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Methods.

Ki67 in breast cancer may have prognostic significance. The tumor suppressor gene p53 and the marker for cellular proliferation, chemotherapeutic regimens in breast cancer, overall 5-year survival.

**Introduction.**

Despite the improvement of diagnostic methods and chemotherapeutic regimens in breast cancer, overall 5-year survival significantly depends on the stage of the disease. Over expression of tumor suppressor gene p53 and the marker for cellular proliferation Ki67 in breast cancer may have prognostic significance.

**Methods.** We evaluated 675 patients diagnosed with breast cancer at UF Health Jacksonville between January 2000 and June 2007 with up to 5-year follow up. The aim of the study was to determine whether immunohistochemical (IHC) assessment of Ki67 and p53 may predict outcome, the “hazard” of dying. Cox’s proportional hazards models were used to control for age (< 50 vs. ≥ 50), race (white vs. other), lymph node group (negative vs. positive), ER (estrogen receptor) group (negative vs. positive), PR (progesterone receptor) group (negative vs. positive), and tumor type.

**Results.** When only p53 was considered in the model, the hazard of dying was significantly higher for p53 positive compared to p53 negative (HR = 1.32, 95 % CI 1.02, 1.70, p = 0.036). When only ki67 was considered in the model, the hazard of dying was significantly higher for ki67 positive compared to ki67 negative (Hazard ratio = 1.64, 95 % CI 1.08, 2.49, p = 0.021). Neither of the two markers, nor their interaction was significant when all variables were considered in the model.

**Discussion.** This study confirms the expression of p53 and Ki67 as strong individual indicators of patient outcome. However, when controlling for the other variables, the two markers are not independent predictors. Future studies that will include these markers might help design targeted therapy.

**Plexiform fibromyxoma of the gallbladder**


We report the unusual case of a plexiform fibromyxoma, occasionally assessed in a lithiasic gallbladder. The full thickness assessment of the gallbladder wall revealed an intra-mural, well demarked multinodular tumor (1 cm), consisting of a plexiform growth of spindle cells, included within a fibromyxoid stroma with a rich microvascular network. The tumor cells featured no nuclear atypia, nor mitotic activity. At the immunohistochemical profiling, the spindle shaped cells unequivocally featured vimentin, SMA, HHF35, collagen IV, and CD34; no cells expressed CD117, PDGFRA, CD10, desmin, GFAP, EMA, and S-100. Faint STAT6 nuclear expression was observed in isolated tumor cells. The molecular profiling did not reveal any CKIT and PDGFRA genes mutations. The uncommon site of the tumor presentation and its aberrant CD34 expression both confer to the reported case a unique place among the myxoid tumors of the gastrointestinal tract.

A population of 1136 HPV DNA-HR positive women: expression of p16 INK4a / Ki67 Dual-Stain Cytology and cytological diagnosis. Histological correlations and cytological follow up

P. Rossi, L. Borghi, R. Ferro, R. Mencarelli

**Objective.** The objectives of this study were to evaluate, in a selected HR-HPV positive population, the clinical performance of the p16/ki67 immunostaining in all the cytological diagnoses, as a reflex test of triage HPV-cytology, and assess the usefulness of p16/ki67-staining to classify CIN1 according to its risk of progression/regression in order to plan a personalized follow-up.

**Methods.** Our analysis was in consecutive cases of 1136 women aged 25-64 years, asymptomatic, HR-HPV DNA HC2 tested positive in a HPV-screening program, from February to December 2011. All the women had a cervical sample, in the Thin Prep, used for cytological diagnosis and for p16/Ki67 dual-staining. Histological correlations were 442. We studied the follow-up of two years of 387 cases, especially the biological behaviour of 316 low-grade lesions.

**Results.** p16/Ki67 dual-staining increases the VPP CIN2+ and NPV CIN2+, especially in atrophy/dystrophy, in ASC-US and LSIL. In follow-up of 387 cases, 71 CIN2+ and 316 CIN1, 69 CIN2+, after surgical treatment, had a negative follow up; two cases of CIN2 (p16/ki67-) without invasive treatments, had a spontaneous regression. Among the 316 CIN1, progression was observed in 10 women (4 p16/Ki67+ and 6 p16/Ki67-); regression in 260 women (64 p16/ Ki67+ and 196 p16/Ki67-); 46 women had a persistent LSIL (9 p16/Ki67+ and 37 p16/Ki67-). It seems no significant differences in the biological behaviour in relation to the expression of the two biomarkers.

**Conclusions.** p16/Ki67 immunostaining increases sensitivity of cytology in some diagnostic categories. After follow up of two years, a personalized and adequate treatment does not seem still possible. Further studies and trials are required to improve the management of the cervical lesions in HPV-based screening strategies.

**Case reports**

**Solitary thyroid metastasis from colon cancer: fine-needle aspiration cytology and molecular biology approach**

M. Onorati, P. Uboldi, C.L. Bianchi, M. Nicolà, G.M. Corradini, S. Veronesi, A.I. Fasci, F. Di Nuovo

Thyroid gland is one of the most vascularized organs of the body, nevertheless clinical and surgical series report an incidence of secondary malignancies in this gland of only 3 %. Colorectal carcinoma metastatic to the thyroid gland is not as uncommon as previously believed, in fact the number of cases seems to be increased in recent years due to the more frequent use of fine-needle aspiration cytology (FNAC) guided by ultrasonography. Although kidney, breast and lung metastases to the thyroid are frequent, metastasis from colon cancer is clinically rare with 52 cases reported in the literature in the last 5 decades and three cases described as solitary thyroid metastasis from the colon cancer without any other visceral metastases.

To the best of our knowledge, we report the fourth case of solitary, asymptomatic thyroid metastasis from colon cancer without involvement of other organs. We discuss the importance of FNAC to detect metastatizing process as a compulsory step of the diagnostic and therapeutic management algorithm, combined with a molecular biology approach. A review of the last 5 decades literature, to update the number of cases described to date, is also included.

**Tuberous sclerosis: histological analysis with confocal laser scanning microscope of gingival angiofibromatosis**

G. Favia, A. Tempesta, L. Limongelli, E. Maiorano

**Introduction.** Tuberous sclerosis (TS) is an autosomal dominant neuro-cutaneous syndrome characterized by multiple hamartomas in various organs, especially on skin and central nervous system. The most common features of TS include facial angiofibromas, hypomelanotic cutaneous macules, shagreen patches in the lumbar area, cerebral cortical tubers, sub-ependymal nodules, sub-ependymal giant cell astrocytomas, cardiac rhabdomyomas, and renal angiomyolipomas. Fre-
厥ently oral manifestations such as fibrous hyperplasia, angiofibromas and dental enamel pitting are also observed. The aim of this case report was to describe the histological aspects of oral diffuse hyperplastic angiofibromatosis, never reported in the English literature and analyzed by Confocal Laser Scanning Microscope (CLSM), and to highlight the surgical implications of these aspects such as use of Diode Laser.

**Case report.** A 14-years-old female patient with TS diagnosis came to our attention for diffuse gingival hyperplasia on the mandible. Clinical examination highlighted epidermal hamartomas on the whole body, especially on the face and scalp. Pathologic hyperplastic tissue was removed by pulsed diode laser at the power of 5-6W, and the surgical samples were sent for conventional and CLSM histopathological examination. After laser excision, wounds healed quickly without complications. At CLSM examination collagen fibres, showing intense fluorescence and with variable spatial orientation, and variably sized blood vessels were noticed suggesting the diagnosis of gingival angiofibromatosis, a still unreported finding in TS patients.

**Conclusions.** CLSM analysis allows to highlight some unusual histopathological features of TS; diode laser is very effective for the treatment of gingival angiofibromatosis.

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**An incidentally diagnosed epithelioid trophoblastic tumor in hysterectomy**

A. Usubutun, I. Selcuk, G. Boyraz, Z.S. Tuncer

Epithelioid trophoblastic tumor is a rare non-molar gestational trophoblastic disease. A 40-year-old multiparous woman was incidentally diagnosed with epithelioid trophoblastic tumor after hysterectomy. Hysterectomy specimen revealed multiple small, tan to yellow nodules measuring 0.3-0.8 cm just below the endometrium. In the microscopic examination uniform neoplastic cells with varying cellularity were accompanied by necrotic zones and eosinophilic hyaline material. Immunohistochemically neoplastic cells were diffusely stained with CK7, inhibin-alpha, p63, hPL, and CD146. There was no staining with beta-HCG, SMA, PLAP, or h-caldesmon. Ki-67 proliferative index was approximately 10% and cyclin E was stained in approximately 10% of the neoplastic cells. Although immunohistochemical studies are helpful in classifying gestational trophoblastic lesions, borderline values can cause diagnostic confusion between neoplastic and reactive lesions, particularly in inadequate endometrial biopsies.
The potential of ki67 and p53 assessment in development of individualized targeted therapy in breast cancer patients

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Key words
Breast cancer • P53 • Ki67

Introduction

Breast cancer is a major public health problem for women across the globe, being the most common type of cancer in women. Despite the improvement of diagnostic methods and chemotherapeutic regimens in breast cancer, overall 5-year survival significantly depends on the stage of the disease. However, over the last decades, understanding of the tumor biology has been greatly improved by molecular research that allows application of a tumor’s molecular features for selection of personalized therapy and prediction of short and long term therapy 1. Traditionally, there has been a division of breast cancer into hormone receptor positive and negative groups to guide treatment with endocrine and chemotherapy, respectively. However, within the group of hormone receptor positive patients, a subgroup of patients appeared to respond poorer to this somewhat targeted therapy, with early relapses and poorer survival 2. Studies have shown that Ki-67 can be used for subtyping and distinguishing breast cancer and is therefore of greatest importance, considering the implications for treatment protocols, especially when combined with other markers such as p53 2,3. In addition, utilizing immunohistochemistry as surrogate marker for molecular testing (gene profiling) to sub-classify breast cancer is an important factor in terms of cost containment. Ki67 is an immunohistochemical marker of proliferating cells which is widely used in various types of malignancy to assess proliferation rate. It is a fairly large

Results

When only p53 was considered in the model, the hazard of dying was significantly higher for p53 positive compared to p53 negative (HR = 1.32, 95% CI 1.02, 1.70, p = 0.036). When only ki67 was considered in the model, the hazard of dying was significantly higher for ki67 positive compared to ki67 negative (Hazard ratio = 1.64, 95% CI 1.08, 2.49, p = 0.021). Neither of the two markers, nor their interaction was significant when all variables were considered in the model.

Discussion

This study confirms the expression of p53 and Ki67 as strong individual indicators of patient outcome. However, when controlling for the other variables, the two markers are not independent predictors. Future studies that will include these markers might help design targeted therapy.
nuclear protein (395kD) that is present during all active cell cycle phases, except for G0. Naturally, proliferation status correlates tightly with tumor aggressiveness, and therefore, Ki67 labeling index is a commonly used prognostic indicator in breast cancer. In DCIS (Ductal Carcinoma in Situ), Ki67 positivity is associated with a higher risk of developing DCIS local recurrence after breast conserving therapy.

P53 is a tumor suppressor gene which regulates cell cycle progression in response to various stimuli; alteration of p53 function is seen in tumor development and progression in various organ systems. Positivity for these markers generally infers a worse prognosis, greater probability of failure with endocrine therapy in hormone receptor positive patients and poorer survival.

An Iranian study suggested that Ki67 may have more significant prognostic strength in terms of survival than p53; however, this study was fairly small, and a larger scale study is needed to substantiate these findings. There is also an urgent need to improve prognostic classifiers in breast cancer. Most recent decisions for breast cancer patients are made on the basis of prognostic and predictive factors. Molecular studies of breast cancer continue to unveil the biological heterogeneity of the disease which has opened new perspectives for personalized therapy. Overexpression of tumor suppressor gene p53 and the marker for cellular proliferation Ki67 in breast cancer may have prognostic significance.

The aim of our study was to investigate the correlation between Ki67 and p53 positivity with patient outcome in a large retrospective study of 675 patients with 5 year follow up, to potentially predict patient outcome and open new horizons in the development of individualized targeted therapy.

Materials and methods

We evaluated 675 patients diagnosed with breast cancer with 5 year follow up at UF Health Jacksonville between January 2000 and June 2007. The expression of Ki67 and stability of p53 were determined by immunohistochemistry. The aim of the study was to determine whether immunohistochemical (IHC) assessment of Ki67 and p53 may predict outcome.

The primary outcome variable was the ‘hazard’ of dying. Hazard is the instantaneous probability of dying given that patients have survived up to a given point in time or the risk for death at that moment. First, three Cox’s proportional hazards models were fit: one with p53, ki67, and interaction p53*ki67 as predictors, one with only p53 predictor, and one with only ki67 as predictor. Second, models were fit to determine the joint effects of p53 group and ki67 group of patients, controlling for age (< 50 vs. ≥ 50), race (white vs. other), lymph node group (negative vs. positive), ER (estrogen receptor) group (negative vs. positive), PR (progesterone receptor) group (negative vs. positive), and tumor type (DCIS group vs. other, Lobular carcinoma vs. other, Infiltrating ductal carcinoma vs. other, and other tumors vs. DCIS+Lobular carcinoma + Infiltrating ductal carcinoma). The best subset selection method was performed to assess the best predictive model using all the other variables. The criterion used to determine the “best” subset is based on the global score chi-square statistic. For two different models, each having the same number of explanatory variables, the model with the higher score chi-square statistic is considered to be better. Then, each of the markers p53 and ki67 were added in the best model previously found.

Results

Patients’ demographic and baseline characteristics are listed on Table I. Seventy percent of the patients were 50 years or older, and 53% were white (53%). P53 was negative for 74% of the sample, and Ki67 was negative for 43% of the patients. Assessment of positive results was based on the standard of practice (for p53 scoring >10% nuclear staining was considered positive, for Ki67 ≥ 20% is positive, 10 to 20% borderline and 1 to 9% negative). Figure 1 presents patterns of staining for Ki67 and p53.

First, the joint effect of p53, ki67, and the interaction between p53 and ki67 on the probability of survival was assessed. The reference level for both markers was chosen to be the “negative” level. None of the two markers, nor their interaction was significant (p = 0.050, p = 0.242, and p = 0.252, respectively). When only p53 was considered in the model, the hazard of dying was significantly higher for p53 positive compared to p53 negative (HR = 1.32, 95% CI 1.02, 1.70, p = 0.036). Figure 2 is the survival plot that contains two curves, one for p53 positive and one for p53 negative. It can be seen that, overall, the probability of survival is higher for p53 positive compared to p53 negative. When only ki67 was considered in the model, the hazard of dying significantly higher for ki67 positive compared to ki67 negative (HR = 2.9, 95% CI 1.59, 5.27, p = 0.001).

The primary outcome variable was the ‘hazard’ of dying. Hazard is the instantaneous probability of dying given that patients have survived up to a given point in time or the risk for death at that moment. First, three Cox’s proportional hazards models were fit: one with p53, ki67, and interaction p53*ki67 as predictors, one with only p53 predictor, and one with only ki67 as predictor. Second, models were fit to determine the joint effects of p53 group and ki67 group of patients, controlling for age (< 50 vs. ≥ 50), race (white vs. other), lymph node group (negative vs. positive), ER (estrogen receptor) group (negative vs. positive), PR (progesterone receptor) group (negative vs. positive), and tumor type (DCIS group vs. other, Lobular carcinoma vs. other, Infiltrating ductal carcinoma vs.

### Table I. Patients’ demographic and characteristics (N = 675).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50 years or older</td>
<td>515 (76)</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>355 (53)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>Yes</td>
<td>18 (3)</td>
</tr>
<tr>
<td>P53</td>
<td>Positive</td>
<td>175 (26)</td>
</tr>
<tr>
<td>Ki67</td>
<td>Negative</td>
<td>292 (43)</td>
</tr>
<tr>
<td></td>
<td>Borderline</td>
<td>90 (14)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>293 (43)</td>
</tr>
<tr>
<td>Tumor</td>
<td>DCIS</td>
<td>86 (13)</td>
</tr>
<tr>
<td></td>
<td>IDC</td>
<td>533 (79)</td>
</tr>
<tr>
<td></td>
<td>Lobular carcinoma</td>
<td>34 (5)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>22 (3)</td>
</tr>
<tr>
<td></td>
<td>Non-emergent</td>
<td>211 (33)</td>
</tr>
<tr>
<td>ER</td>
<td>Negative</td>
<td>166 (30)</td>
</tr>
<tr>
<td>PR</td>
<td>Negative</td>
<td>213 (39)</td>
</tr>
<tr>
<td>ER and PR</td>
<td>Negative</td>
<td>163 (30)</td>
</tr>
</tbody>
</table>

DCIS = Ductal Carcinoma in Situ, IDC = Invasive Ductal Carcinoma
was significantly higher for ki67 positive compared to ki67 negative (Hazard ratio = 1.64, 95 % CI 1.08, 2.49, \( p = 0.21 \)). There was no difference between ki67 borderline compared to ki67 negative (Hazard ratio = 0.82, 95 % CI 0.53, 1.26). Figure 3 presents the survival curves for ki67 groups.

