



PATHOLOGICA

Journal of the Italian Society of Anatomic Pathology
and Diagnostic Cytopathology,
Italian Division of the International Academy of Pathology

Società Italiana di Anatomia Patologica
e Citopatologia diagnostica
Divisione Italiana della International
Academy of Pathology



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Innovazione e sostenibilità al servizio del malato

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Milano
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02

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The journal welcomes contributions concerned with experimental morphology, ultrastructural research, immunocytochemical analysis, and molecular biology. Reports of work in other fields relevant to the understanding of human pathology may be submitted as well all papers on the application of new methods and techniques in pathology. The official language of the journal is Italian. Articles from foreign authors will be published in English.

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Mercoledì 23 settembre 2015						
ORARIO	AULA ROSSA 1	AULA BLU 1	AULA BLU 2	AULA GIALLA 1	AULA GIALLA 2	AULA GIALLA 3
12.00 - 14.00	Registrazione Partecipanti					
14.00 - 15.00	Cerimonia inaugurale e Lettura Magistrale in AULA ROSSA 1					
15.00 - 16.00	Gruppo di Studio GIPAM	Patologia fetoplacentare	Patologie correlate all'alimentazione	Patologie Apparato digerente e Pancreas	Simposio non ECM Roche - Novartis	Patologia endocrina
16.00 - 17.00			17.30 - 18.30 Progetto E.A.T.		Patologia polmonare	
17.00 - 18.00			Simposio non ECM Qiagen			
18.00 - 19.30					Patologia polmonare	

Giovedì 24 settembre 2015					
ORARIO	AULA BLU 1	AULA BLU 2	AULA GIALLA 1	AULA GIALLA 2	AULA GIALLA 3
8.00 - 9.00	Gruppo di Studio APEFA	Gruppo di Studio GIPATEC	Gruppo di Studio GIPAD	BIOBANCHE	Patologia Ginecologica
9.00 - 10.00	COMUNICAZIONI Ematopatologia e Apparato Digerente	COMUNICAZIONI Miscellanea 1	COMUNICAZIONI Miscellanea 2		
10.00 - 11.00	Ematopatologia	Patologia Mammaria	Dermatopatologia		
11.00 - 12.00			Simposio non ECM Cepheid		
12.00 - 13.00	Simposio non ECM Thermo Fisher Scientific		Simposio non ECM Leica Biosystems	Uropatologia	COMUNICAZIONI Miscellanea 3
13.00 - 13.45	Lettura Magistrale				
13.45 - 15.00	Lunch				
15.00 - 16.00	Ematopatologia	Patologia mammaria	Dermatopatologia	Uropatologia	Patologia Ginecologica
16.00 - 17.00		16.30 - 17.00 Progetto NORDIC QC-HER2			
17.00 - 18.00		Patologia mammaria			

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Venerdì 25 settembre 2015						
ORARIO	AULA BLU 1	AULA BLU 2	AULA GIALLA 1	AULA GIALLA 2	AULA GIALLA 3	
8.30 - 9.30	Sessione Giovani	Siapec incontra i Coord. Gruppi di Studio	COMUNICAZIONI Patologia Molecolare	Patologia Molecolare	COMUNICAZIONI Testa - Collo	
9.30 - 10.30		Patologia Testa e collo	Gestione organizzazione e rischio clinico	Simposio non ECM Myriad Genetics	Sessione Tecnici	
10.30 - 11.30					Patologia Molecolare	
11.30 - 12.30						
12.30 - 13.30		Assemblea SIAPeC-IAP Premiazioni e Cerimonia di chiusura				

Mercoledì, 23 settembre 2015

Aula Rossa 1 – 14.15-15.00

Lettura Magistrale

Alimenti e malattie intestinali. Non solo celiachia

G.R. Corazza

Paper non received

Aula Blu 1 – 15.00-18.00

Patologia fetoplacentare

MODERATORI: G.Bulfamante (Milano), E. Fulcheri (Genova), L. Resta (Bari)

Influenza dello sviluppo intrauterino fetoplacentare sulla salute e le malattie dell'adulto

L. Avagliano

Ginecologia e ostetricia, Ospedale San Paolo, Milano

Introduction

The “developmental-origin-of-health-and-disease” hypothesis suggests that critical events occurring during fetal life can lead to metabolic syndrome in adulthood¹, including hypertension, glucose intolerance, insulin resistance, dyslipidemia, obesity. During critical conditions in pregnancy, the fetus adapts to its environment and optimizes metabolic responses by reprogramming its genome. This reprogramming favors pregnancy evolution and fetal-neonatal early survival but influences long-term health and potentially causes a predisposition to diseases later in life².

