1. Fluorescence in situ hybridization using a probe for EWSR1 region on chromosome 22q12 revealed a rearrangement of the EWSR1 locus confirming the diagnosis of ES.
Conclusion. The differential diagnosis of ES can be difficult, expecially with Wilms Tumor and clear cell sarcoma that are other two renal neoplasms typically occurring in pediatric age. Cytogenetical demonstration of the rearrangement of the EWSR1 locus by FISH is a fondamental supportive tool to immunohistochemistry in making the diagnosis of ES.

PD44
An alternative fixation

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Objective. The purpose of the study reported here was to determine the effect of the new fixative Greenfix *vs* Formalin or Holland of biopsy specimens and to determine the method that were most consistently associated with good nuclear and cytoplasmatic details, safe contrast and not background.
Materials and methods. Three samples of the same biopsy were fixed with Formalin, Greenfix, Holland. Sections stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), were scored for histopathologic criteria by pathologist. Moreover molecular methods (FISH, PCR) were applied.
Results. The quality levels of the cellular features was determined by good resolution of the nuclear chromatin; reliable histochemical and immunohistochemical results, with more contrast and less diffusion of chromatic positivity, comparable with Holland; excellent contrast ephitelium-stroma and good results in molecular biology.
Conclusions. Formalin-fixed paraffin-embedded archival clinical specimens were invaluable in discovery of prognostic and therapeutic targets for diseases such as cancer. However formalin was very toxic fixative. We suggest that the implementation of new, multipurpose fixatives may further improve the quality and suitability of histochemistry, immunohistochemistry and molecular analysis from fixed tissue specimens, and reduce the toxicity for operators. The alternative fixative Greenfix, has showed minimal impact of nucleic acids equivalent Holland maintaining tissue morphology for diagnosis. Preparations were high quality with a cellular structure comparable to formalin fixation.

PD45
The double labelling technique for combined histochemistry and immunohistochemistry analysis

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Objective. The aim of this study is to develop a double staining technique for simultaneous demonstration of chemical features and cells antigenic markers in histological sections. We remember that the double labelling is more informative of the biological features of the specimens and offer a more pathological diagnosis.
Materials and methods. First, the antibodies specific for CK, CD31, AML, were used for immunohistochemical analysis of skin pathology; LCA, and CD20, CD31, MIB for colorectal adenocarcinomas and LCA, CD31 for hepatic steatosis. In second step the Masson staining was applied to cutanea biopsy, and Alcian-blue or PAS to gastrointestinal tissue. The final visualization of the staining products of both reactions had been performed.
Results. Using this protocol, we show that there is not background, and the reaction products can be easily distinguished. In particular the Masson staining, the historical method for detection of melanin, shows enhance efficiency and selectivity in detectible the granule's pigments also in the double staining method.
Conclusions. The results of historical diagnostic methods for general functions of tissue (the secretion, the pigments, for example), are too actual. We think that the double staining methods promise a good chance for methodological training of young technicians. This method can assure in a right way the evaluation of diagnostic cases with uniformity of views and judgement.