Using the best subset selection method, the “best” model selected was the model including race (\( p = 0.14 \)), lymph node group (\( p = 0.013 \)), PR group (\( p = 0.18 \)), DCIS group (\( p = 0.06 \)), and LC group (\( p = 0.22 \)) as significant predictors. When entered in the model described above, p53 (\( p = 9.65 \)) and ki67 (\( p = 5.77 \)) are no longer signifi-
Discussion

Several studies have shown the cumulative value of including p53 and Ki67 immunohistochemical staining when assessing breast cancer prognosis and risk-stratifying patients. Positivity for these markers generally infers a worse prognosis (e.g., greater probability of failure with endocrine therapy in hormone receptor positive patients) 2, 10. Sarode et al. reported high Ki67 and p53 overexpression were characteristic of HER2 and triple negative subtypes of breast cancer 11. While Ki67 has been established as an important prognostic indicator, there seems to be a lack of uniformity in reporting Ki67 values, with different reasons, such as different techniques, cut-off values, etc. 12. In addition, intratumoral heterogeneity of proliferation can make it very difficult to assess an accurate Ki67 proliferation rate, depending on adequate tissue sampling 12. Standardization of Ki67 reporting values may help strengthen the value of Ki67 as a prognostic factor.

An Iranian study suggested that Ki67 may have a more significant prognostic strength in terms of survival than p53 6; however, this study was fairly small, and a larger scale study would be indicated to substantiate these findings. Other studies demonstrated that the combination of p53 and Ki67 is more accurate than Ki67 alone in predicting the prognosis for patients with hormone receptor positive and Her2-negative breast cancer 4. Our results confirm that both Ki67 and p53 are significantly associated with an adverse clinical outcome in terms of reduced overall survival. When adjusting for clinic-pathological parameters such as race, lymph node status, ER and PR status, the association between outcome and p53/Ki-67 status is no longer significant, indicating that these variables are confounders. This correlates with a study from Cheang et al. reporting that Ki67 was an independent prognostic factor for luminal B type of breast carcinoma, advocating the use of a surrogate IHC panel including ER/PR/HER2 and Ki67 for biological subtyping, independently of standard clinic-pathologic parameters such as age, lymph node status, tumor size and grade 13. Our results indicate that both Ki67 and p53 are single prognostic indicators that may be evaluated separately from other clinic-pathological parameters.

Individually, Ki-67 and p53 are valuable prognostic factors in the setting of breast cancer that can also aid in subtyping different types of breast cancer. However, in our study, they are no longer significant predictors when both markers were entered in the same model, emphasizing their independence as prognostic indicators and indicating that they should be evaluated separately when assessing a panel of immunohistochemical stains as surrogate markers for tumor biology.

Conclusions

This study confirms the expression of p53 and Ki67 as strong single predictors of patient outcome and survival. This in the future might help to design studies for targeted therapy development and improving patient care.

References

We report the unusual case of a plexiform fibromyxoma, occasionally assessed in a lithiasic gallbladder. The full thickness assessment of the gallbladder wall revealed an intra-mural, well demarked multi-nodular tumor (1 cm), consisting of a plexiform growth of spindle cells, included within a fibromyxoid stroma with a rich micro-vascular network. The tumor cells featured no nuclear atypia, nor mitotic activity. At the immunohistochemical profiling, the spindle shaped cells unequivocally featured vimentin, SMA, HHF35, collagen IV, and CD34; no cells expressed CD117, PDGFRA, CD10, desmin, GFAP, EMA, and S-100. Faint STAT6 nuclear expression was observed in isolated tumor cells. The molecular profiling did not revealed any CKIT and PDGFRA genes mutations. The uncommon site of the tumor presentation and its aberrant CD34 expression both confer to the reported case a unique place among the myxoid tumors of the gastrointestinal tract.

Introduction
Myxoid non-GIST tumors of the gastrointestinal tract encompass a heterogeneous group of uncommon non-epithelial lesions, and the different definitions applied to such tumors (e.g. myxoma, fibromyxoma, plexiform fibromyxoma) reflect both the complex phenotype of the lesions and their controversial nosology. In 2007, Takahashi and colleagues defined as “plexiform angio-myxoid myofibroblastic tumor (PAMT)” a multi-nodular benign tumor, mostly arising in the gastro-duodenal district, but also occasionally described in the small and large bowel. We report the unique case of a benign myxoid, plexiform, spindle shaped cell tumor of the gallbladder wall, featuring the histological and immunohistochemical phenotype of the plexiform fibro-myxoid tumor, with aberrant expression of CD34 endothelial marker.

Clinical history
A 55-year-old Caucasian female was referred to the General Surgery Unit of the Padova teaching Hospital for a previously established diagnosis of cholelithiasis. At the admission, no significant past medical history was recorded, and both the physical examination and laboratory tests were unremarkable. The pre-surgical abdominal ultrasound did not reveal any mural thickening. Laparoscopic cholecystectomy was performed, with no post-surgical complications.
bladder’s wall was serially sectioned, and histologically assessed. The newly obtained tissue samples revealed a multi-nodular myxoid lesion, 1 cm large in its wider diameter. Further serial histology sections of the target lesion were obtained for additional histochemical stainings (PAS, Pas after diastase digestion, and Alcian blue), immuno-phenotyping, and molecular profiling. Immunohistochemical profiling was performed on the Bond TM Polymer Refine Detection System (Leica Microsystems, Newcastle upon Tyne, UK). The applied primary antibodies included (source and solutions): Vimentin (Novocastra labs ltd; 1:200), CD10 (Diagnostic Biosystems, Pleasanton, CA; 1:50), CD34 (Thermo Scientific; Waltham, MA 1:100), Collagen IV (Dako, Carpinteria, CA; 1:100), HHF-35 (Cell Marque, Rocklin, California; 1:20); α-smooth muscle actin (SMA, Cell Marque; 1:100); CD117 (Dako; 1:100), desmin (Dako; 1:50); GFAP (Dako; 1:800), S-100 (Histo-Line Laboratories, Milan, Italy; 1:50), AE1/AE3 (Life Technology, Milan, Italy; 1:50), STAT6 (Santa Cruz Biotechnology, Dallas, TX; 1:100); EMA (Dako; 1:100); CD31 (Dako; 1:20), MIB1 (Dako; 1:100), PDGFRα (Santa Cruz Biotechnology; 1:200). Sections were lightly counterstained with hematoxylin. Appropriate positive and negative controls were run concurrently for all the applied antisera.

**Mutational analysis**

DNA profiling was performed on tissue samples obtained from the paraffin block representative of the target lesion. DNA was extracted after enrichment for neoplastic cellularity using manual micro-dissection of 10 consecutive 4-μm FFPE sections, purified using the QIAamp DNA FFPE Tissue Kit (Qiagen), and qualified as reported elsewhere. PCR products of exons 9, 11, 13, and 17 of the PDGFRα gene (primers upon request) were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) and labelled with Big Dye Terminator v3.1 (Applied Biosystems, Monza, Italy). Agencourt CleanSEQ magnetic beads (Beckman Coulter) were used for post-labeling DNA fragment purification, and sequence analysis was performed on an Applied Biosystems 3130xl Genetic Analyzer.

**Results**

On the hematoxylin and eosin stain, the full thickness section of the gallbladder wall demonstrated a multinodular, well circumscribed, myxoid tumor (Fig. 1). The tumor cells population consisted of sparse spindle shaped cells organized in a plexiform pattern, and lying within an abundant myxoid (Alcian blue positive) matrix, which included a rich network of capillary-sized vessel. Tumor cells exhibited oval pale nuclei, and slightly eosinophilic cytoplasm. Neither nuclear atypia, nor (even typical) mitoses were observed. Very rare lymphocytes and plasma cells were dispersed within the myxoid stroma.

The tumor cells consistently expressed vimentin, α-smooth muscle actin, HHF-35, collagen IV, and CD34; no immunostain was demonstrated for CD117, desmin, GFAP, CD10, EMA, and S-100. Some isolated tumor cells showed faint STAT6 nuclear expression. MIB1 labeling index was 0%. The molecular profiling failed in demonstrating any mutations in both the CKIT and PDGFRα genes.

The gallbladder mucosa showed multiple foci of low-grade biliary intraepithelial neoplasia (BilIN-1) co-existing with pyloric- and intestinal-type epithelial metaplasia.

**Discussion**

We report a peculiar case of multi-nodular tumor of the gallbladder wall, consisting of a uniform population of spindle cells, lying in an abundant myxoid matrix. Histologically, the tumor’s nodular growth pattern, its myxoid matrix, the phenotype of the neoplastic cells, and their plexiform arrangement were all consistent with the microscopic phenotype of a benign tumor originally described in the gastric wall by Takahashi and colleagues and by the same Authors defined as “plexiform angiomyxoid myofibroblastic tumors (PAMT)”. Since this initial description, several gastric (and some rare intestinal) cases have been reported. After the original “PAMT” definition, several alternative nomenclatures have been proposed, and phenotypically similar tumors have been labeled as “myofibroblastic tumor” and, more recently, “plexiform fibromyxoma”. Consistently with the original description of the gastric plexiform angiomyxoid myofibroblastic tumor (and consistently with a myofibroblastic/fibroblastic phenotype), the tumor cells consistently expressed vimentin, SMA, and HHF35; unexpectedly, however, the tumor cells also featured moderate/strong immunostain for CD34, which had been reported as negative in the original gastric cases. Among benign myxoid tumors, a SMA/CD34 positive immunophenotype has been consistently associated to superficial angiomyxoma (cutaneous myxoma); such a neoplasia, however, most frequently includes peripheral areas of increased cellularity, focal cellular atypia, and a sparse inflammatory infiltrate, which were not observed in our case. Moreover, this tumor is peculiarly located within the skin and is frequently characterized by the presence of abnormal epithelial structures, such as epidermoid cysts, thin strands of squamous epithelium, and small buds of basaloid cells, which have been suggested to have an etiopathogenetic role for these neoplasms. Of note, CD34 expression has been described only in a single case of gastric fibromyxoma case: this tumor, however, did not feature SMA expression.

Within the gastrointestinal tract, a phenotypically benign myxoid lesion evocates a wide spectrum of differential diagnoses, particularly: spindle shaped cell myxoid GIST, leiomyoma, perineurioma, schwannoma, desmoids fibromatosis, solitary fibrous tumor, inflam-
Plexiform fibromyxoma of the gallbladder

In the present case, the myxoid GIST (a relative rare variant of the GISTs’ family) has been ruled out for the concurrence of a distinctive plexiform pattern, the lack of CD117/PDGFRA expression, and the absence of any CKIT/PDGFRA genes mutation. The diagnosis of inflammatory fibroid polyp, and/or inflammatory myofibroblastic tumor was basically ruled out because of the absence of any inflammatory cell population (eosinophils, in particular). The lack of S-100 expression excluded the neurogenic origin (plexiform neurofibroma, or schwannoma) of the lesion, whereas the lack of EMA expression discharged the diagnosis of perineurioma. The inconsistent STAT6 nuclear expression was not conclusive for a case of myxoid solitary fibrous tumor.

In summary, we describe the first case of plexiform fibromyxoma of the gallbladder, featuring aberrant CD34 expression. This benign tumor, which has been never

Fig. 1. Representative histological features of the present case. The nodular and plexiform appearance of the tumor are recognizable at H&E (A) and Alcian Pas (B) stain and at immunohistochemical analysis for CD34 (C). Note the small and medium size tumor nodules dissecting the gallbladder muscular wall (H&E stain, D; Alcian Pas stain, E). (F) Tumor cells showed a moderate/strong CD34 immunoreaction. (G-I) Representative H&E stain images at different magnifications demonstrating the paucicellular tumor composed by spindled cells intermingled in a myxoid matrix. (Original magnifications 2x; 10x; 20x; and 40x)
reported among the non-epithelial gallbladder tumors, further expands the constellation of the myxoid tumors arising in the gastrointestinal tract.

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A population of 1136 HPV DNA-HR positive women: expression of p16 INK4a / Ki67 Dual-Stain Cytology and cytological diagnosis. Histological correlations and cytological follow up

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Key words
HR-HPV (High Risk- Human Papilloma Virus) • p16/Ki67 +/- (p16 INK4a /Ki67 Immunostaining positive/negative) • HC2 (Hybrid Capture 2 - Qiagen, Hilden, Germany) • VVP CIN2+ (Positive Predictive Value for CIN2 or more) • NPV CIN2+ ((Negative Predictive Value for CIN2 or more)

Summary
Objective. The objectives of this study were to evaluate, in a selected HR-HPV positive population, the clinical performance of the p16/ki67 immunostaining in all the cytological diagnoses, as a reflex test of triage HPV-cytology, and assess the usefulness of p16/ki67-staining to classify CIN1 according to its risk of progression/regression in order to plan a personalized follow-up.

Methods. Our analysis was in consecutive cases of 1136 women aged 25-64 years, asymptomatic, HR-HPV DNA HC2 tested positive in a HPV-screening program, from February to December 2011. All the women had a cervical sample, in the Thin Prep, used for cytological diagnosis and for p16/Ki67 dual-staining. Histological correlations were 442. We studied the follow-up of two years of 387 cases, especially the biological behaviour of 316 low-grade lesions.

Results. p16/Ki67 dual-staining increases the VPP CIN2+ and NPV CIN2+, especially in atrophy/dystrophy, in ASC-US and LSIL. In follow-up of 387 cases, 71 CIN2+ and 316 CIN1, 69 CIN2+, after surgical treatment, had a negative follow up; two cases of CIN2 (p16/ki67-) without invasive treatments, had a spontaneous regression. Among the 316 CIN1, progression was observed in 10 women (4 p16/Ki67 + and 6 p16/Ki67-); regression in 260 women (64 p16/Ki67 + and 196 p16/ Ki67-); 46 women had a persistent LSIL (9 p16/Ki67 + and 37 p16/Ki67-). It seems no significant differences in the biological behaviour in relation to the expression of the two biomarkers.

Conclusions. p16/Ki67 immunostaining increases sensitivity of cytology in some diagnostic categories. After follow up of two years, a personalized and adequate treatment does not seem still possible. Further studies and trials are required to improve the management of the cervical lesions in HPV-based screening strategies.

Introduction
Epidemiological evidence shows that cervical cancer is related to sexual activity and associated to HR-HPV infection. Several authors compared specificity and sensibility of HPV test, cytology, and biomarkers to identify and manage high lesions of the cervix 1-8 27. Many methods have been proposed and studied to improve the performance of HPV testing, providing different levels of evidence. As confirmed by “European guidelines for quality assurance in cervical cancer screening Second Edition Supplements. 2015”, the validity relative to all new strategies triage may be: a) Cross-sectional because this is relevant for the decision of referring women to colposcopy; b) Longitudinal, to assess the risk of CIN2, CIN3, and cancer over time 9.

Numerous biomarkers proteins have been identified to increase the specificity of HPV-HR testing 1 24-7 10-12. Many of these proteins are involved in cell cycle regulation, such as p16 protein, signal transduction, DNA replication, and cellular proliferation. The altered expression of these proteins is a consequence of the binding of the HR-HPV E6 and E7 oncoproteins to host regulatory proteins, resulting in the degradation of the p53 tumor suppressor gene product and the inactivation of the retinoblastoma protein leading to the deregulation of the cell cycle 213 14.

The Ki67 antigen, a high molecular weight non-histonic protein, is generally accepted as the most reliable marker
of proliferating cells. It is expressed in all phases of the cell cycle, except G0. The interaction of E6 and E7 HPV DNA in the host cell disturbs the cell cycle, expressing themselves by the abnormal expression of proteins, including the Ki-67. In normal cells, the expression of p16 and Ki67 is mutually exclusive. The objectives of this study were to evaluate the clinical performance of the p16/Ki67 immunostaining in all the cytological diagnoses, as a reflex test of triage HPV-cytology, to identify samples of patients with high-grade cytological diagnoses, as a reflex test of triage HPV-cytology. A positive result was, within the same cell, a brown nuclear and cytoplasmatic staining and red nucleolar staining indicative of p16 and Ki67 expression. The p16/Ki67 immunostaining was conducted using the CINtec®PLUS Kit (Roche mtm laboratories, Heidelberg, Germany) by monoclonal mouse anti-Human p16INK4a antibody, Clone E6H4TM, and monoclonal rabbit anti-Human Ki-67 antibody, Clone E6H4TM, and Fast Red Chromogen solution. Two Biologists analyzed all cases, individually and without knowing the cytological diagnosis. A positive result was, within the same cell, a brown nuclear and cytoplasmatic staining and red nucleolar staining indicative of p16 and Ki67 expression. The presence of one or more double-immunoreactive cells was regarded as a positive test outcome, irrespective of morphology. Slides without any double-stained cells were called negative for p16/Ki67 dual-staining. A positive result for only one of the biomarkers (p16 or Ki67), in the same cytological slide, was regarded as a positive control of the immunocytochemical reaction. The investigation with p16/Ki67 dual-staining did not alter the screening protocol. In fact, all the women with abnormal cytology were called for colposcopy and, in line with current clinical practice, gynecologists were aware of Cytology and HPV test results but blinded to any dual-stained cytology results.