The placenta is the key organ for the normal evolution of pregnancy. The placenta is the provider of oxygen and nutrients

from the mother to the fetus and allows waste removal from the fetus to the mother. Moreover, the placenta acts as fetal protective tool, buffering the effect of damaging insults during pregnancy and regulating acid-base balance. Since its role in nutrients transport, immunity regulation and hormonal production, the placenta is one of the main players in fetal programming by changing the pattern or amount of exchange of maternal-fetal nutrients³. Both intrauterine nutrient restriction and intrauterine nutrient excessive supply may predispose for the development of adult metabolic syndrome: the placenta acts as a nutrient sensor, matching fetal growth rate to the availability of nutrient resources, by adapting its transport function⁴.

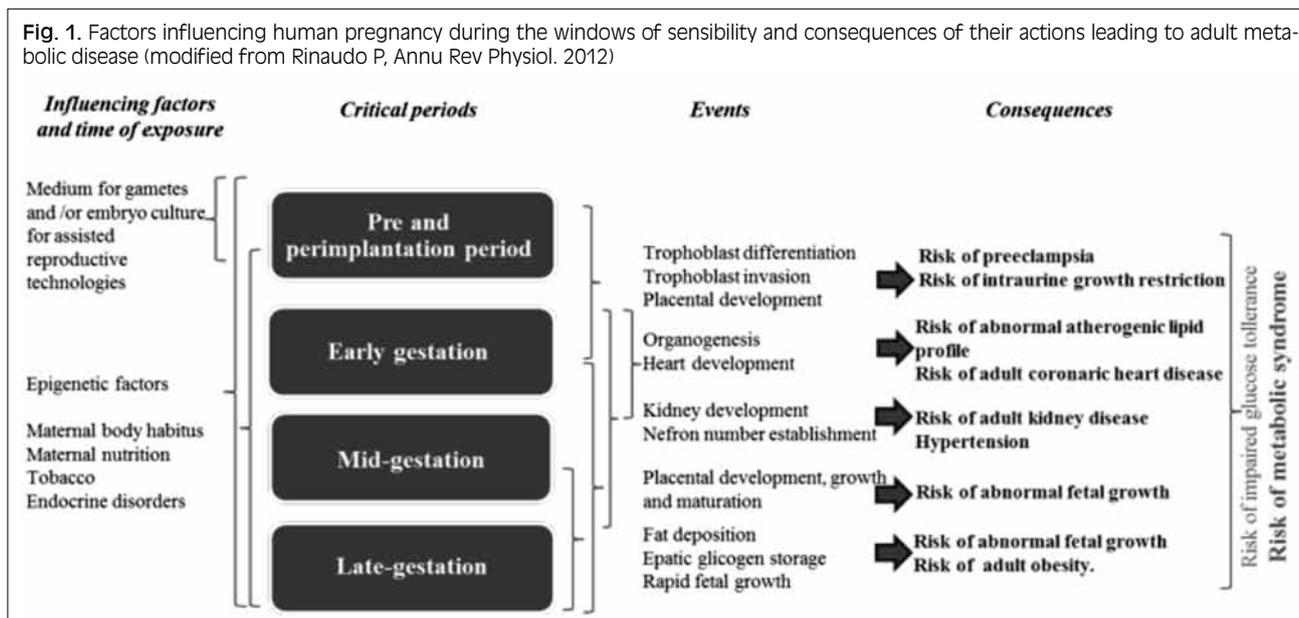
Methods

Review of the literature.

Results

Factors influencing the intrauterine fetal-placental development and intra-uterine life are summarized in figure 1; the possible consequences of their actions are exposed.

Fig. 1. Factors influencing human pregnancy during the windows of sensibility and consequences of their actions leading to adult metabolic disease (modified from Rinaudo P, Annu Rev Physiol. 2012)



Conclusion

Pregnancy is a crucial period of embryo-fetal development, during which the intrinsic and extrinsic factors can act influencing the post-natal health.