Among all the 464 women with cytological abnormalities, 442 women agreed further study and were referred to colposcopy. At least one biopsy for further diagnostic was taken in all women. All histological samples were fixed in 10% buffered formalin and embedded in paraffin wax by conventional techniques. Serial section were stained with haematoxylin-eosin and classified by two certified pathologists.

The immunohistochemical investigation with primary monoclonal mouse antibody clone E6H4 TM (p16) and primary monoclonal mouse antibody clone MM1 (Ki67) were carried out on histological sections.

Materials and methods

We conducted our analysis in consecutive cases of 1136 women aged 25-64 years, asymptomatic, HR-HPV DNA HC2 tested positive in a HPV-screening program, from February to December 2011. All the women had a cervical sample. The cytological specimen, collected in the Thin Prep® Pap Test (Hologic, Marlborough, Massachusetts Inc.), was used for cytological diagnosis and for p16/Ki67 dual staining. Thin-layer cytology slides were prepared using TP2000 slide Processor, according to manufacturer’s protocol, stained according Papanicolaou method. The cytological diagnoses were made according to the Bethesda 2001 Cervical Cytology Classification System.

A second cytology slide was prepared from residual liquid-based material, using TP2000 slide Processor. Ten cases were excluded in this second examination because of the minimum squamous cellularity criteria as specified in the Bethesda System 2001. The p16/Ki67 immunostaining was conducted using the CINtec®PLUS Kit (Roche mtm laboratories, Heidelberg, Germany) by monoclonal mouse anti-Human p16INK4a antibody, Clone E6H4TM, and monoclonal rabbit anti-Human Ki-67 antibody, Clone 274-11 AC3. Two Chromogen solutions were necessary: DAB Chromogen (3',3'-diaminobenzidine Chromogen solution) and Fast Red Chromogen solution. Two Biologists analyzed all cases, individually and without knowing the cytological diagnosis. A positive result was, within the same cell, a brown nuclear and cytoplasmatic staining and red nuclear staining indicative of p16 and Ki67 expression. The presence of one or more double-immunoreactive cells was regarded as a positive test outcome, irrespective of morphology. Slides without any double-stained cells were called negative for p16/Ki67 dual-stain cytology. A positive result for only one of the biomarkers (p16 or Ki67), in the same cytological slide, was regarded as a positive control of the immunocytochemical reaction. The investigation with p16/Ki67 dual-staining did not alter the screening protocol. In fact, all the women with abnormal cytology were called for colposcopy and, in line with current clinical practice, gynecologists were aware of Cytology and HPV test results but blinded to any dual-stained cytology results.

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The immunohistochemical investigation with primary monoclonal mouse antibody clone E6H4 TM (p16) and primary monoclonal mouse antibody clone MM1 (Ki67) were carried out on histological sections.

Results

All 1136 women had a cytological diagnosis with the following results: 662 had negative for intraepithelial lesion or malignancy, 177 had ASC-US, 236 had LSIL, 28 had ASC-H, 20 had HSIL, 3 had AGC and 10 had malignancy. Among all the 464 women with cytological abnormalities, 442 women agreed further study and were referred to colposcopy. At least one biopsy for further diagnostic was taken in all women. All histological samples were fixed in 10% buffered formalin and embedded in paraffin wax by conventional techniques. Serial section were stained with haematoxylin-eosin and classified by two certified pathologists.

The immunohistochemical investigation with primary monoclonal mouse antibody clone E6H4 TM (p16) and primary monoclonal mouse antibody clone MM1 (Ki67) were carried out on histological sections.

Histological correlations

Among all the 464 women with cytological abnormalities, after three to eight month, 442 patients have accepted the invitation to more deepening examination. They were referred to colposcopy and biopsy sampling for further diagnostic follow-up in according to the screening protocol. In this study, biopsies were done in 155 women with cytological diagnosis of ASC-US, in all the
patients with LSIL, ASC-H, HSIL and AGC, respectively 236, 28, 20 and 3 cases. The women did not come for more deepening examinations were 22.

The histological diagnoses were obtained: 55 negative; 316 condyloma/low-grade intraepithelial neoplasia CIN1; 47 high-grade intraepithelial neoplasia CIN2; 22 high-grade intraepithelial neoplasia CIN3; 2 Endocervical Adenocarcinoma in Situ.

For the cyto-histological correlation analysis we considered one endpoint CIN2+ or more (CIN 2+), according to the current screening programs.

The Positive Predictive Value for CIN2+ (PPV CIN2+) in the cytological diagnoses was: ASC-US 9.7%; LSIL 9.3%, ASC-H 50%, HSIL 90.0%. The Negative Predictive Value for CIN2+ (NPV CIN2+) was calculated only for categories cytological of ASC-US and LSIL, which resulted to be respectively 90.3% and 90.7%.

In addition we evaluated the clinical performance of p16/ki67 as a test reflex of triage HPV-cytology to identify samples of patients with high-grade lesions. So, we calculated the hypothetical PPV and NPV of cytological categories based on p16/Ki67 double staining positivity (p16/Ki67 +) or negativity (p16/Ki67 -). The Positive Predictive Value for CIN2+ (PPV CIN2+) of ASC-US p16/Ki67 + was 45.5%, of LSIL p16/Ki67 + was 43.2%; of ASC-H was 59.1%, of HSIL was 90, 0%.

Similarly, the Negative Predictive Value for CIN2+ (NPV CIN2+) of ASC-US p16/Ki67 - was 96.9%, of LSIL p16/Ki67 - was 97.2%.

In Figure 2 and Figure 3, the cytological and histological cases, that expressing the two biomarkers.

**Follow-up**

This study has considered cytological follow up of 387 cases, including 71 high-grade lesions and 316 low-grade lesions, until 31 December 2013, two years after the first diagnosis.

As regards the 71 women with high-grade lesions, excisional or ablative therapeutic interventions to remove the abnormal tissue were made on 69 cases (45 CIN2; 22 CIN3; 2 Endocervical Adenocarcinoma in Situ), according to the screening protocol. All these women have a negative follow up, through periodic checks with vaginal cytology specimens.

Only two women did not undergo treatments because pregnant and their high-grade lesions (CIN2) regressed spontaneously. Both had, as first diagnosis, ASCUS p16/Ki67 -.

In accordance with the screening protocol, the 316 low-grade lesions were followed with periodic checks, without surgical ablative treatment. Their evolution for two
years after the first diagnosis has been studied, evaluating the following outcomes: progression was defined as a histological diagnosis of cervical intraepithelial neoplasia grades 2-3, regression as a negative cytology, and persistence as a cytological result of low-grade squamous intraepithelial lesion.

Overall 10 low-grade lesions progressed to high-grade lesions with a histological diagnosis of CIN2+, 260 regressed spontaneously in two years, and 46 resulted persistent low-grade lesions.

In order to understand the possible significance of p16/Ki67 as markers of progression / regression, we subdivided the 316 low-grade lesions according to the results of Immunohistochemistry on the first cytological specimen and we have separately studied their biological behavior. In this group of 316 cases, we had identified 77 cases p16/Ki67 + at initial cytological analysis and 239 cases p16/Ki67 -.

After two years, among the 77 low-grade lesions p16/Ki67 +, 4 (5.2% of the total of p16/Ki67 +) progressed to CIN2+, 64 (83.1%) regressed and 9 (11.7%) were persistent low-grade lesions.

Similarly the study of the 239 low-grade lesions p16/Ki67 – showed 6 (2.5% of the total of p16/Ki67 -) progressed to CIN2+, 196 (82.0%) regressed and 37 (15.5%) were persistent low-grade lesions (Tab. I).

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Tab. I. Results of the biological behavior of 316 low grade lesions. Follow up of two years.

<table>
<thead>
<tr>
<th></th>
<th>IIC results</th>
<th>TOTAL CASES (% of the total p16/Ki67 results*)</th>
</tr>
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<tbody>
<tr>
<td>Progression to CIN2+ (10 cases)</td>
<td>p16/Ki67 positive</td>
<td>4 (5.2%**)</td>
</tr>
<tr>
<td></td>
<td>p16/Ki67 negative</td>
<td>6 (2.5%****)</td>
</tr>
<tr>
<td>Regression to a normal cytology (260 cases)</td>
<td>p16/Ki67 positive</td>
<td>64 (83.1%***)</td>
</tr>
<tr>
<td></td>
<td>p16/Ki67 negative</td>
<td>196 (82.2%***)</td>
</tr>
<tr>
<td>Low-grade lesion persistent (46 cases)</td>
<td>p16/Ki67 positive</td>
<td>9 (11.7%*)</td>
</tr>
<tr>
<td></td>
<td>p16/Ki67 negative</td>
<td>37 (15.5%***)</td>
</tr>
</tbody>
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* The percentage value refers to the total number of cases CINteo®PLUS positive** or CINteo®PLUS negative***

Fig. 2. Cytological cases expressing the two biomarkers.
Discussion

In the earlier part, the study examines the clinical performance of the p16/ki67 immunostaining in all the cytological diagnoses, as a reflex test of triage HPV-cytology.

The immunocytochemical investigation with CINtec® PLUS, in effect, can increase the sensitivity of a single cytology test for the detection of cervical intraepithelial neoplasia of grade 2 or higher in the different diagnostic categories, by increasing of the Positive Predictive Value for CIN2+ and, even more, the Negative Predictive Value for CIN2+.

In women HPV positive/Pap cytology negative, p16/ki67 dual-stained cytology may identify underlying CIN2+. Many authors consider the dual-stained cytology as a biomarker combination indicative of transforming HPV infections 16, even if the morphology seems still not atypical. In our experience, evaluating the cost / benefit ratio in the cases with negative cytology, the immunocytochemical investigation with CINtec® PLUS can be a valuable support in the clinical picture of atrophy / dystrophy (menopause) in which the differential diagnosis among atypical squamous metaplasia and high-grade lesion needs to be done, avoiding a false negative diagnosis. Similarly, the investigation with the double-staining is not necessary in the cytological diagnosis of ASC-H and HSIL, because the sensitivity of cytology is already high and does not require further examination. Women with ASC-H (atypical squamous cells, high-grade squamous lesion cannot be excluded), HSIL (high grade squamous intraepithelial lesion), or a more severe finding at cytology triage should be referred to colposcopy without further observation or testing 9.

In agreement with literature, the results of this study show that p16/Ki67 dual-staining cytology may be useful in women with cytological diagnosis of ASC-US or LSIL.

In cases p16/ki67+, the Positive Predictive Value for CIN2+ (PPV CIN2+) of ASC-US was 45.5%, of LSIL was 43.2%; of ASC-H was 59.1%, of HSIL was 90.0%, of ASC-H was 59.1%, of HSIL was 90.0%,
with an important percentage variation of +35.6% for ASC-US and +33.3% for LSIL.
In cases p16/ki67-, it seems to be more significant the Negative Predictive Value for CIN2+ (NPV CIN2+) for ASC-US and LSIL that is respectively 96.9% and 97.2%, with a percentage variation of + 6.6% for ASC-US and + 6.5% for LSIL. These values are similar to NPV 96%, referred in the literature. Because of the high NPV, the number of colposcopy could potentially decrease for this group of women, but some authors describe, however, few women, with low-grade lesions p16 negative, which may progress to CIN 3.
In glandular lesions, the double-immunostaining can be an aid in the differential diagnosis among Endocervical Adenocarcinoma in Situ (AIS) and benign endocervical glandular lesions, as well as ProExC. The morphology-independent interpretation of p16/Ki67 dual-staining cytology testing, has a higher reproducibility compared to the p16 single-staining cytology approach, which needs of further morphological interpretation.
In this study, the diagnostic agreement in the interpretation of the results of Immunohistochemistry was 100% between the diagnoses made individually by two or more biologists and pathologists.
Various combinations of cytological, molecular and / or histopathological test results must be integrated in order to determine the risk of an individual woman for precancer / cancer and – according to the level of risk – its proper management.
In the second part of our study, we evaluated the usefulness of p16 / Ki67-staining CIN1 to classify according to its risk of progression / regression in order to plan a personalized follow-up. So we studied the follow-up of two years on a group of 387 women, evaluating the biological behavior of cervical lesions without surgery. Most of high-grade lesions are more likely to persist rather than regress. Cases of spontaneous regression are described among the high-grade lesions p16/ki67-, more frequently among CIN2, indicating the presence of a small subset of HSIL with low proliferative activity. Unfortunately, studies about spontaneous regression of CIN2 are difficult because of surgical treatment (conization) of these cervical lesions. In this study 69 cases high-grade lesions (45 CIN2; 22 CIN3; 2 Endocervical Adenocarcinoma) after surgical treatment, have a negative follow-up, through periodic checks with vaginal cytology specimens.
Only two women did not undergo treatments because pregnant and their high-grade lesions (CIN2) regressed spontaneously. They had a first cytological diagnosis of ASCUS p16/Ki67-.
The study about spontaneous regression of high-grade lesions requires additional clinical trials.
The majority of HR-HPV infection induces low grade lesions, which spontaneously regress, without treatment, within one to two years of exposure and less than 10% eventually progress to high-grade lesion or invasive cancer.

Many studies support a correlation between protein biomarkers, especially p16 expression and distribution pattern into the cell (diffusely or focally positive), and disease progression in low grade lesions. Statistical analyses showed a significant association between diffuse p16 staining and progression to CIN3, as well as between p16 negativity and regression at follow up. Many studies show that CIN1 lesions p16 – rarely progress and may benefit from a less intensive follow up; but few women, with p16 negative expression, may progress to CIN 3.

In our follow up study of 316 low-grade lesions, only 10 lesions progress to high-grade lesions with a histological diagnosis of CIN2 after two years since the first diagnosis. Among these, the percentage of low-grade lesions, which in the first cytological analysis was p16/ki67 + is 5.2%; while the percentage of low-grade lesions p16/ki67 - which has progressed to a high-grade lesion, is 2.5%. 260 cases have a spontaneous regression, without the need for further treatments. The percentage of low-grade lesions, which in the first cytological analysis was p16/ki67 + and which later regress, is not very different from that p16/ki67 -. They are respectively of 83.1% and 82.2%.
The management of persistent lesions isn’t yet standardized. Some women cannot psychologically tolerate periodic controls for a long time, and they prefer a decisive excision treatment, to prevent the development of more serious lesions.
In this study, 46 low-grade lesions are persistent. The different percentages of low-grade lesions, which in the first cytological analysis was p16/ki67 + or p16/ki67 -, slightly higher in cases p16/ki67 -, do not seem significant because of the limited number of cases. p16/ki67 dual-staining cytology testing does not seem to be an indicator for the persistence of the low-grade lesion. Other authors studied the possible correlation between additional biomarkers, such as p16, Ki67, E-cadherin, ProExC but the used biomarkers are not helpful to differentiate between persistent CIN and no persistent lesions. A longer follow-up, perhaps, may be useful to better understand the biological behavior of these lesions.
In the oncogenesis of cervical carcinoma many events are necessary. The induction of chromosomal instability, accumulations of mutation and the status of the individual host’s immune system influence the biological behavior, regression or progression, of cervical lesions. According to literature, we think that currently available data are not yet sufficient to recommend performance of these and other triaging markers for management and follow up of HPV positive women. A review of the emerging evidence and an update of the current recommendations is required in the near future, to reduce over-treatment and plan a personalized follow-up.
References


Case report

Solitary thyroid metastasis from colon cancer: fine-needle aspiration cytology and molecular biology approach

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Key words

Solitary thyroid metastasis • Fine needle aspiration cytology • Colon cancer

Summary

Thyroid gland is one of the most vascularized organs of the body, nevertheless clinical and surgical series report an incidence of secondary malignancies in this gland of only 3%. Colorectal carcinoma metastatic to the thyroid gland is not as uncommon as previously believed, in fact the number of cases seems to be increased in recent years due to the more frequent use of fine-needle aspiration cytology (FNAC) guided by ultrasonography. Although kidney, breast and lung metastases to the thyroid are frequent, metastasis from colon cancer is clinically rare with 52 cases reported in the literature in the last 5 decades and three cases described as solitary thyroid metastasis from the colon cancer without any other visceral metastases.

To the best of our knowledge, we report the fourth case of solitary, asymptomatic thyroid metastasis from colon cancer without involvement of other organs. We discuss the importance of FNAC to detect metastasizing process as a compulsory step of the diagnostic and therapeutic management algorithm, combined with a molecular biology approach. A review of the last 5 decades literature, to update the number of cases described to date, is also included.