References

- ¹ Barker DJ, Osmond C. *Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales*. *Lancet* 1986;1:1077-81.
- ² Gluckman PD, Lillycrop KA, Vickers MH, et al. *Metabolic plasticity during mammalian development is directionally dependent on early nutritional status*. *Proc Natl Acad Sci USA* 2007;104:12796-800.
- ³ Rinaudo P, Wang E. *Fetal programming and metabolic syndrome*. *Annu Rev Physiol* 2012;74:107-30.

- ⁴ Jansson T, Powell TL. *Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? A review*. *Placenta* 2006;27 Suppl A:S91-7.

La placenta: da "scatola nera" della gravidanza a elemento predittivo della salute post-gravidica del figlio e della madre

Il ruolo della diagnostica anatomopatologica

G. Bulfamante

Paper not received

Aula Blu 2 – 15.00-18.30

Patologie correlate all'alimentazione

MODERATORI: V. Villanacci (Brescia), U. Volta (Bologna)

Gli aspetti clinici e laboratoristici nel malassorbimento e nelle intolleranze alimentari. Dove inizia e finisce il ruolo del clinico e del laboratorio

U. Volta

Department of Medical and Surgical Sciences, University of Bologna

Adverse reaction to foods can be classified in two main subsets, i. e. toxic and non-toxic. Apart from those of toxic origin, which are mainly caused by food toxins, the non-toxic forms, including both food allergy and intolerance, are determined by a particular individual reaction towards commonly tolerated dietary components. The underlying pathogenic mechanisms are immune-mediated or due to enzymatic deficiencies.

Gluten, a complex of proteins present in wheat and other cereals, is responsible for different immune-mediated adverse food reactions including coeliac disease (CD), non coeliac gluten sensitivity (NCGS) and wheat allergy (WA). Until a few years ago, CD was known as a rare food intolerance, confined to young children, characterised by a severe malabsorption and a flat intestinal mucosa¹. Nowadays, CD is regarded as a systemic autoimmune disorder, very common in the general population (1 in 100 individuals), with a possible onset at any age (from infancy to elderly) and a multiform presentation. The identification of CD is challenging since it can onset not only with diarrhoea and weight loss, but also with non classical gastrointestinal symptoms such as constipation, bloating and recurrent abdominal pain, and with extra-intestinal manifestations such as anaemia, raised transaminases, osteoporosis, recurrent miscarriages, aphthous stomatitis and associated autoimmune disorders, as well as without any detectable symptom. Over the last 20 years, the diagnostic accuracy of serology for CD has progressively increased with the development of highly reliable tests such as IgA anti tissue transglutaminase (tTGA) and anti-endomysial (EmA) and IgG anti deamidated gliadin peptide antibodies (DGP). CD is closely related to a well-defined HLA profile, characterized by the positivity for HLA-DQ2 and -DQ8. Genetic testing is particularly useful for identifying among first degree relatives of coeliacs those who are predisposed to develop the disease. Moreover, it can be of help when there is a discrepancy between serology and histology. According to the ESPGHAN guidelines, the finding of high tTGA titers (>10 times the

cut-off value), confirmed by EmA and genetic positivity, allows to diagnose CD in symptomatic children and adolescents without the need of small bowel biopsy. The increasing application of antibody markers in the clinical practice has led to the discovery of a very high number of "borderline" cases, characterised by celiac-type autoimmune serology and mild intestinal lesions or normal small intestinal architecture, that can be classified as potential CD (PCD). Therefore, it is evident that the "old CD" with flat mucosa is only a part of the spectrum of gluten-sensitive enteropathy. It is possible to speculate that serology can identify CD in its early stages before the appearance of a severe intestinal damage. In these cases with a positive serology, but with mild or absent intestinal lesions the detection of HLA-DQ2 and/or -DQ8 can be of help to reinforce or exclude the diagnosis of PCD.