Introduction

Colon cancer is one of the most common cancers with a high propensity to metastasize to the regional lymph nodes, the liver and the lung. Colon cancer metastasis to the thyroid gland is uncommon. In fact, they have been assessed originating mainly from renal, lung and breast carcinoma. In this regard, routine evaluation of thyroid nodules by FNAC in patients with primary known cancer may increase detection of metastatic lesions with increasing frequency. In this report we describe a case of colon carcinoma metastatic to the thyroid gland without involvement of other organs, diagnosed by FNAC 26 months from the primary malignancy. Combined cytology with histology, immunostaining and molecular biology analysis has been the best and complete diagnostic and therapeutic approach to the metastatic disease.

Case report

In January 2010, a 51-year-old woman underwent a left colectomy. A moderately differentiated colon adenocarcinoma was histologically diagnosed (pT3N2a). From March 2010 to August 2011 the patient received 12 cycles of chemotherapy with FOLFOX4 (Oxaliplatin + 5 fluorouracil + folic acid). The patient has been free from disease for 26 months when she noted a slight enlargement of the right lobe of the thyroid so that in October 2013 she underwent a routine ultrasound examination that revealed a solid and hypoechogenic nodule with an unclear border, measuring 10 mm in maximum diameter. Enlargement of cervical lymph nodes was not evident. The laboratory data revealed no abnormalities in CEA levels and in thyroid function. Ultrasonography-guided FNAC of the thyroid nodule was performed using a 22-gauge needle attached to a disposable 10-ml plastic syringe. Thyroid aspiration cytology was hypercellular and showed...
Solitary thyroid metastasis from colon cancer: fine-needle aspiration cytology and molecular biology approach

Clusters of malignant tall columnar cells with a high degree of cell overlapping with some colloid globules and necrosis intermingled to neoplastic cells (Fig. 1a). Based on these cytological features and the clinical history, a diagnosis of thyroid metastasis from colon cancer was formulated. At the end of March 2014, a total thyroidectomy was performed. On gross examination, the right lobe of the thyroid was slightly enlarged and the cut surface showed a whitish, solid nodule. Microscopically, the malignant cells showed cribriform glandular structures covered by cuboidal cells (Fig. 1b). The immunohistochemical positivity for Cytokeratin 20 (CK20) (Fig. 1c) and CDX2 (Fig. 1d), associated to the negativity for TTF1 and Cytokeratin 7 (CK7) confirmed the intestinal etiology as diagnosed by FNAC. Tissue samples from primitive (colon) and metastatic (thyroid) tumors were microdissected and DNA purification was performed using QIAamp DNA FFPE Tissue Kit (Qiagen) according manufacturer’s instructions. Molecular analyses revealed the presence of the same p.G13D mutation (G > A) in codon 13 of exon 2 of KRAS gene in both tumors, determining the substitution of a Glycine with an Aspartic Acid, in both surgical specimens (Fig. 2). According to this molecular data, following surgery, the patient underwent chemotherapy with FOLFIRI (irinotecan + 5 fluorouracile + folinic acid) without the addiction of cetuximab. The post-operative course was uneventful and the patient is still alive 18 months after thyroidectomy, without any other evidence of disease.

Discussion

The number of clinical papers reporting carcinomas metastatic to the thyroid is extremely limited. In 1960, among the 58 cases collected by Elliot ¹, the most common metastatic tumor to the thyroid gland was renal carcinoma (33 cases) followed, in order, by gastrointestinal tract, lung and breast cancer.

According to a report by Wychulis et al ², in 1964 there were only 10 cases (0.05 %) of tumor metastasis to the thyroid out of 20,262 cases involving thyroid surgery. In 1987 Ivy ³ reported the Mayo Clinic statistics, accord-
ing to which, only 30 cases of thyroid metastasis were observed over a 36 years period. Most of these cases were renal, breast, and lung cancers, which had a similar rate of occurrence. According to Mayo Clinic reports\(^4\), in 1997 43 cases were observed in a 10 years period, and the most frequent primary tumors were again renal, breast, and lung cancer. Conversely, there are few clinical reports of metastasis of colon cancer to the thyroid. Regarding the reason why thyroid metastasis from other primitive tumors are generally uncommon, two hypotheses were formulated by Willis\(^5\) and supported for a long time. One “mechanical” hypothesis refers to the extremely abundant supply of arterial blood to the thyroid, and its fast blood flow, which make difficult the adhesion and implantation of tumor cells in the gland. Another “chemical” explanation could be that the high oxygen saturation and high iodine content of thyroid tissues inhibits the growth of tumor cells. Regarding the latter, Smith et al.\(^6\) argued that, when an organic disorder exists in the thyroid tissue, a decrease in arterial blood flow and a low-oxygen or low-iodine state results, thus increasing the susceptibility to metastasis. Also, the route of metastasis is believed to be haematogenous, through portal, pulmonary, and large veins, therefore metastasis to the lungs or liver often occur prior to thyroid metastasis, although the latter sometimes occur directly without prior involvement of lung or liver.

As a route in this case, the existence of a vertebral venous system has long been suggested, and this theory can explain metastasis to the thyroid from mammary glands, kidneys or pelvic organs\(^7\). Thyroid gland metastases from colonic carcinoma are clinically rare probably because FNAC guided by ultrasonography was not used as preoperative diagnostic procedure in past years. The number of clinical cases with metastasis of colon cancer to the thy-

**Fig. 2.** Sequencing electropherograms (forward + reverse) of colon cancer and metastasis to the thyroid gland showing the mutation in codon 13 of the KRAS gene.
<table>
<thead>
<tr>
<th>No.</th>
<th>Reported year and authors</th>
<th>Age/Sex</th>
<th>Primary site</th>
<th>Free interval after colectomy</th>
<th>FNA</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CURRENT CASE</td>
<td>51/F</td>
<td>LC</td>
<td>45 m</td>
<td>Done</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>COZZOLINO et al.²² 2010</td>
<td>66/M</td>
<td>SC-R</td>
<td>6</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>HYUN et al.²³ 2010</td>
<td>59/F</td>
<td>AC</td>
<td>6 m</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>YOUN et al.²² 2006</td>
<td>85/M</td>
<td>R</td>
<td>21 m</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>CAVANNA et al.²² 2006</td>
<td>55/F</td>
<td>SC</td>
<td>24 m</td>
<td>Done</td>
<td>2 DoD</td>
</tr>
<tr>
<td>6</td>
<td>MATTAVELLI et al.²³ 2006</td>
<td>52/F</td>
<td>R</td>
<td>30 m</td>
<td>Done</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>HANNA et al.²³ 2006</td>
<td>28/F</td>
<td>COLON NOS</td>
<td>-</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>FADARE et al.²³ 2005</td>
<td>59/F</td>
<td>SC</td>
<td>-</td>
<td>Done</td>
<td>Alive</td>
</tr>
<tr>
<td>9</td>
<td>RIDDLE et al.¹¹ 2005</td>
<td>75/F</td>
<td>SC</td>
<td>85 m</td>
<td>Not Done</td>
<td>12 DoD</td>
</tr>
<tr>
<td>10</td>
<td>HAKER et al.¹² 2005</td>
<td>77/M</td>
<td>-</td>
<td>84 m</td>
<td>Not Done</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>KOKLU et al.¹³ 2005</td>
<td>64/F</td>
<td>Concurrent</td>
<td>Done</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>MALANI et al.¹⁴ 2005</td>
<td>49/F</td>
<td>R</td>
<td>12 m</td>
<td>Not Done</td>
<td>7 DoD</td>
</tr>
<tr>
<td>13</td>
<td>PHILLIPS et al.¹⁵ 2005</td>
<td>81/F</td>
<td>COLON NOS</td>
<td>-</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>POON et al.¹² 2004</td>
<td>64/M</td>
<td>R</td>
<td>12 m</td>
<td>Done</td>
<td>18 DoD</td>
</tr>
<tr>
<td>15</td>
<td>FLUITA et al.¹¹ 2004</td>
<td>28/F</td>
<td>R</td>
<td>Concurrent</td>
<td>Done</td>
<td>6 DoD</td>
</tr>
<tr>
<td>16</td>
<td>WITT et al.¹⁰ 2003</td>
<td>71/M</td>
<td>R</td>
<td>7 m</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>ROSSI et al.³ 2003</td>
<td>42/M</td>
<td>LC</td>
<td>-</td>
<td>Not Done</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>PERINU et al.¹¹ 2003</td>
<td>43/M</td>
<td>LC</td>
<td>24 m</td>
<td>Not Done</td>
<td>Alive</td>
</tr>
<tr>
<td>19</td>
<td>KUMAMOTO et al.⁸ 2003</td>
<td>66/F</td>
<td>AC</td>
<td>3 m</td>
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<td>4 AWD</td>
</tr>
<tr>
<td>20</td>
<td>YAMADA et al.²⁰ 2003</td>
<td>60/F</td>
<td>R</td>
<td>3 m</td>
<td>Done</td>
<td>4 DoD</td>
</tr>
<tr>
<td>21</td>
<td>OOSAWA et al.⁸ 2002</td>
<td>58/M</td>
<td>R</td>
<td>-</td>
<td>Done</td>
<td>-</td>
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<td>22</td>
<td>AKIMARU et al.⁸ 2002</td>
<td>67/M</td>
<td>AC</td>
<td>6 m</td>
<td>Done</td>
<td>4 DoD</td>
</tr>
<tr>
<td>23</td>
<td>KANAYA et al.¹¹ 2001</td>
<td>80/F</td>
<td>AC</td>
<td>1,5 m</td>
<td>Done</td>
<td>OTHER DEATH</td>
</tr>
<tr>
<td>24</td>
<td>BOLEAS et al.¹¹ 2001</td>
<td>80/F</td>
<td>COLON NOS</td>
<td>7 m</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>SHIGA et al.¹² 2001</td>
<td>36/F</td>
<td>R</td>
<td>3 m</td>
<td>Done</td>
<td>2 AUVÉ</td>
</tr>
<tr>
<td>26</td>
<td>SHINOHARA et al.² 2000</td>
<td>66/F</td>
<td>TC</td>
<td>4 m</td>
<td>Not Done</td>
<td>4 AWD</td>
</tr>
<tr>
<td>27</td>
<td>KIM et al.⁸ 1999</td>
<td>68/F</td>
<td>SC</td>
<td>2 m</td>
<td>Done</td>
<td>6 AWD</td>
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<tr>
<td>28</td>
<td>TAZUKE et al.¹³ 1998</td>
<td>61/F</td>
<td>R</td>
<td>2 m</td>
<td>Not Done</td>
<td>Alive</td>
</tr>
<tr>
<td>29</td>
<td>TAKASHIMA et al.¹³ 1998</td>
<td>67/M</td>
<td>R</td>
<td>2 m</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>NAKAMURA et al.²² 1997</td>
<td>70/F</td>
<td>AC</td>
<td>5 m</td>
<td>Done</td>
<td>6 DoD</td>
</tr>
<tr>
<td>31</td>
<td>YOSHIMATSU et al.¹⁹ 1996</td>
<td>50/F</td>
<td>AC</td>
<td>0,5 m</td>
<td>Done</td>
<td>5 DoD</td>
</tr>
<tr>
<td>32</td>
<td>MASUDA et al.¹⁹ 1996</td>
<td>38/F</td>
<td>R</td>
<td>4 m</td>
<td>-</td>
<td>5 DoD</td>
</tr>
<tr>
<td>33</td>
<td>MASUDA et al.¹⁹ 1996</td>
<td>73/M</td>
<td>R</td>
<td>1 m</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>34</td>
<td>OSIN et al.¹² 1996</td>
<td>70/F</td>
<td>SC</td>
<td>Concurrent</td>
<td>Done</td>
<td>AWD</td>
</tr>
<tr>
<td>35</td>
<td>MASE et al.¹³ 1993</td>
<td>-</td>
<td>SC</td>
<td>4 m</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>SHIBUTANI et al.²² 1992</td>
<td>52/F</td>
<td>SC</td>
<td>3 m</td>
<td>Done</td>
<td>8 DoD</td>
</tr>
<tr>
<td>37</td>
<td>MAEDA et al.²² 1992</td>
<td>66/F</td>
<td>AC</td>
<td>1 m</td>
<td>Done</td>
<td>2 AWD</td>
</tr>
<tr>
<td>38</td>
<td>MATSUSAKO et al.¹ ¹ 1991</td>
<td>78/F</td>
<td>AC</td>
<td>1 m</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>39</td>
<td>JINGU et al.¹ ² 1990</td>
<td>48/F</td>
<td>R</td>
<td>2,5 m</td>
<td>-</td>
<td>10 DoD</td>
</tr>
<tr>
<td>40</td>
<td>CRISTALLINI et al.¹ ² 1990</td>
<td>64/F</td>
<td>-</td>
<td>4 m</td>
<td>Done</td>
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</tr>
<tr>
<td>41</td>
<td>MESKO et al.¹ ² 1990</td>
<td>59/F</td>
<td>R</td>
<td>2 m</td>
<td>Done</td>
<td>-</td>
</tr>
<tr>
<td>42</td>
<td>RICAUD et al.¹ ² 1987</td>
<td>68/F</td>
<td>-</td>
<td>2 m</td>
<td>-</td>
<td>3 DoD</td>
</tr>
<tr>
<td>43</td>
<td>RICAUD et al.¹ ² 1987</td>
<td>77/M</td>
<td>R</td>
<td>4 m</td>
<td>-</td>
<td>1 DoD</td>
</tr>
<tr>
<td>44</td>
<td>LESTER et al.¹ ² 1986</td>
<td>55/F</td>
<td>AC</td>
<td>2,5 m</td>
<td>Not Done</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>IVY et al.¹ ² 1984</td>
<td>72/M</td>
<td>-</td>
<td>192 m</td>
<td>Not Done</td>
<td>&lt; 12 DoD</td>
</tr>
<tr>
<td>46</td>
<td>ITO et al.¹ ² 1983</td>
<td>34/M</td>
<td>R</td>
<td>2 m</td>
<td>Done</td>
<td>7 DoD</td>
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<tr>
<td>47</td>
<td>ISHIDA et al.¹ ² 1982</td>
<td>70/F</td>
<td>R</td>
<td>Concurrent</td>
<td>Not Done</td>
<td>3 DoD</td>
</tr>
<tr>
<td>48</td>
<td>MAKE et al.¹ ² 1974</td>
<td>68/M</td>
<td>SC</td>
<td>4 m</td>
<td>Done</td>
<td>8 DoD</td>
</tr>
<tr>
<td>49</td>
<td>WYCHULIS et al.¹ ² 1964</td>
<td>37/F</td>
<td>R</td>
<td>0,5 m</td>
<td>-</td>
<td>10 DoD</td>
</tr>
<tr>
<td>50</td>
<td>ELLIOTT et al.¹ ² 1960</td>
<td>56/F</td>
<td>R</td>
<td>Concurrent</td>
<td>-</td>
<td>3 DoD</td>
</tr>
<tr>
<td>51</td>
<td>SKLAROFF et al.¹ ² 1954</td>
<td>73/F</td>
<td>R</td>
<td>7 m</td>
<td>-</td>
<td>2 DoD</td>
</tr>
</tbody>
</table>

NOT AVAILABLE; F: female; M: male; m: months; AWD: alive with disease; DoD: dead of disease; FNA: fine-needle aspiration; RC: right colon; LC: left colon; AC: ascending colon; R: rectum; TC: transverse colon; SC: sigmoid colon
roid, to the best of our knowledge, was about 52 (obtained by systemic review of publication), including the present one (Tab. 1) 1-23. For our diagnosis, FNAC was performed similarly to ordinary thyroid testing, and was considered a useful procedure because it could diagnose metastasis from colon cancer as other cases. Therefore, the final treatment policy should be determined considering the level of metastasis to other organs, the patient’s general condition, the presence or absence of local pressure symptoms, etc. In the present case, there was no evidence of metastases other than in the thyroid, so the thyroid was surgically ablated to reduce the dissemination and prevent local pressure symptoms that were expected to occur in the future. Although thyroid gland metastasis from gastrointestinal tract cancer is mainly associated with the spread to other organs, in our case the presence of a solitary metastasis supports the theory of isolated spread to the thyroid via vertebral venous system bypassing the lung and the liver and associated with a better prognosis for the patient, as in our case. Moreover it is important to keep in mind that the thyroid gland can be a site of metastases for a variety of tumors when evaluating a thyroid nodule 24-26, especially in a patient with a prior history of malignancy. For this reason physician should consider the possibility of thyroid gland metastasis when he is performing routine follow-up examinations for recurrence of colorectal cancer until proven otherwise. In the presence of a thyroid nodule, the first approach to the diagnosis must be the use of FNAC guided by ultrasonography. In fact the number of cases seems increased in recent years and this increase may be related to more frequent use of FNAC in any suspected case. The management of thyroid metastases should depend on the individual situation, FNAC helps to evaluate the right treatment avoiding unnecessary thyroidectomy in patients with a poor prognosis. In our case combined cytology together with histology, immunostaining and molecular biology analysis, has proved to be the most complete and efficient diagnostic and therapeutic approach to improve the disease free survival. In medical centers with a wide range of clinical cases and a consolidated experience, FNAC remains a fundamental tool and plays a pivotal role in the diagnostic management and in the follow-up of malignancies because it is sensitive, repeatable, not invasive, and inexpensive. Finally molecular biology analysis performed on cytological specimen could help for an immediate therapeutic approach.

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**CASE REPORT**

**Tuberous sclerosis: histological analysis with confocal laser scanning microscope of gingival angiofibromatosis**

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**Key words**

Tuberous Sclerosis Complex • Confocal Laser Scanning Microscopy • Diffuse Angiofibromatosis • Diode Laser

**Summary**

**Case report.** A 14-years-old female patient with TS diagnosis came to our attention for diffuse gingival hyperplasia on the mandible. Clinical examination highlighted epidermal hamartomas on the whole body, especially on the face and scalp. Pathologic hyperplastic tissue was removed by pulsed diode laser at the power of 5-6W, and the surgical samples were sent for conventional and CLSM histopathological examination. After laser excision, wounds healed quickly without complications. At CLSM examination collagen fibres, showing intense fluorescence and with variable spatial orientation, and variably sized blood vessels were noticed suggesting the diagnosis of gingival angiofibromatosis, a still unreported finding in TS patients.

**Conclusions.** CLSM analysis allows to highlight some unusual histopathological features of TS; diode laser is very effective for the treatment of gingival angiofibromatosis.

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**Introduction.** Tuberous sclerosis (TS), also known as Epiloia or Bourneville-Brissaud syndrome, is a rare multisystemic disease characterized by hamartomas in various organs, mainly affecting skin and central nervous system. The most common features of TS include facial angiofibromas, hypomelanotic cutaneous macules, shagreen patches in the lumbar area, cerebral cortical tubers, subependymal nodules, subependymal giant cell astrocytomas, cardiac rhabdomyomas, and renal angiomyolipomas. Frequently oral manifestations such as fibrous hyperplasia, angiofibromas and dental enamel pitting are also observed. The aim of this case report was to describe the histological aspects of oral diffuse hyperplastic angiofibromatosis, never reported in the English literature and analyzed by Confocal Laser Scanning Microscope (CLSM), and to highlight the surgical implications of these aspects such as use of Diode Laser.

**Case report.** A 14-years-old female patient with TS diagnosis came to our attention for diffuse gingival hyperplasia on the mandible. Clinical examination highlighted epidermal hamartomas on the whole body, especially on the face and scalp. Pathologic hyperplastic tissue was removed by pulsed diode laser at the power of 5-6W, and the surgical samples were sent for conventional and CLSM histopathological examination. After laser excision, wounds healed quickly without complications. At CLSM examination collagen fibres, showing intense fluorescence and with variable spatial orientation, and variably sized blood vessels were noticed suggesting the diagnosis of gingival angiofibromatosis, a still unreported finding in TS patients.

**Conclusions.** CLSM analysis allows to highlight some unusual histopathological features of TS; diode laser is very effective for the treatment of gingival angiofibromatosis.

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surgical implications of these aspects such as the use of Diode Laser.

Case report

A 14-years-old female patient, who had been previously diagnosed with TS at 7 months of age, was referred to the Complex Operating Unit of Odontostomatology of the University of Bari. The diagnosis of sporadic TS had been formulated based on the patient’s clinical history showing seizures, mental retardation, and cardiac anomalies. Phenobarbital, Levetiracetam and Rufinamide were administered to control epileptic seizures.

General clinical examination highlighted epidermal hamartomas on the whole body, especially on the forehead and scalp (Fig. 1), while intra-oral examination showed poor oral hygiene and diffuse mandibular gingival enlargement; the hyperplastic gingival tissue involved not only anterior sector, as generally reported in literature, but the molar region as well. The hyperplastic gums appeared pale pink, and covered the corresponding teeth until the occlusal surface or the incisal margins. Orthopantomographic X-rays showed a radiolucent lesion in the region of 3.6 (Fig. 2).

Pathologic hyperplastic tissue removal was made under general anaesthesia with concurrent local infiltration of mepivacaine, using a diode laser in pulsed mode (t-on 300ms/ t-off 400ms), at the power of 5-6W; no sutures were applied on the treated areas (Fig. 3).

The post-operative course was uneventful and gingivectomy allowed for better oral hygiene and prevented complications such as bleeding, dental inclusions and retentions.

Conventional histological examination showed thickened and acanthotic epithelium with elongated rete ridges, densely packed, whirly collagen fibres, fibroblasts, and variably sized vascular structures, without chronic inflammatory cells. At CLSM examination, the collagen fibers, showing intense fluorescence, also manifested variable spatial orientation, due to cross-links among the bundles, typical of fibromatosis. Also, variably sized blood vessels and large and polygonal interstitial cells displayed fluorescence of lower intensity were noticed. The vascular component consisted of small groups of venous-like structures, frequently showing dilated lumina, thin walls and plump endothelial lining (Fig. 4). These findings were consistent with periodontal angiofibromatosis.

The parents of the patient released informed consent for the use of such data for scientific publication, and on di-
agnostic and therapeutic procedures; this study was performed in accordance to the principles of the Declaration of Helsinki and has been approved by our institution ethical committee (Study n° 4576 – Prot. 1443/C.E.).

Discussion

TS is an autosomal dominant neurocutaneous syndrome, generally diagnosed in the first 15 months of life, which manifests with great phenotypic variability. This disease was first described by von Recklinghausen in 1862 and more recently, by Bourneville, Pringle, and Vogt 4.

TS is caused by inactivating mutations of Tuberous Sclerosis Complex 1 (TSC1) and Tuberous Sclerosis Complex 2 (TSC2), which encode for “hamartin-tuberin complex” acting as tumour suppressor genes. One-thirds of TS cases are inherited from an affected parent and de novo mutations have been implicated in two-thirds of all cases. Affected patients with familial TS may show phenotype variability due to genetic mosaicism 2.

The TSC phenotype is widely variable with some pathognomonic features present at birth and others developing later during the patient’s life. The second International Tuberous Sclerosis Complex Consensus Conference (Washington 2012) 2 revisited the clinical diagnostic criteria published subsequent to the first International TS Consensus Conference in 1998 5-7.

The Table I reports the major and minor TS diagnostic criteria; the diagnosis is definite when 2 major features or 1 major feature with ≥2 minor features are present or after the identification of a TSC1 or TSC2 pathogenic mutation; possible when 1 major feature or ≥2 minor features 2.

Ventricular tachycardia, paroxysmal arrhythmia, Wolff-Parkinson-White syndrome, epileptic crises, autism, learning disorders, abnormal behaviour, dyspnea and spontaneous pneumothorax are all possible consequences of the presence of the lesions in different organs 8-10.

TS oral manifestations are quite frequent and consist of benign, well-delimited, fibrous nodules of the anterior gingiva, lips, palate, or tongue, variably known as gingival fibromas, oral angiofibromas, or oral fibrous papules 2 11. These lesions tend to appear in late childhood, can be normal-colored, as gingival fibromatosis, or red, suggesting a reactive lesion such as pyogenic granuloma.

Although a prevalence of 11% has been reported, the real frequency of these lesions may be significantly higher. Lygidakis and Lindenbaum 12 found oral fibromas in 46% of TS patients, while Araujo et al 4 detected angiofibromas of the anterior region of the gums, dorsal tongue, buccal mucosa, and lip in 5 patients.

It has been suggested that oral fibromas might not be directly related to TS but rather resulting from drugs ad-

<table>
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<th>Tab. I. Tuberous Sclerosis Diagnostic Criteria 2.</th>
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<td><strong>Genetic diagnostic criteria:</strong></td>
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<td>The identification of either a TSC1 or TSC2 pathogenic mutation in DNA from normal tissue</td>
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<td><strong>Clinical diagnostic criteria:</strong></td>
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<td><strong>Major features</strong></td>
</tr>
<tr>
<td>1. Hypomelanotic macules (≥3, at least 5-mm diameter)</td>
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<td>2. Angiofibromas (≥3) or fibrous cephalic plaque</td>
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<td>3. Ungual fibromas (≥2)</td>
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<td>4. Shagreen patch</td>
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<td>5. Multiple retinal hamartomas</td>
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<td>6. Cortical dysplasias</td>
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<td>7. Subependymal nodules</td>
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<td>8. Subependymal giant cell astrocytoma</td>
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<td>9. Cardiac rhabdomyoma</td>
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<td>10. Lymphangioleiomyomatosis (LAM)</td>
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<td>11. Angiomyolipomas (≥2)</td>
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<td><strong>Minor features</strong></td>
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<td>1. “Confetti” skin lesions</td>
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<tr>
<td>2. Dental enamel pits (&gt;3)</td>
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<tr>
<td>3. Intracranial fibromas (≥2)</td>
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<td>4. Retinal achromic patch</td>
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<td>5. Multiple renal cysts</td>
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<td>6. Nonrenal hamartomas</td>
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ministration for the neurological manifestations of the disease. At this regard, phenytoin, often administered to control epileptic seizures in such patients, is a well known inducer of gingival hyperplasia. Lygidakis found oral fibromas in up to 46% of patients with TS, many of whom had never received phenytoin or any other type of anti-seizure medication. Consequently, gingival fibromas have been thereafter considered as typical clinical signs of TS, especially when affecting multiple sites, such as the tongue, palate or lips. Damm et al. reported maxillary bone involvement with the presence of fibrous tumors in TS. Furthermore, dental enamel pitting is observed in up to 100% of patients with TS. Dental pits can be observed in the general population, but at lower frequencies and with fewer lesions than in TS.

In our study, the patient showed pale pink enlargements of anterior and posterior lower gums and, in view of her therapeutic regimen including Phenytoin, Levetiracetam and Rufinamide but not phenytoin, her gingival hyperplasia was considered unrelated to treatment. In the current case, gingival outgrowth caused teeth displacement, spacing, bleeding and bad oral hygiene; consequently, gingivectomy and gingivoplasty were performed by pulsed diode laser that also allows effective haemostasis and coagulation without bleeding, as well as an excellent incision, due to its penetration in depth, estimated around 0.5–3 mm. The clotting capability of the diode laser provided a clear view of the surgical site and a precise extension of the incision. Hence, this procedure may be considered very effective for oral soft tissue surgery. Moreover, other advantages are reduced necessity of anaesthesia and faster wound healing, with less discomfort for the patient. Furthermore laser excision does not induce tissue artifacts, retractions, deformation or detachment.

The surgical specimen underwent conventional histological examination that showed thickened and anodontic epithelium with elongated rete ridges, densely packed, whirly collagen fibres, fibroblasts, and variably sized vascular structures, without chronic inflammatory cells.

CLSM analysis showed variably sized blood vessels and large and polygonal interstitial stem-like cells displaying fluorescence of lower intensity which may represent a distinctive intra-oral alteration of TS. The vascular component consisted of small groups of venous-like structures with dilated lumina among the collagen fibres with variable spatial orientation, typical of fibromatosis, thus suggesting the diagnosis of hyperplastic angiofibromatosis. The presence of the polygonal interstitial stem-like cells in association with the lack of chronic inflammation confirmed that angiofibromatosis could not be considered a reactive event, but a structural feature of the disease. This pathologic vascular component suggests the use of diode laser photocoagulation in order to reduce lesions dimensions before laser excision.

Conclusions

This case report represents a never-reported clinical finding of TS, periodontal angiofibromatosis, that could help to achieve the correct diagnosis of TS in patients with an equivocal clinical presentation. The diagnosis was obtained thanks to CLSM histological examination allowing the identification of additional features, such as low fluorescence areas and a typical vascular component. The presence of the vascular component confirmed the effectiveness of diode laser for the treatment of such lesions in view of cutting precision, clotting capability, without intra- or post-operative complications and faster wound healing.

ABBREVIATIONS

TS, Tuberous Sclerosis; TSC, Tuberous Sclerosis Complex; CLSM, Confocal Laser Scanning Microscopy.

References
An incidentally diagnosed epithelioid trophoblastic tumor in hysterectomy

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Key words
Gestational trophoblastic disease • Epithelioid trophoblastic tumor • Immunohistochemistry • Placental site nodule • Differential diagnosis

Summary
Epithelioid trophoblastic tumor is a rare non-molar gestational trophoblastic disease. A 40-year-old multiparous woman was incidentally diagnosed with epithelioid trophoblastic tumor after hysterectomy. Hysterectomy specimen revealed multiple small, tan to yellow nodules measuring 0.3-0.8 cm just below the endometrium. In the microscopic examination uniform neoplastic cells with varying cellularity were accompanied by necrotic zones and eosinophilic hyaline material. Immunohistochemically neoplastic cells were diffusely stained with CK 7, inhibin-alpha, p63, hPL, and CD146. There was no staining with beta-HCG, SMA, PLAP, or h-caldesmon. Ki-67 proliferative index was approximately 10 % and cyclin E was stained in approximately 10 % of the neoplastic cells. Although immunohistochemical studies are helpful in classifying gestational trophoblastic lesions, borderline values can cause diagnostic confusion between neoplastic and reactive lesions, particularly in inadequate endometrial biopsies.

Introduction
Gestational trophoblastic diseases can be categorized into molar and non-molar lesions. Molar lesions are complete hydatidiform, partial hydatidiform mole and invasive mole. In contrast, exaggerated placental site (EPS), placental site nodule (PSN), choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT) are non-molar lesions 1. The PSN and EPS are benign non-neoplastic trophoblastic lesions of intermediate trophoblasts, whereas the ETT and PSN are malignant lesions arising from intermediate trophoblasts. Since the initial description by Shih and Kurman in 1998, only less than 100 cases of epithelioid trophoblastic tumor (ETT) were reported in the literature 2-3. Pathogenesis of these trophoblastic lesions is not clear; however, previous studies suggested that the PSN and ETT arise from chorionic intermediate trophoblasts, and the PSTT and EPS arise from implantation site trophoblasts. Since the PSN and ETT share a common origin, it has been proposed that the PSN can be the precursor lesion of the ETT 4.

In the present study, a case of ETT incidentally diagnosed after hysterectomy is reported. The case had common morphologic features with PSN and common immunohistochemical features with PSTT. We discussed the value of immunohistochemistry in differential diagnosis and pathogenesis of ETT.

Case report
A 40-year-old multiparous (G5P5) woman was admitted to our outpatient clinic with symptoms of chronic pelvic pain and intermittent vaginal spotting. She had been suffering from spotting for 5 months. Her last delivery was uncomplicated after full term pregnancy 2 years ago. Gynecologic examination revealed an enlarged uterus in three month-gestational size and the ultrasonography...
showed multiple intramural leiomyomas ranging from 2 to 3 cm in diameter. The serum β-hCG level was not determined preoperatively. Since the patient reported failure of medical therapy to alleviate her symptoms, surgical intervention was planned. The patient underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy.

Hysterectomy specimen was measured 160 grams with dimensions of 15x10x5 cm. The macroscopic evaluation revealed multiple small, tan to yellow nodules measuring 0.3-0.8 cm, localized to a certain area in the uterine corpus without any relation to endocervix. These tumor nodules were in the endometrium and the tumor superficially invaded the myometrium (Fig. 1A). Microscopic appearance of the tumor and the relation of the neoplasm with the endometrium and myometrial invasion can be seen in Figure 1B. Endometrial thickness was 0.1 cm and multiple intramural leiomyomas were also recorded. No other pathology was observed in the uterus or the ovaries.

In the microscopic evaluation, there were varying sizes of nodules consisting of large uniform polygonal cells with mononucleated eosinophilic cytoplasms (Fig. 2A-C). Uniform neoplastic cells with a clear cytoplasm and a few multinucleated neoplastic cells were also present. Most of the nodules were found to be highly cellular; however, some were less cellular (Fig. 2C). Nodules generally had a well-delineated pushing border (Fig. 2A), but focal infiltrating zones between smooth muscles were also present. Large necrotic zones and eosinophilic hyaline material were accompanying the lesion (Fig. 2B). Mitotic activity was less than 1 in 10 HPF and there was no lympho-vascular invasion. Although lesions were localized in the endometrium, superficial myometrial invasion was also present (Fig. 2A).