A malabsorption syndrome can be observed in other disorders which can cause a non-gluten dependent villous atrophy and require a differential diagnosis with CD, including Whipple disease, eosinophilic enteritis, giardiasis, autoimmune enteropathy, common variable immunodeficiency and drug enteropathy (NSAIDs, sartans). Autoimmune enteropathy, a life-threatening disorder destroying the intestinal wall, can be distinguished from CD for the finding of anti enterocyte autoantibodies, whereas the diagnosis of Whipple disease, a disorder much more frequent in men, can be confirmed by the demonstration of *Tropheryma Whippelii* by PCR in body fluids and in small bowel biopsy. Common variable immunodeficiency, characterized by decreased levels of at least two serum immunoglobulin (Ig) isotypes and recurrent infections of the respiratory and gastrointestinal tracts, represents a serious pitfall for the differential diagnosis with CD since this condition can falsely mimic CD but in some cases it is always associated with CD. Giardiasis, a protozoan disorder causing severe diarrhea, weight loss and abdominal pain, can be diagnosed for the finding of the protozoa in the stools and in small biopsy specimens. Notably, positivity for tTGA at low titer has been sometimes observed in patients with giardiasis as a confounding factor for CD. Eosinophilic gastroenteritis displays a severe malabsorption syndrome with diarrhea, abdominal pain and severe anemia, and it can be identified by the increased number of circulating eosinophils (not always present), but with a higher accuracy by the eosinophilic small intestinal infiltration. Recently, a malabsorption syndrome with diarrhea and weight loss has been detected in patients with high blood pressure treated by sartans

(olmesartan). Similar effects were observed in patients treated by means of NSAIDs.

The spectrum of gluten related disorders has recently acquired a new syndrome defined as NCGS². Both innate and adaptive immunity seem to play a pathogenic role in this syndrome which recognizes a wide spectrum of gastrointestinal symptoms (IBS-like) and extra-intestinal manifestations, triggered by gluten in patients without CD and WA. Symptoms disappear in a few hours or days after gluten withdrawal and recur rapidly after gluten ingestion. Despite the absence of a severe intestinal damage, patients with NCGS can present laboratory signs of malabsorption including low levels of ferritin, vitamin D-25-OH, folic acid and vitamin B12, although to a lesser extent than those observed in CD. Besides gluten, other wheat proteins as well as fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) may contribute to this syndrome. This syndrome occurs mainly in young women, being rare in children. Epidemiological data are still scant and approximate with a prevalence ranging from 0.6% to 6%, based on primary or tertiary care center estimates. No biomarker is available, but half of patients tests positive for IgG anti-gliadin antibodies, which disappear quickly after gluten-free diet together with symptoms. Differently from CD, genetic markers are still undefined. Although currently limited to a research setting, double-blind, placebo-controlled, cross-over trial strategy is recommended to confirm the diagnosis.

WA is a food allergy occurring more frequently in childhood (0.4%-1%) and disappearing in about 70% of cases at the age of 14 years³. Wheat ingestion elicits typical IgE-mediated reactions of immediate onset, including also intestinal symptoms, i.e. diarrhea and abdominal pain, together with urticaria, angioedema, bronchial obstruction or, in severe cases, systemic anaphylaxis. Six major foods – milk, egg, soy, wheat, peanut, and tree nuts - account for over 80% of the reactions in food allergies.

As for non-immune-mediated food intolerances, the most frequent form is lactose intolerance, which can be defined as congenital or acquired deficiency of lactase, the enzyme required for digesting lactose⁴. Lactose intolerance is much more common in African Americans and Asians (up to 80%) than in Caucasians (up to 50%). Clinical picture is characterized by flatulence, abdominal pain and diarrhea. The diagnosis relies on lactose breath test and genetic testing. Fructose intolerance can manifest in two forms: a genetic aberration termed “hereditary fructose intolerance”, resulting from a deficiency of the hepatic enzyme fructose-1-phosphate aldolase, affecting 1 in 20000 individuals in western countries, or an incomplete fructose absorption (more common in adults and referred to as fructose malabsorption) in which the capacity of the gut to transport fructose across the intestinal epithelium is exceeded⁵. The clinical picture is characterized by vomiting, diarrhea, hypoglycemia and liver/kidney impairment. Diagnostic tests are the fructose breath test and genetic testing.