Morphologic features resembled intermediate trophoblastic lesions as well as an epithelioid leiomyoma. In the immunohistochemical studies, neoplastic cells were diffusely stained with CK 7 (invitrogen 1/75), inhibin-alpha (Novocastra 1/100), p63 (Biocare 1/100), hPL (Therma 1/250), and CD146 (Mel-CAM) (Novo 1/20). There was no staining with beta-HCG (Therma...
An incidentally diagnosed epithelioid trophoblastic tumor in hysterectomy

Epithelioid trophoblastic tumor is a very rare pathological entity with limited number of reported cases in the literature. Most of the reported cases are in reproductive period and commonly present with vaginal bleeding. The age of the patients is between 15 and 48 years with a mean of 36.1 years. Tumor mostly ensues after a full term pregnancy. It can also follow spontaneous abortion and hydatidiform mole. The presented patient was in late reproductive years and multiparous. She had symptoms of pelvic pain and vaginal spotting similar to the cases reported in the literature. The gestational event may be related to the development epithelioid trophoblastic tumor was a full term pregnancy 2 years ago. The interval between the gestational event and the development of the tumor was reported 1-18 years with a mean 6.2 years in previous studies.

Serum human chorionic gonadotropin levels are usually elevated in the epithelioid trophoblastic tumor, but in contrast to choriocarcinoma, levels generally do not exceed 2,500 mIU/ml. An increased preoperative β-hCG level can be a predictor of a large tumoral mass. However, preoperative value of β-hCG was not available in the presented case. Since the tumoral lesion was in microscopic size at pathological examination, an increased level of β-hCG was not expected. Although macroscopic examination of the hysterectomy specimen is very important for small focal ETTs, the case we presented here had macroscopic small, unremarkable tumor nodules located in the uterine corpus. Incidental tumor nodules can easily be overlooked without careful inspection.

The ETT is composed of mononuclear intermediate trophoblasts and has a nodular proliferation pattern. Although the intermediate cells of the tumor containing eosinophilic, hyaline-like material and necrotic debris that form nests and cords is characteristic, microscopic features can resemble a squamous cell carcinoma or an epithelioid leiomyoma. Immunohistochemistry can be very helpful for the differential diagnosis in most cases. Epithelioid trophoblastic tumors are positive for immunohistochemical markers such as cytokeratins, epithelial membrane antigen, hPL, p63, and also inhibin-alpha. In our case, the immunohistochemical staining resulted in diffuse positivity with inhibin, CK7, and p63; and focal staining with hPL and CD146. This result proves the trophoblastic nature of the lesion, and points the chorionic intermediate trophoblasts as the origin.

The PSN is supposed to be the precursor lesion of the ETT and has similar morphologic and immunohistochemical features with ETT. Sometimes it can be hard to differentiate these two lesions. Placental site nodule do not contain necrosis, it is less cellular with sharp borders and has a low proliferative index which is generally <10%. Epithelioid trophoblastic tumors usually have a Ki-67 index between 10% and 25%. The presented case had both morphologic and immunohistochemical features indicating both an ETT and a PSN. Having cellular large nodules with tumor cells infiltrating the myometrium with focal calcifications supported the ETT diagnosis. Sometimes cyclin E can be helpful for the differential diagnosis of PSN and ETT. Although, our case had characteristic morphologic fetaures for ETT showing cellular large nodules with tumor cells infiltrating the myometrium with focal calcifications and necrosis, immunohistochemical studies showed borderline values for Ki-67 and cyclin E. The pathologist must be aware of the variability of these markers, particularly when working on inadequately small endometrial biopsies. The other related issue is a new entity called ‘atypical epithelioid trophoblastic lesion’ which was described for lesions that have borderline microscopic Ki-67 and cyclin E features between PSN and ETT. Although some suggestions exist that the ETT can develop from a placental site nodule, all nodules observed in the pre-
sented case were larger than the nodules seen in the PSN and showed the characteristic features of ETT. Epitheloid trophoblastic tumors are generally benign in nature, and hysterectomy or local excision of the tumor can be the sufficient treatment. Chemotherapeutic agents used for the treatment of other GTDs may not be useful for the treatment of ETTs, but the rates of metastasis and death are 25% and 10%, respectively. Lung, liver and vagina are the three most common metastatic organs. Multifocal disease in uterus, serosal involvement, necrosis, high mitotic index, cytologic atypia, and vascular invasion are all signs of a poor prognostic, metastatic ETT. In our patient the mitotic index and Ki-67 index were low, and the β-hCG level was negative after hysterectomy; and the evaluation of the patient postoperatively revealed no metastasis on computed tomography.

References

Letter to the editor-in-chief

A new contemporary prostate cancer grading system: message to the Italian pathologists

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In 2005, the Gleason grading system underwent its first major revisions. Some of the changes were proposals that Gleason score 2-4 should not be assigned to cancer on needle biopsy. The consensus conference also came up with extremely stringent criteria for cribriform Gleason pattern 3 consisting of small glands with regular contour, regular distribution of lumina, and uniform round lumens. The 2005 ISUP consensus conference agreed with the original Gleason system that fused glands, irregular cribriform glands, and the hypernephromatoid pattern were designated as Gleason pattern 4. Whereas the original Gleason pattern 4 consisted of only irregular cribriform glands, the 2005 consensus conference concluded that ill-defined glands or acini with poorly formed glandular lumina also warrant the diagnosis of Gleason pattern 4. Whether glomeruloid glands and mucinous carcinoma were Gleason pattern 3 or 4 in the 2005 conference was not resolved. In November, 2014, 65 prostate cancer pathology experts, along with 17 clinicians including urologists, radiation oncologists, and medical oncologists from 19 different countries gathered in a consensus conference to further update the grading of prostate cancer. Changes from the 2005 conference were: 1) Gleason patterns 1-2 are virtually never made; 2) Gleason pattern 4 consists of cribriform (regardless of morphology) and glomeruloid glands along with fused, and poorly formed glands; 3) Hypernephromatoid pattern should no longer be used; and 4) Mucinous carcinoma should be graded based on its underlying morphology, whereby some contain Gleason pattern 3 and some pattern 4. The 2015 schematic Gleason diagram displays these updates. A contemporary prostate cancer grading photomicrograph montage was recently created by this author (Fig. 1) to more completely show the various patterns within each grade (Fig. 1) and to correlate with the new grading system described below.

Despite modifications in 2005 and 2014, there are still problems with the Gleason system. It ranges from 2 to 10, yet in current practice it is rare for a score lower than 6 to be reported. Some men think that they have an intermediate prognosis tumor when they are told that they have a Gleason score 6 out of 10, which contributes to fear of undergoing active surveillance. Gleason scores have also been incorrectly grouped together for both treatment and prognosis purposes. For example, the widely used D’Amico prostate cancer risk classification system considers Gleason score 7 as a single score without distinguishing 3 + 4 versus 4 + 3. In 2013 based on data from Johns Hopkins Hospital, we proposed 5 prognostically distinct Grade Groups (Tab. I). The way that tertiary patterns are factored in with these grade groups are that on needle biopsy with 3 + 4 = 7 with a lesser amount of 5 it is called 3 + 5 = 8 (Grade Group 4), and 4 + 3 = 7 with lesser amount of 5 it is called 4 + 5 = 9 (Grade Group 5). On radical prostatectomy, 3 + 4 = 7 with <5% pattern 5 is graded as 3 + 4 = 7 with tertiary 5 (Grade Group 3 with minor high grade pattern), and 3 + 4 = 7 with >5% pattern 5 is called 3 + 5 = 8 (Grade Group 4). On radical prostatectomy, 4 + 3 = 7 with <5% pattern 5 is called 4 + 3 = 7 with tertiary 5 (Grade Group 4 with minor high grade pattern), and 4 + 3 = 7 with >5% pattern 5 is called 4 + 5 = 9 (Grade Group 5). This new system was subsequently validated in a multi-institutional study of >20,000 radical prostatectomy specimens, >16,000 needle biopsy specimens, and over 5,000 biopsies followed by radiation therapy. There was broad (90%) consensus for the adoption of this new prostate cancer Grading system in the 2014 consensus conference and was accepted by the World Health Organization (WHO) for the 2016 edition of Pathology and Genetics: Tumours of the Urinary System and Male Genital Organs. The new grades would, for the foreseeable future, be used in conjunction with the Gleason system [ie. Gleason score 3 + 3 = 6 (Grade Group 1)].

In summary, the new grading system has the following advantages: 1) reduces grades of prostate cancer down to the lowest number of grades, where each has a different prognosis; 2) is simple with only 5 grades, so that it
Fig. 1. From left to right:
1st row: Closely packed uniform sized and shaped large glands; Large variably sized and shaped glands, some with infolding; Uniform medium sized glands; Variably sized glands.
2nd row: Occasional tangentially sectioned glands amongst well-formed small glands; Occasional tangentially sectioned glands amongst well-formed glands with open lumina; Back-to-back discrete glands; Branching glands.
3rd row: Large irregular cribriform glands with well-formed lumina; Irregular cribriform glands with slit-like lumina, glomeruloid structures, and fused glands; Irregular cribriform glands with small round lumina; Small round cribriform glands.
4th row: Poorly-formed glands with peripherally arranged nuclei; Small poorly-formed glands; Small poorly-formed glands; Fused poorly-formed glands.
5th row: Sheets of cancer; Sheets of cancer with rosette formation; Small nests and cords of tumor with scattered clear vacuoles; Individual cells.
6th row: Nests and cords of cells with only vague attempt at lumina formation; Solid nests of cancer; Solid nests with comedonecrosis; Cribriform glands with central necrosis.

Discrete Well-formed Glands (Gleason Patterns 1-3)

Cribriform/Poorly-formed/Fused Glands (Gleason Pattern 4)

Sheets/Cords/Single Cells/Solid Nests/Necrosis (Gleason Pattern 5)
will be easier for patients and clinicians to understand; and 3) logically and in line with other grading systems starts with grade is 1 as the lowest grade, in contrast to Gleason scores which in current practice begins with 6.

**References**


II MEETING NAZIONALE
Gruppo Italiano di Paleopatologia

L’AQUILA, AUDITORIUM DEL PARCO
31 OTTOBRE 2015 ore 9:00
INGRESSO LIBERO
Coevolution of Diet and Brain in *Homo spp.*

V. SALFI

*Quality Engineering, Pescara*

The story begins in Africa 6-7 mya, and includes about 24 of known human and pre-human species, among which *H. sapiens* on the one hand, and chimpanzees on the other were the only survivors. From the findings in the archaeological excavations of the African savannas to the modern laboratories of biomechanical analysis, radiometric dating and molecular genetics, paleontologists are furiously redesigning the tree; or, better, the bush of the human family!

Chimps and humans with Encephalization Quotient (EQ) equal to 2.0 and 5.8, respectively, show average DNA sequence divergence of 6.5 mya and share upwards of 98.5 percent of their DNA; considering substitutions of one base pair for another, the results indicate that only about 1.2 percent of the genomes are different. Humans and chimps, which have about 22,000 genes each, have essentially the same genes, but differ for when and where the genes turned on and off. When considering duplications and rearrangements of larger sections of the genetic code as well, it was found an additional 2.7 percent difference between the two genetic blueprints.

The Rift Valley in East Africa is considered the cradle of mankind, namely the place where it has evolved and diversified our species starting from *Hominini* of genuses *Sahelanthropus* (6.9 mya), *Orrorin* (6.0 mya), *Ardipithecus* (5.6-4.4 mya), *Australopithecus* (4.1-2.5 mya), *Paranthropus* (2.6-1.4 mya), to those of genus *Homo*, as *H. habilis* (2.3-1.5 mya), *H. ergaster/erectus* (1.8-1.0 mya) and the first *H. sapiens* (0.2-0.15 mya). Australopithecines with brains of 500-550 cc and EQ of 2.5-3.0, were bipedal arboreal, mostly vegetarian insulinsensitive, lived in a warm, moist environment in which carbohydrate derived from fruit and berries was an important source of energy. Their Megadontia Quotient (MQ) was equal to 2.0-2.7, about 2-3 times that of the chimpanzee (0.9). Brain and reproductive tissues by our ancestors hominids developed strict requirements for the specific glucose as an energy source.

With the first severe glaciation, 2.5 mya, global temperatures fell dramatically and resulted in drying of moist African forest, open woodland and savannah. Hominids that were unable to use grasslands became increasingly carnivorous. The first stone tools in the fossil record coincide with the existence of *H. habilis* (MQ=1.9; EQ=3.6), suggesting that they may have supplemented a vegetarian diet with scavenged meat. Later *H. erectus* (MQ=1.0; EQ=4.0) is known to be an active hunter and was the first species to make stone tools and use fire systematically. Low glucose intake associated with a low carbohydrate, high-protein carnivorous diet, in the course of at least seven glacial periods, which dominated the last two million years of human evolution, led to insulin resistance thus becoming a survival and reproductive advantage. When food energy was abundant, but dietary carbohydrate scarce, those with greater inherent insulin resistance were able to redirect glucose from maternal use to fetal metabolism, increasing birth weight and survival of offspring. Eating meat favored the evolution of primitive human teeth, brain and behavior. Hunting and gathering along with the fire have been the strategy of the genus *Homo* ancestral subsistence starting from about 1.8 mya with the *H. erectus*, a cooked meat eater, *carnicottivorius*, active preferential, and with the ultimate appearance of *H. sapiens* 200 kya. From *Australopithecus* spp. to *H.erectus*, and finally *H.sapiens* a primary metabolic organ-selective divergence took place: the resulting brain increase stole energy both from muscles and from stomach and intestines which reduced their size accordingly.

A brain increased and thus a larger skull involved in females a larger birth canal, and the anticipated birth of the fetus, whose brain was continuing its growth postpartum so as to force human groups to be less mobile, at least until a newborn was not considered surplus and then deleted, perhaps cannibalized. Regular consumption and culturally accepted human flesh is a practice that is often resorted to in human prehistory. *H.antecessor* 0.8 mya at Gran Dolina in Spain, *H.neanderthalensis* 250 kya at Saccopastore (Rome) in Italy and 100 kya at Moula-Guercy (Ardèche) in France. There has been at least 3 waves of immigration from Africa. Various samples of *H.erectus* spread from Africa and colonized the Euroasia, 2-0.7 mya. The second radiation by *H.heiderbergensis* happened 750 - 135 kya. Modern man walked on these footsteps, having also had development in Africa, about 200 kya, and then (70 kya) also migrated to Eurasia, and besides Australia (45 kya) and Beringia (25 kya), and lastly the Americas (15 kya).

In East Africa at some point between 195 and 123 kya, the size of the populations of first *H.sapiens* have collapsed due to climatic conditions that made our ancestors’ African homeland uninhabitable. This would have resulted in a migration to the South around 150 kya. The southern coast of Africa would have been one of the few spots where humans could survive during this climate crisis, because it harbors an abundance of shellfish and edible plants. Everyone alive today is descended from a group of people from a single region of shelter who survived this catastrophe. Excavations of a series of sites have recovered items left behind by what it may have been that progenitor population. The Blombos Cave (Southern Cape coastline, South Africa) contains deposits currently dated between 100 and 70 kya, which mostly contain engraved ochre, engraved bone ochre processing kits, marine shell beads, highly refined bone and bifacial points. So a “Big Bang” of intelligence happened along the Costa of Extreme South 70-80 kya, which was followed by a migration back to N-E and the worldwide diffusion of *H.sapiens*.

In Europe and Levant the early modern humans (Cro-Magnons) encountered the Neandertals. Both species have coexisted for thousands of years during the harsh winters of last Ice Age in the competition for resources in steady decline and have crossed each other with the formation of hybrids, of which the males were probably sterile. This, along with inadequate technological knowledge would lead to the disappearance of Neandertal populations 30 kya. *H.sapiens* would suffer the same fate, but survived and have become rampant, thanks to a more suitable clothing, better tools like projectile weapons, and ‘hyperprosocial’ behavior, i.e. genetically encoded propensity to cooperation among unrelated individuals. From 11 populations in Africa, Europe and Americas, the expansion lineages were identified and the historical demographic variations were reconstructed: major population expansions in three continents began before Neolithic time, i.e. 15-11 kya in Africa, 13 kya till now in Europe and 12-8 kya in America. All the expansions began post-LGM (last glacial maximum, 23-17 kya) as the temperature started to rise, before the advent of agriculture. But 12.9 kya it occurred the cold and dry Younger Dryas, a relatively sudden decline of 2-6 degrees Celsius that lowered the bearing capacity of the hunting and gathering and forced the progressive use of true agricultural practices to get more food per unit of territory.
although with much more time and work, and less nutritional quality. The archaeological and radiometric data show that various forms of domestication of plants and animals arose independently in seven locations of the globe. In the so-called Fertile Crescent region eight species of plants, including barley, wheat, barley, peas, lentils, flax and olive were domesticated 10-9.7 kya. In the same area they were also domesticated four big mammals, such as goats (9 kya), sheep, oxen, pigs (8.7-8 kya). The encephalization process, the foundation of our global success, with the advent of agriculture has led to lifestyles incompatible with several of our ancestral genes. Indeed the modern human genome is a temporal mosaic: structure and organization of the brain have evolved quite recently, 10 kya of ~400 generations, while several genes, including those related to the nutrition, have remained mostly the Paleolithic, 2 mya of ~80,000 generations. Nutritional standards of the populations of the Neolithic were generally lower than those of the Upper Paleolithic, and life expectations were shorter, in part due to illness. The average height dropped from 178 cm to 165 cm for men and 168 cm to 155 cm for women, and so it was until the 20th century when the average human height has recovered to pre-Neolithic. With the domestication of plants and animals there was determined (i) the sharp reduction (3-2 times) of micronutrients, fibers and other co-nutrients, as well as long chain omega-3 (LCo-3) (ii) the occurrence of more or less heavy food intolerance, (iii) the generation of various infective diseases, inherited from oxen (measles, tuberculosis, smallpox), pigs (influenza, pertussis), ducks and chickens (flu, malaria).

The large human brain, as a final outcome of millions of years of evolutionary experiments, is presenting ~100 billion (10^11) neurons, ~100 billion (10^11) non-neurons, ~100 trillion (10^14) synapses, more than 105 km of interconnections, and estimated capacity of 1.25 terabytes of data storage (10^12). All the aforementioned is based on morpho-functional units more or less full of fibers also very long, as well as on a massive vascular network, everything made with phospholipidic membranes rich in long chain polyunsaturated acids, LC-PUFA. As a result the brain is made of unsaturated fat (60% of its dry weight)! If the muscles are mainly made of protein, and then to feed it takes the amino acids, to feed the brain they are required fatty acids, such as docosahexaenoic acid (22:6o-3), DHA, and arachidonic acid (20:4o-6), AA, which, therefore, are 'Brain Food'! Endogenous biosynthesis of AA and DHA from vegetable precursors cis-linoleic acid (18:2o-6), LA, and alpha-linolenic acid (18:3o-3), ALA, is relatively low and does not keep up with the growing body of an animal to higher growth. In animals with largest size versus the smallest, in the membranes of liver cells, precursors LA and ALA derivatives increase compared to AA and DHA; the small amount of DHA that the body can produce is used in the nerve membranes of the brain and the retina so they have limited growth. Thus, for the evolution of H.sapiens, a source of pre-formed DHA and even AA would have conferred a significant advantage in the context of neural and vascular development, respectively, constituting oneself the specific and unique requirement.

Moreover, the evolution of the visual and nervous system had occurred in the early proto-ocean environment some 600 mya. The first visual systems used vitamin A as the photon sensitive molecule with DHA as the main constituent of the lipid support for the protein and photo-transduction system. These molecules would have been present in abundance, having been produced by the blue-green algae which dominated the proto-oceans for some 2.5 billion years previously. As systems evolved differentiated functions, transmission of the electrical impulse to a specialised target was carried out at junctions/synapses exploiting specialised neurotransmitting molecules. Like the photon receptor, the lipid architecture of the synaptic membrane used DHA. Therefore, the marine food chain consistently provided the necessary DHA for the origin and evolution of simple and then the advanced neural and visual systems. It also provided necessary AA for efficient vascular system development which is essential for the provision of the disproportionately high energy requirement needed by the brain. H.sapiens is unlikely to have evolved a large, complex, metabolically expensive brain in an environment which did not provide abundant dietary LC-PUFA. Conversion of 18-carbon PUFA from vegetation to AA and DHA is accepted quantitatively insufficient due to a combination of high rates of PUFA oxidation for energy, inefficient and rate limited enzymatic conversion and substrate recycling. The littoral marine, as well, as inland lacustrine food chains provided evolving human populations consistently greater amounts of pre-formed LC-PUFA than the terrestrial food chain.

D.F.Horrobin (1998) proposed that at some point of human evolution specific alterations have taken place with multi-factorial metabolic changes that have resulted in an expansion of the cerebral function beyond the only mass increase: mutations in certain genes <100 kya before the spread the H.sapiens from Africa. For millions of years (!) the increased size of the brain does not have guaranteed to our initial ancestors special creativity and intelligence. The turning point must have been acquiring better neural micro connectivity. Sudden emergence of creativity, art, religion, war, began 50-100 kya, not connected with the increase of cerebral mass per se. The brain micro connectivity depended largely on the availability of phospholipids, major components of the brain itself. In the metabolism of phospholipids related enzymes and proteins play a strategic key role. The turnover of the axons and dendrites requires large amounts of LC-PUFA and amino acids, that only a rich food animal nutrition, as meat, fish, shellfish, and eggs, can provide.

Biochemical variations related to changes in the metabolism of LC-PUFA may be identified today in the families of successful creative men where it is schizophrenia. Madness, inventiveness, and leadership co-exist in the same families in all populations sapiens: incidence = 0.5-1.5% according to WHO criteria, in all races and in all continents, albeit at variable course from case to case. Therefore, changes in the biochemistry of phospholipids and LC-PUFA would be responsible for both schizophrenia and our humanity.

F.H.Previc (2009) interpreted the differences between modern humans and prehistoric in terms of increases in brain levels of dopamine, part of a general physiological adaptation caused by increased consumption of meat 2 mya in H.habilis, still pushed on in H.erectus, H.heidelbergensis, H.sapiens from diets of meat and shellfish, climatic conditions, from ecological demands and social competition. The high dopaminergic activity of the human brain may have been responsible for the evolution of the mind itself, given that dopamine is crucial to short-term memory, cognitive transfer, conceptual abstraction, and other features of an advanced intelligence. The “high-dopamine” personality is characterized by high intelligence, a sense of personal destiny, a religious/cosmic preoccupation, an obsession with achieving goals and conquests, an emotional detachment that in many cases leads to ruthlessness, and a risk-taking mentality.

In order to flush out the underlying mechanisms of human
Diet between the Neolithic and the Bronze Age in Italy: the isotopic evidence

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The questions that archaeologists face when it comes to food production and consumptions in past societies are normally confined to timing, waves of dispersion of crops/animals and adoption vs. abandonment of food practices. However, faunal and floral assemblages in archaeological contexts are usually highly fragmented and are biased towards the preservation of larger mammalian bones, rather than smaller mammals, fish and plant remains. The development of isotopic analysis of human and animal bone collagen offers an applicable and informative approach for directly assessing past diet, while focusing both on determining chronological changes in diet at the population level (corresponding to gross economic transitions), and examining dietary variability within specific populations.

In particular, stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopes measured in skeletal tissues vary according to the ecosystem where the food is acquired and can help discriminate between groups of plants eaten, as well as marine vs. terrestrial diet and position along the food web.

The isotopic investigation presented here focussed on several sites from northern and southern regions of the Italian peninsula dated to the Neolithic and the Bronze Age. The goal of our study was to explore diet across time, while directly assessing possible levels of dietary complexity. We have analysed over 500 human and 100 animal bone samples that represent, so far, the largest isotopic dataset for Italian recent prehistory.

Our isotopic record seems to reveal a generally homogeneous dietary pattern, mostly based on the consumption of C3 plant resources with a limited contribution of animal proteins to the human diet.

Surprisingly, we have noticed very little variation at the transition between the Neolithic and the Bronze Age, with differences in the isotopic ranges circumscribed either geographically or chronologically, within the two phases.

In particular, for the Neolithic the only significant difference in the isotopic range seems to appear at Passo di Corvo, in the Apulian Tavoliere, where high nitrogen ratios have been associated with herding practices and (possibly unintentional) malnouring effect. For the Bronze Age, the most striking evidence comes from sites in the Po Plain, where carbon values reveal the consumption of C4 plants (i.e., millets), which appears to be unprecedented for this region of Europe.

References


Burials of Casal Bertone (Rome, I-III century AD): analysis of some cases of metabolic disease

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The purpose of this study is to investigate the skeletal remains of the population of Casal Bertone, located in the Eastern suburbs of Rome, regarding skeletal lesions that may be indicative of child metabolic disease. Overall the sample consists of 72 inhumed subjects. The demographic composition

Diversity, genetic research led by the fixity of the genome have given way to the ‘epigenome’ analysis, i.e. the study of the whole dynamic of the relations that the genome itself generates not only with the rest of the organism but with the environment. Where, when and how genes are turned ‘epigenetically’ is probably behind many of the differences between human groups. L.Carmel, D.Gokham, S.Päälö, and others (2014), called Lords of Paleogenetics, reconstructed, first, the ‘epigenome’ of Neandertal and Denisovan, an extinct Siberian relative of same Neandertal, versus modern H.sapiens, designing ~2000 DNA methylation maps, by harnessing the natural degradation processes of methylated and unmethylated cytosines. Differentially methylated regions (DMRs), i.e. genomic regions with different methylation statuses are regarded as possible functional regions involved in gene transcriptional regulation. Among those genetic pattern changes, many are expressed in brain development, in large part involved in neurological and psychiatric disorders. Numerous changes were also observed in the immune and cardiovascular systems, whereas the digestive system remained relatively unchanged. Additionally, there are substantial methylation changes in the HOXD cluster that may explain anatomical differences between archaic and present-day humans.

In conclusion, this is not just a matter of genes (present or absent), but which of them are turned on and which, instead, are silenced (or have never turned on). The difference in the final evolution of Man and his prodigious brain was a game of larger mammalian bones, rather than smaller mammals, where the food is acquired and can help discriminate between groups of plants eaten, as well as marine vs. terrestrial diet and position along the food web.

The questions that archaeologists face when it comes to food production and consumptions in past societies are normally confined to timing, waves of dispersion of crops/animals and adoption vs. abandonment of food practices. However, faunal and floral assemblages in archaeological contexts are usually highly fragmented and are biased towards the preservation of larger mammalian bones, rather than smaller mammals, fish and plant remains. The development of isotopic analysis of human and animal bone collagen offers an applicable and informative approach for directly assessing past diet,
of a skeletal sample reveals a high mortality rate for infants (0-6 years-45.8%). This report considers abnormal lesions found in two infants at least. The individual T.71 (18-24 months of age) is represented by the right lower limb, ilium and ulna. Flaring and swelling of distal metaphyses, fraying bone margins growth plate, porosity growth plate, cupping deformities of growth plate metaphyses are present. The tibia and the fibula show medial bending deformities. Angulation of femoral neck and angulation of knee are visible as well. The described features lead to a likely rickets diagnosis. The specimen T.78 (6-12 months of age) exhibits bilateral porosity on the external surface of the sphenoid’s greater wing, on the endo-ectocranial surface and also on the orbital roof. Other recorded features are abnormal porosity at the palate, on the coronoid process of the mandible, around the infraorbital foramen of the maxilla and along the alveolar process, which could be associated with the bleeding gums phenomenon. Postcranial features include: slight porosity and new bone formation on the supra- and infraspinous fossae of the scapulae. Subperiosteal hemorrhages are inferred from the presence of porosity and hypertrophic new bone formation on any limb bone. The other skeletal features consist of: prominent frontal and parietal bossing, slight medial angulation of the mandibular ramus and the enlargement of costochondral rib junctions. Lastly the cortex of the distal and proximal metaphyses of the limbs is irregular and porous and the growth plates display porous frayed and flared surfaces. The metaphyses of long bones show bending and cupping. The skeleton reveals a set of lesions indicating that this individual probably suffered from rickets and scurvy.

References

Health status and isotopic variability: possible correlation in the metabolic disorders in the community of Piazza Madonna di Loreto (Rome, VII- VIII century AD)

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Paleonutrition of the rural Italian population from the Middle Ages to the Contemporary Age: isotopic analysis of some Tuscan skeletal samples

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The studies on paleodiet through stable isotope evidence of carbon (δ¹³C) and nitrogen (δ¹⁵N) content in bone collagen represent a line of investigation widely practiced in archaeology and anthropology. The application of this method in prehistoric American and European skeletal series, as well as in historical age groups, has provided new investigative tools to reconstruct environment, food economies, access to resources and social characterization of human groups in the past. This method was recently applied by the Division of Paleopathology of University of Pisa, in collaboration with the second University of Naples, in several samples from rural Tuscan cemeteries. These skeletal series are different in chronology, related to contexts of the Medieval (11th-14th century) and Post Medieval Ages (19th century), from the inner Apennine and the hilly Tuscany. The comparison of sites with different settling characteristics (Parish cemeteries, graveyards of Castle), as well as within a site with individuals occupying different spatial hierarchical positions (in proximity or away from the church), provides useful data to interpret the diet as social indicator. Our analysis also offers some insights to interpret correctly the meaning of results in relation to the material characteristics of burials, settlements and of the written sources. Finally isotope models allow us to advance some hypotheses on food and diet in different human groups.

References

Dentoalveolar diseases and dietary habits in the social upper classes of the Italian Renaissance: the Guinigi family from Lucca

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Teeth and their pathologies are very important when studying
the life-style, social behaviour, health condition and diet of ancient populations. Many articles in paleoanthropological literature describe dentoalveolar diseases in the Antiquity, mainly in the low-class societies, and only a few reports regard the oral conditions of the social upper classes. The purpose of this research is to examine the dental condition of an upper-class family of the Italian Renaissance, in terms of dietary habits and food resources. The research was carried out on the skeletal remains of the Guinigi family from Lucca (Tuscany), dated back between the end of the 14th and first half of the 17th century. The study of dentoalveolar diseases was performed on 45 individuals and 325 teeth, equally distributed between males and females, and isotopic analysis of $^{13}$C and $^{15}$N was performed on 13 samples. The frequency of dentoalveolar diseases was very high in the upper class samples, and varied from 27% to 60% of the teeth/alveoli affected, while the frequencies were lower (16-20%) in the rural samples. Caries was extraordinary frequent in the Guinigi family with a prevalence of 70.8% in females and 43.5% in males, while ante-mortem tooth loss and abscesses were more frequent in males, whose life span was higher. Different factors may promote tooth decay, but dietary habits, as well as physiological or behavioural factors, certainly play an important role in caries development, and may explain the differences observed between sexes. The results of isotopic analysis indicated a diet based on higher protein intake with respect to the lower social classes, with a good presence of vegetables, but gave no indication about cariogenic foods. A large consumption of not complex sugars may be responsible, at least in part, for the high frequency of caries among the wealthy classes and in particular in the Guinigi family. It is well known that expensive and elaborate foods, including sweets, sugar cane and honey, adorned the banquet tables of Renaissance Princes. Moreover, some members of the Guinigi family, in the middle of the 16th century, founded a company for sugar cane refining and trade, probably due to the consumption of very large quantities of this elitarian food.

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Pulmonary antracnosis on natural mummies of XVI-XVIII century AD from Roccapelago (MO, Italy)

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Roccapelago is a small town of the Apennines; during the restoration of the local parish church it was found a burial crypt containing the remains of 300 individuals who lived between the sixteenth and seventeenth century AD, of which at least 60 in natural mumification. Natural mumification was made possible by the unique location of the crypt built on the ruins of the ancient fortress of Roccapelago and equipped