In latest years it has been observed the growing introduction in the clinical practice of alternative tests for food intolerance such as the cytotoxic test, the provocation/neutralization sublingual or subcutaneous test, the heart-ear reflex test, the kinesiology, the biorisonance, the electro-acupuncture, and the hair analysis, or immunological tests (immunocomplex or specific food IgG). These tests have not been validated and they are not evidence-based. Physicians should alert patients about the risk of an indiscriminate use of these test in the diagnosis of food allergy. In fact the use of an incorrect diet could be dangerous causing nutritional deficiencies.

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Diseases related to food ingestion Malabsorption, histopathological aspects. Where to begin and where to finish the role of the pathologist. Food intolerances, all that is not celiac. Where to begin and where to finish the role of the pathologist.

C. Baronchelli, V. Villanacci, S. Manenti, M. Salemme
Anatomia Patologica, Spedali Civili Brescia, Italy

In recent years, on the base of the refinement and dissemination of instrumental methods and laboratory tests there has been a significant increase in the number of diagnoses of celiac disease and related disorders at the same time in the first instance food intolerances similar to celiac disease.

The audits provide an active participation and cooperation from the pathologist in close contact with the clinician and the laboratory technician in the exact definition and diagnosis of such conditions. The food-related diseases session provides an overview on the right approach to the execution of biopsies but also a precise definition of the histopathological criteria that allow an accurate diagnosis of celiac disease in particular that should be remembered is a treatable but incurable. We will therefore describes the current classifications in use as well as the most recent proposals associated with the precise criteria that allow a differential diagnosis with other conditions and above all the complications of the disease such as Collagenous Sprue, Refractory Celiac disease, Ulcerative Jejunitis and Lymphoma.

In the category of food intolerance it is underlined the growing role of eosinophils, in the pathogenesis and diagnosis as a crucial element in defining these conditions and the recent amount of Non Celiac Gluten Sensitivity.

The session ends with a display of some emblematic cases of pathological conditions above.

Una finestra sulla prevenzione: il progetto italiano E. A. T.

L. Morricone

Paper not received

Aula Gialla 1 – 15.00-19.00

Patologie apparato digerente e pancreas

Neoplasie esocrine del pancreas: approccio multidisciplinare

MODERATORE: G. Zamboni (Verona)

Radiologia

R. Pozzi Mucelli

Paper not received

Chirurgia

C. Bassi

Paper not received

Patologia

P. Capelli

Paper not received

Neoplasie endocrine del pancreas

MODERATORE: C. Capella (Varese)

Patologia

S. La Rosa¹, R. Maragliano², S. Uccella², F. Sessa²,
C. Capella²¹*Dept. of Pathology, Ospedale di Circolo, Varese, Italy*²*Dept. of Surgical and Morphological Sciences, University of Insubria, Varese, Italy*

Pancreatic neuroendocrine neoplasms (PanNEN) are composed of cells showing neuroendocrine differentiation and expressing typical markers of the diffuse neuroendocrine system like synaptophysin and chromogranin A. The account for about 1-2% of pancreatic neoplasms with an annual incidence of 0.2-2 cases per million population. However, their incidence has been increased over the last 40 years like the general incidence of gastrointestinal NENs with a documented high prevalence. Such finding likely reflects a better knowledge and recognition of these tumor types by both oncologists and pathologists, as well as the improvement of diagnostic tools, such as endoscopy, serum determination of chromogranin A, and search for the 2A subtype of somatostatin receptors either in vivo or in tissue samples. For these reasons it is not surprising that the increased incidence of PanNENs is mainly due to the diagnosis of nonfunctioning neoplasms.

PanNENs are extremely rare in infancy. The mean age of patients is 50 years (range between 30 and 60 years). The 5-years and 10-years overall survival is 82.7% and 73.6%, respectively. In about 5-10% of cases PanNENs arise in the context of hereditary syndromes: MEN1 syndrome, von Hippel Lindau (VHL) syndrome, and tuberous sclerosis. In these cases patients are younger.