Pulmonary antracnosis on natural mummies of XVI-XVIII century AD from Roccapelago (MO, Italy)
with ventilation slots. These subjects underwent numerous anthropological and paleopathological investigations including biopsy of lung tissue. A total of 24 individuals were subjected to biopsy of the alleged lung tissue through pre existing solutions of continuity of the rib cage. From each individual was obtained a tissue sample that was in turn divided into two fragments of different size. The fragments of greater size were rehydrated according to Sandison 1955, routinely processed as a standard surgical biopsy from a living patient, avoiding formalin fixation. All these samples have been embedded in paraffin, cut at the rotary microtome in 5 mm-thick histological sections, routinely stained with hematoxylin and eosin. The fragment of smaller size were also rehydrated then fixed absolute alcohol for 7 days, and included in vacuum-impregnated epoxide resin acc. to Fulcheri 2001. All these samples have been cut with rotary microtome using tungsten blades to get 2 mm-thick histological sections stained with hematoxylin and eosin, Pearsall, Masson trichrome, PAS and Von Kossa. In 12 cases it was biopsied the right hemithorax, in 6 cases the left hemithorax, in 4 cases the paracervical tissue, in one case the abdomen and in one case the paratracheal tissue. In a total of 18 cases (75%) it was possible to recognize lung tissue. In the remaining 6 cases: 2 cases it was not possible to recognize the original tissue, in two cases only fibrous tissue not compatible with lung tissue was observed and in the remaining two cases the sample was heavily contaminated by post vital larvae or spores. Among the subjects in which it was possible to recognize lung tissue in about 1/3 of the study population (7 cases; 29.16%) was observed massive deposition of a black, non-reflective, intratissutal and acellular pigment, often nodular shaped, constantly surrounded by dense fibrous tissue more evident with the Masson Trichrome staining. The morphological findings appeared diagnostics for a pneumoconiosis secondary to a massive pulmonary anthracosis. In one of these subjects we also observed the deposition of a pigment intensely positive for Pearsall staining indicative of previous bleeding events and consistent with a particularly serious form of pneumoconiosis antracotica. All these findings are consistent with the parish registers and the geography of the site, located at about 1100 meters above sea level and even today, surrounded by lush hardwood forests, confirming that it represented an important location for the production charcoal until the first half of the XX century. The observation of deposition anthracotic pigment in the lungs (the so-called anthracotic tattoo) is a common autopsy finding of our days and already paleopathologically described at in populations exposed to the fumes of braziers or outbreaks. Only if massive and accompanied by fibrosis or repeated bleeding is possible to consider pneumoconiosis, which is a known professional pathology of coal workers. This series is one of the first and most conspicuous paleopathological documentation of an occupational lung disease. The town of Saluzzo, Piedmont, in the northwest of Italy, was an important and independent marquisate from 1175 to 1548, when the France annexed its territories because the last mar- grave Gabriele died without sons. In the medieval heart of the city there is the church of San Giovanni, designated to hold the burials of the noble families and six marquises (Federico I, Federico II, Tommaso III, Ludovico I, Ludovico II and Gabriele), but the building underwent several extensions and restorations during the centuries so at present is impossible to identify the place of the medieval graves. We performed GPR (Ground Penetrating Radar) scansion on the entire surface of the church that revealed many structural anomalies and the certain presence of some sepultures and chamber tombs on the whole area. Then we concentrated our study on the apse where is placed the well preserved monumental cenotaph of Ludovico II (1438-1504), a masterpiece of ‘flamboyant gothic’, built in 1508. We lifted up the marble hatch on the ground in front of the monument and inspected the crypt, closed for at least seventy years and never photographed nor analyzed. The room was unexpectedly big (10x7 meters for 3 meters high) and accurately built. We found however a significantly altered context especially in modern times, with five lithic sarcophagi of the noble ‘Del Carretto’ family (XIX century-early XX century); in the opposite corner we noticed instead a trunk that contained scattered bones and, at least, seven skulls. We hypothesize that this ossuary was the result of a cleaning made in the crypt in modern times in order to hold new graves. At present it is not possible to identify and date those buried, but future detailed investigations (e.g. carbon 14 and isotopes) will certainly provide more information and, perhaps, identify the marquises of the Middle Ages. References Gabrielli N. Arte nell’antico marchesato di Saluzzo. Istituto Bancario San Paolo, 1974. Lobetti-Bodoni A. La Cappella del Santo Sepolcro (Coro della Chiesa di San Giovanni in Saluzzo). Tomba dei Marchesi di Saluzzo. Saluzzo: Lobetti-Bodoni 1898. Vaccetta G. 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ries. One of the arks revealed the natural mummy of Ferdinandino Orsini, 5th Duke of Gravina, identified by an epigraph with his name and date of death (1549), in good condition, with the exception of the face, completely skeletonized. The skull suffers from an extensive destructive lesion that afflicted the medial wall of the orbit right, the root of the nose and, partly, the ethmoid without osteitic reaction. The histological examination performed on the bone showed wide lacunae with, inside, epithelial-like cells, partially necrotic, positive for the immunohistochemical stain for PanCK. The border between the bone and the surrounding neoplasia were clear; the brownish fleshy appearance mass had darker margins (like a palisade) and was separated from the bone by clefting artifacts.

In our opinion, the pathology that affected Orsini 500 years ago was the basal cell carcinoma in an advanced stage, in fact it is the most frequent form of skin cancer and occurs predominantly on the sun-exposed skin of adults. Microscopically the tumour tends to infiltrate the subcutaneous tissue with a peripheral palisade surrounded by loose of stroma and cleft-like retraction spaces of artifactual nature. It grows in a slow and indolent fashion, but can ulcerate and may invade skull, nares, orbit or temporal bone with wide osteolitic lesion, enough to deserve the Latin name of ‘ulcus rodens’, i.e. erosive ulcer. Immunohistochemically, the cells are positive for keratin and distant metastases are very rare.

This case is very important because it represents one of the only four cases of malignant soft tissue tumor diagnosed in paleopathology.

References

From L’Aquila to Europe. Bodies and burials of the Franciscan Observance leading figures, 600 years after its introduction in Abruzzo region (1415)

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Due to a progressive decline of the Franciscan Order, a movement called Regular Observance took place in XIV century. An increasing number of monks left the friaries to live in poverty and hermitage. In a couple of centuries, they became the leading part of the Order. In 1415, some friars moved to L’Aquila to build the small convent of S. Giuliano, from which the Observance spread throughout Abruzzo region, as far as Italy and Europe.

The greatest exponent of the Observance, Saint Bernardino da Siena (1380-1444), visited L’Aquila twice, in order to promote reconciliation of the opposing parties. Here he died and his body was embalmed to be displayed inside a new basilica. The artificial mummy underwent at least 4 recognitions, but no detail is available about his diseases and the embalming technique adopted. Saint Giovanni da Capestrano (1386-1456) defended Bernardino from the charge of heresy, built the San Salvatore Hospital (1445-1457) and guided a Crusade against the Ottomans in eastern Europe. He died during the following epidemic. His remains are traditionally known to be destroyed by the Turks in 1526, but some Author supposed they may be still preserved and ascribed to an orthodox Saint. Saint Giacomo della Marca (1393-1476) also had oratorial skills and received inquisitional and diplomatic commissions in Eastern Europe from the Pope. He organized the Mount of Piety to lend money to the poor without interests. He died in Naples and his body was embalmed by the procedures used for Aragonese kings. Since 2001 his artificial mummy is preserved in Monteprandone (Marche region) and the fifth recognition held in 2008 evidenced well-developed muscular insertions, confirming the historical reports on his strong walking activity.

In the outskirts of L’Aquila are also preserved the human remains of the Blessed Bernardino da Fossa (1421-1503, skeletal remains), Vincenzo da L’Aquila (1435-1504, natural mummy) and Timoteo da Monticchio (1444-1504, skeletal remains). The mummy of the Blessed Antonia da Firenze (1401-1472) is an interesting example of female mummy and its recognition is scheduled in the near future. A systematic search for additional minor figures in Abruzzo region and a survey of their remains is in progress.

References

Paleopathological study of Mammutthus meridionalis of Madonna della Strada (L’Aquila)

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A skeleton of a male, 50-55 years old Mammutthus meridionalis, dated to the Pleistocene and conserved at the Spanish Fort in L’Aquila (Italy), showed a broken left tusk, in association with the presence of a deep (15x20 cm) bone erosion, involving the dental alveolus and the premaxillary bone, in close proximity to the maxillary sinus and the nasal cavity. During gross examination, small samples from three representative areas of the eroded bone were obtained. Thin sections were made and the specimens were examined under plane and polarized light, using a high resolution microscope with an incorporated digital camera. Microscopical study revealed the intra vitam origin of the lesions, characterized by the presence of woven bone fibers, typical of the early phases of bone remodeling, and lamellar bone with diluted and remodeled Haversian systems. The gross and histological findings were consistent with an osteomyelitis with bone sequestration, caused by a localized blunt trauma or, more likely, resulting from an ascending,
post-traumatic chronic pulpitis, due to the tusk fracture occurred during an accident or interspecies fights. The histological exam excluded the involvement of granulomatous inflammation (e.g. tuberculosis) or neoplasia. A disease process of at least several months in duration may be hypothesized, as suggested by the histologically visible bone remodeling. A long survival of the animal after tusk loss may also be supposed, since alteration of masticatory function with altered molar teeth consumption and postural changes (i.e. atlantauxial fusion), resulting from asymmetric weight distribution, were observed.

In this study, the application of (paleo)histological techniques proved to be fundamental in order to establish the nature of bone lesions detected on archeological samples, also providing a good case for studying skull trauma and shedding light on the life history of these large mammals.

References

Application of nanoparticles in consolidation treatments of archeological bones

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Archeological bones may undergo conservation treatments to reinforce their mechanical features and save these materials from decay or to allow the completion of research analyses. Nevertheless, the materials used for the conservation may produce alterations on the original find, negatively interfering on subsequent studies, such as bone surface topography as well as on the analysis of some components of the bone tissues (such as isotopes and DNA). The loss of mechanical properties is often caused by demineralization processes, so that one simple and compatible way to strengthen demineralized bones could be the in situ growth of calcium carbonate, in form of aragonite crystals - having strong mechanical strength thanks to their acicular shape. The in situ growth of aragonite crystals could be the in situ formation of aragonite crystalline phase. SEM images underlined the formation of a superfi-}

Enlarged vascular foramina and lytic lesions in vertebral bodies: a diagnostic dilemma

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Among the skeletal material from the sites of Alghero, Messu- mundu and Sant’Antioco di Bisarcio (Sassari, Sardinia) and dated back to the period comprises between the 13th and the late 16th century 5 subadult individuals aged between 5 and 15 years and a mature male showed peculiar osteolytic phenomena of the vertebral bodies. These lesions have the appearance of enlarged vascular foramina, affecting several vertebrae mainly of the thoracic and lumbar spine, sometimes with involvement of the sacrum; on the same vertebral body several lesions are generally visible. In the literature similar features have been attributed to brucellosis or tuberculosis. As for the Sardinian skeletal material, an imaging study on the vertebrae of the adult individual was carried out in order to evaluate the appearance of the lesions within the body. Computed Tomography evidenced internal irregular elongated cavitations, sometimes joined together; erosive rounded lesions, whose presence is not detectable externally, were also showed.
The molecular analysis has so far been performed on the subadult from Sant’Antiocho di Bisarcio, but at initial analysis the DNA resulted degraded. Therefore, the nature of these lesions remains unclear, as it is not sure if they should be referred to tuberculosis, brucellosis or other pathological conditions [hemolytic anemias (eg. Thalassemia), lymphomas, multiple myeloma and infection by Echinococcus]. Further molecular analyses will be carried out on the remains belonging to the other five individuals in an attempt to clarify the etiology of the above mentioned lesions.

References

Evidence of syphilis in a noble burial discovered in Piedmont dating back to the eighteenth century

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Ancient human remains were discovered in a burial context inside a crypt of the San Giovanni Battista church (Racconigi, Cuneo). No information is available about the origin of the burials or the dating of the bones. Historical documents suggest that the crypt dates back to when the church was built (1719-1730). The hypogeum has a roughly square shape. Access is through an opening in the ground floor of the church. Anthropological analyses show that the bones belonged to four individuals in primary burials: three adults and one subadult. In particular, an adult (1/A) of indeterminate age was found prone and represented almost exclusively by the lower limbs; a subadult (2/A), aged 10-13 years old, was found almost completely in a supine position; an adult male (3/A), aged 58-72 years old, that was skeletal in almost all districts even though only partially preserved, was found lying on his right side; an adult male (4/A) aged 58-72 years old, almost completely preserved, was found in a supine position. Some interesting paleopathological findings were observed, in particular, lesions reflecting treponematosis. Macroscopic changes in the teeth and bones typical of venereal and congenital syphilis were detected in the two adults (1/A-4/A) and in the subadult (2/A). The most characteristic cranial lesion is the pattern of scarring (caries sicca) seen on the frontal bone of adult male 4/A. Deforming osteomyelitis of the tibia and fibula were observed in adult 1/A. Hutchison’s incisors were detected in subadult 2/A.

Although paleopathology must basically describe and observe rather than diagnose and deduce on the basis of the macroscopic examination of the skeletal remains alone, it is equally true that in certain cases, like in individual or privileged burials, one can very carefully attempt to achieve a conclusive view, as in this case. Signs of an infectious disease, such as syphilis, were observed in three of the individuals that were found in the crypt of the Church in Racconigi and whom we may hypothesize were related to each other.

References

The skulls of Borgo Cerreto (Perugia): medical, surgical, and anatomical activity of Baronio Vincenzi (XVII century)

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In the Sixties of the last century the vault of a 17th century private chapel was opened, revealing three isolated skulls with evidence of surgical and anatomical activity. The chapel was built by Baronio Vincenzi, who lived and practiced medicine in Borgo Cerreto, a village in the province of Perugia, between the 16th and the 17th century. The skull bc 01 belongs to an adult male, aged 25-35 years. It shows a hole on the left front-parietal region (30 x 31 mm), that can be identified as the result of a skull trepanation. The margins of the lesion are regularly smoothed and inclined internally and the diploic tissues result almost completely obliterated by a cicatricial bone. A bone splinter (10 x 8 mm), completely reabsorbed, can be observed on the right side of the hole. These findings are the proof of a long survival of the subject. X-ray examination confirms a regular process of ossification, without infection. Trepanation was performed with a Hippocratic trypanon, largely used in cranial surgery of Modern Age. The specimen bc 02 is without skullcap and the right upper part of the face; it belongs to an adult male, 25-30 years aged. The cuts were produced by a bone saw with a thin blade. The choice of these regions suggests the willingness to study the basal skull, the right eye cavity and the paranasal sinuses. The skull bc 03 consists only in a skullcap of an adult individual, which shows the signs of a bone saw. In conclusion, the recovery of a trepanned skull, at present the first specimen of this type recovered so far in Umbria, together with two others skulls with the signs of post-mortem examination, inside the Vincenzi family vault can be probably related to the professional activity of Baronio. He was an experienced surgeon and a skilled anatomist, who certainly experienced the empirical surgery of the nearby surgical School of Preci, famous throughout Europe for the treatment of urinary bladder stones, cataract as well as the ability in skull trepanation.

References

Mi è difficile crederci, l’avevo sentito solo qualche giorno prima per sapere come stava e per metterci d’accordo sulla conclusione dell’ultima perizia per il dr Guariniello.

Pier-Giacomo Betta lascia sua moglie Patrizia, sua figlia Beatrice, tantissimi amici, colleghi e soprattutto tanti pazienti che lo hanno amato per tutte le sue battaglie contro il cancro e in particolar modo nelle aule dei Tribunali, per dare giustizia ai malati di Mesotelioma. Per tutti, fin da subito, un grande vuoto.


Tutto questo, però, non gli bastava, conoscere solo la realtà italiana era troppo limitante, e nel periodo 1982-1986 è anche “visiting fellow at Division of Surgical pathology” (Department of Pathology, College of Physicians & Surgeons, Columbia University of New York, USA).


Dal 2007 è stato anche membro del gruppo di lavoro di “Citologia urinaria” dell’Assessorato della Salute Regionale Piemonte.


Tutti titoli di cui sono venuta a conoscenza per lo più dopo la sua morte, essendo lui, sì ricercatore apprezzato da tutti sia a livello nazionale che internazionale, ma anche una persona umile, di alta professionalità e nello stesso tempo discreta.

Ho iniziato a lavorare con Piero dopo la morte del prof. Mollo, che era stato per me “…docente lucido e rigoroso […] sempre attento a sottolineare il significato e l’utilità clinica dello studio anatomopatologico accurato, soprattutto ai fini prognostici e per scopi terapeutici” (Pathologica 06, vol. 101, December 2009), e con il dr Betta ho potuto allargare il mio orizzonte conoscitivo. Ho scoperto in lui quello che ogni assistente e aiuto vorrebbe trovare nel proprio Direttore: capacità di ascolto, grande preparazione e conoscenza sia in ambito patologico, che in ambito clinico, legale e igienico ambientale.

Non solo ho trovato un modello e un vero amico, ma, nell’anno della sua malattia, ho potuto conoscere anche la sua famiglia, a lui tanto cara, prendendo ancora di più coscienza che i veri affetti sono quelli che ti crescono e ti danno la capacità di affrontare la malattia. Mi scaldava il cuore quando diceva: “Riesco ad affrontare la mia malattia perché ho una famiglia stupenda”. Aveva ragione: è il mondo delle relazioni vere, autentiche, quello che ti salva, alla fine, quando ti sembra che tutto sia finito.

Tornando alla sua esperienza professionale, ha dedicato molto della sua attività e conoscenza allo studio del mesotelioma, il tumore causato dalla fibra di amianto, e ha partecipato, come membro della Commissione, al-

Come presidente della Lilt è stato promotore di vaste campagne di prevenzione del cancro in ogni forma, puntando a uno stile di vita sano in cui credeva e che metteva personalmente in pratica. Non solo: una piacevole sorpresa e immensa gioia è stato per me apprendere, al momento del suo funerale, che il sabato mattina andava come volontario ad una trasmissione della radio… sempre per poter essere di aiuto agli altri, ai suoi malati. Anche quando la malattia è sopraggiunta, il suo spirito è sempre stato rivolto alle richieste che gli giungevano; mai una volta l’ho visto preoccupato per se stesso, ma solo e sempre per i compiti che si era preso con grossa responsabilità, che voleva portare a termine per non creare problemi agli altri. Credendo nella possibilità di cura, si è messo nelle mani dei colleghi con piena fiducia; la loro professionalità, che mi ha lasciato il dottor Betta. Non solo è stato un Dirigente medico di prim’ordine del nostro Nosocomio nell’ambito dell’anatomo-patologica, con significative collaborazioni internazionali e con il diretto coinvolgimento nella ricerca sul mesotelioma maligno, ma è stato anche uno straordinario presidente provinciale della Lilt. Questi ‘campi d’azione’ trascendono l’esperienza del singolo individuo e, ora che Piergiacomo Betta non è più tra noi, ci fanno comprendere davvero l’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità che ci lascia: un’eredità.


Grazie Piero

Donata Bellis