PanNENs are a heterogeneous group of neoplasms showing different clinical, morphological, immunohistochemical and molecular features. Following the criteria proposed in the 2010 WHO classification, PanNENs are divided into the following 4 categories, independently of the clinical functional status: i) grade 1 neuroendocrine tumor (NET G1, < 2 mitoses x 10HPF, <2% Ki67); ii) grade 2 neuroendocrine tumor (NET G2, 2-20 mitoses x 10 HPF, 3%-20% Ki67);

iii) neuroendocrine carcinoma (NEC, >20 mitoses x 10 HPF, Ki67>20%) which, by definition, is grade 3; iv) mixed adononeuroendocrine carcinoma (MANEC) which includes the following entities: mixed ductal-neuroendocrine carcinoma, mixed acinar-neuroendocrine carcinoma, and mixed ductal-acinar-neuroendocrine carcinoma. With the exception of MANEC, which shows peculiar morphological features, the classification of the other three tumor types is mainly based on the morphological and proliferative features. G1 and G2 NETs, which correspond to well differentiated neuroendocrine tumors/carcinomas of the 2000 WHO classification, are composed of monomorph cells with mild atypia, rare mitoses, "salt and pepper" nuclear features and finely granular eosinophilic cytoplasm. Atypical nuclei with evident nucleoli can be observed in some cases, especially in G2 NETs which often show histological signs of aggressiveness, including vascular and perineural invasion. The architectural pattern of growth is more frequently trabecular even if, in several cases, pseudoglandular and acinar structures or solid nests may be observed. A diffuse structure, characterized by solid sheets of cells, is much more frequently found in NECs which are composed of atypical cells with necrosis and high mitotic index. The differential diagnosis between NETs and NECs is crucial because of the relevant clinical and prognostic implications. Indeed, surgery is the first therapeutic choice for NETs even when they are metastatic at diagnosis. Conversely, chemotherapy is the first therapeutic choice for NECs. These two neoplastic categories show a very different prognosis: the 5-years survival is 80% for NETs, while the mean survival of patients with NECs is about 11 months.

In addition to PanNETs showing the above described morphological features, there are PanNETs with unusual morphological characteristics. Oncocytic NETs are composed of cells with abundant eosinophilic cytoplasm. Clear cell NETs are composed of cells with abundant clear and vacuolated cytoplasm with numerous lipid vacuoles giving a typical foamy appearance. These NETs may give rise to diagnostic difficulties, especially when diagnosed on small biopsy specimens, and need to be differentiated from adrenal neoplasms,

clear cell carcinoma or signet ring cell carcinoma. Although clear cell NETs have been first described in VHL patients, they have been also successively observed in patients without hereditary syndromes or in MEN1 patients. Pleomorphic cell NETs are composed of cells with marked nuclear atypia which, however, is not associated with a worse prognosis. This specific subtype needs to be differentiated from high grade ductal adenocarcinomas or undifferentiated carcinomas. The morphological features do not predict the hormonal production that can be detected using immunohistochemistry with antibodies directed against the four islet hormones (insulin, glucagon, somatostatin, and pancreatic polypeptide) and, in specific clinical settings, against ectopic hormones like gastrin, serotonin, calcitonin, growth hormone releasing factor (GRF), VIP, ACTH, and PTH. The choice of the antibody depends on the clinical picture.

In the last ten years, immunohistochemical markers of aggressiveness, including cytokeratin 19 and CD117, have been proposed but their prognostic role remains to be elucidated. For this reason, their use in the routine diagnostic pathway is not recommended.

Tumor staging represents a useful tool to the prognostic stratification of patients and, for this reason, it needs to be included in the pathology report. A TNM staging system for PanNENs was proposed for the first time in 2006 by the European Neuroendocrine Tumor Society (ENETS TNM). Successively, the International Union for Cancer Control (UICC) developed a TNM staging system, which is now endorsed by both the American Joint Cancer Committee and the World Health Organization (AJCC/WHO 2010 TNM). However, the tumor definition and derived stages of the ENETS TNM and the UICC/AJCC/WHO 2010 TNM staging systems are definitely different. Specifically, the UICC/AJCC/WHO 2010 TNM is the same as for the ductal adenocarcinoma and is not meant for high-grade neuroendocrine neoplasms. A recent European investigation enrolling 1072 resected PanNENs demonstrated that the ENETS TNM staging system is superior to the UICC/AJCC/WHO 2010 TNM staging system in stratifying patients in different prognostic categories.

Biologia Molecolare

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Pancreatic neuroendocrine tumor (PanNET) is a relatively rare heterogeneous disease. The lack of suitable pre-clinical models has significantly hampered the development of novel biomarker and targets. Thus, complete surgical resection has represented the only potentially curative, and only few therapeutic options have been available treatment for many years. The recent biologic understanding of PanNET molecular landscape is of the utmost importance for research aimed at finding innovative, tailored therapies for this disease. Somatostatin analogues have been demonstrated to have the potential not only to control symptoms of hormone hypersecretion but also have the ability to slow tumor growth in patients with advanced tumors. More recently, exome-wide sequencing efforts have identified the chromatin remodeling pathway (MEN1, DAXX, ATRX) as a major driver of these tumors. However, those studies also evidenced the existence of a significant proportion of PanNETs for which no recurrent genetic alterations can be identified. Deregulation of druggable pathways, including Akt/mTOR, has been used to design targeted-therapies that have proven efficacy in recent clinical trials. The next step will be the correlation of these molecular findings with PanNET-dedicated clinical trials to identify predictive biomarkers for the planning of tailored surgical or medical management of PanNET patients.

Terapia medica

N. Fazio

Paper not received

Aula Gialla 2 – 16.00-17.20

Patologia polmonare

MODERATORI: O. Nappi (Napoli), G. Rossi (Modena)

La classificazione WHO 2015 dei tumori polmonari: Highlights

G. Pelosi

Paper not received

Seminario integrato dei Gruppi di Studio GIPP e GYM

MODERATORE: P. Graziano (San Giovanni Rotondo)

Immunoistochimica delle lesioni non neoplastiche del polmone

M. Chilosi

Paper not received

Immunoistochimica dei markers diagnostici e predittivi nei tumori: un update

C. Doglioni

Paper not received

Valutazione immunoistochimica del Ki67 nei tumoreneuroendocrini polmonari: problematiche e criticità

R. Monaco

Paper not received

Seminario: problematiche dei trapianti polmonari

MODERATORE: A. Cancellieri (Bologna)

Il ruolo del patologo nel trapianto del polmone

F. Calabrese

Istituto di Anatomia Patologica, Università degli Studi di Padova

Lung transplantation is now an accepted treatment for a variety of end-stage of pulmonary conditions. The follow-up and care of lung recipient requires the involvement of skilled multidisciplinary team and pathologist plays a key role in different transplant times.

In the first phase- pre-transplant time- the pathologist can make an important contribution to the final diagnosis of some respiratory disorders particularly those with clinical/radiological controversial features (e.g. in the field of idiopathic interstitial lung disease)¹. No less important is the support that the pathologist can provide in the exclusion of complications (such as lung cancer) which can be several types of different types of end-stage lung diseases (it has been estimated a rate ratio of 4.96 for developing lung cancer in IPF patients compared to the general population).

In the transplant time accurate pathological diagnosis of explanted lungs has enabled more accurate diagnoses to be made in many cases and effectively constitutes an audit of the thoracic medical practice of both the referring and specialist

transplant centers. Significant discrepancies between the final pathological diagnosis on the explanted lungs and the referral diagnosis was reported by two experienced lung transplant centers^{2,3} Both Authors have highlighted the importance of unexpected explant pathology on recipient management.

Surely the phase where the pathologist plays the most important diagnostic contribution is the post-transplant time. The pathologist has a role to play in this process in terms of biopsy diagnosis of, and research into, the pulmonary and systemic complications (Table 1) and in contributing to clinical audit through postmortem examination. Transbronchial biopsy and bronchoalveolar lavage are the specimens routinely used for immunological and infective complications, respectively⁴. The pathologist must ensure that the clinical requirement for a rapid biopsy reporting service, including an on-call service, can be met. In addition to standard technology, ancillary techniques including immunohistochemistry and molecular analyses such as in situ hybridization and polymerase chain reaction (PCR) should be performed in order to better characterize various complications such as new forms of rejection (e.g. antibody mediated rejection)⁵, post transplant lymphoproliferative diseases and several forms of respiratory viral infection⁶.

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Tab.1 Frequent Complications of Lung Transplantation Relevant to the Pathologist

Instantaneous (<2 days)
Reimplantation injury
Hyperacute rejection
Haemorrhage or other surgical complications
Post operative (<30 days)
Infection
Multiorgan failure
Acute rejection
Early (1-6 months)
Infection
Acute rejection
Drug toxicity
Lymphoproliferative disorders
Graft-versus-host disease
Long-term (>6 months)
Infection
Obliterative bronchiolitis/or other signs of chronic lung allograft dysfunction
Drug toxicity
Acute rejection
Neoplasia
Recurrent disease

Rigetto, infezione, altre entità: ruolo della biopsia trans-bronchiale

L. Delsedime

Paper not received

Complicanze neoplastiche frequenti e rare nel polmonetraplantato: dall'istologia ai biomarcatori

P. Morbini

Paper not received

Biopsia trans-bronchiale post-trapianto "pitfalls": presentazione di un caso

N. Nannini

Azienda ospedaliera di Padova, Italy

Case presentation

A 16-year-old female underwent lung transplant for cystic fibrosis in June 2003. The recipient presented cough, dyspnea and asthenia 10 months after transplantation. At high resolution computed tomography multiple bilateral nodular consolidations were seen. All microbiological analysis (viral, bacterial and fungal infections) were negative. At the fourth scheduled trans-bronchial biopsy (TBB) and bronchoalveolar lavage (BAL) no rejection (AOB0) and/or infections (viral, bacteria, fungi) were found. In the TBB only a bronchoalveolar lymphoid tissue (BALT) hyperplasia was detected. Due to significant respiratory function deterioration a CT fine needle biopsy was performed. At histology/immunohistochemistry, biopsy appeared as necrotic tissue infiltrated by medium/large nucleolated lymphoid cells (CD20+: 95%) with scattered accompanying small lymphocytes (CD3+: 5%). Molecular analysis, using polymerase chain reaction (PCR) and in situ hybridization (ISH) for Epstein Barr Virus (mRNA/EBER), showed a strong positivity for the virus in many B lymphocytes. PCR for V-D-J immunoglobulin heavy chains clonality showed a biclonal B cell proliferation. Thus the final diagnosis was of large B cells post-transplant lymphoproliferative disorder (PTLD).

Discussion

PTLDs constitute a spectrum of lymphoproliferation that follows immunosuppression for solid organ transplantation and bone marrow transplantation¹. Specific risk factors include the type of transplant, young age*, number of rejection episodes, multiagent immunosuppression, use of T-cytolytic therapy and pretransplant EBV seronegativity* -as in our case-². The overall incidence of PTLDS for lung and heart-lung recipients is about 9%³. The majority of cases occur in the first four years following transplantation and are associated with active or latent EBV infection*-as in our case-⁴. The source of EBV

Tab. I. Categories of PTLD, taken from WHO 2008 [6]

The pathological diagnosis of PTLD is made on evaluation of cell morphology and immunophenotype, immunoglobulin clonality (PCR for heavy chain gene rearrangement is the gold standard) and detection of EBV DNA or RNA (ISH for EBER on paraffin sections).

Early lesions¹ Plasmacytic hyperplasia Infectious mononucleosis- like lesion	9971/1
Polymorphic PTLD	9971/3
Monomorphic PTLD² (Classify according to lymphoma they resemble)	
B-cell neoplasms Diffuse large B- cell lymphoma Burkitt lymphoma Plasma cell myeloma Plasmacytoma-like lesion Other ³	
T-cell neoplasm Peripheral T-cell lymphoma, NOS Hepatoplenic T-cell lymphoma Other ³	
Classical Hodgkin lymphoma-type PTLD²	
¹ Some mass-like lesions in the post-transplant setting may have the morphologic appearance of florid follicular hyperplasias or other marked but non-IM-like lymphoid hyperplasias. ² ICD-O codes for these lesions are the same as those for respective lymphoid or plasmacytic neoplasm. ³ Indolent small B-cell lymphomas arising in trans- plant recipients are not included among the PTLD	

in early PTLD is usually primary infection in the recipient, especially in children⁵, although PTLD of donor origin may rarely occur³. PTLD is thought to result from uncontrolled proliferation of EBV-immortalized B cells owing to loss of the cytotoxic T-cell control because of immunosuppression for the prevention and treatment of acute allograft rejection⁴. Disease regression may occur once underlying immunosuppression is reduced or reversed¹. The morphology of PTLD ranges from plasma cell hyperplasia and infectious mononucleosis-like changes to appearances identical with large B-cell lymphomas*-as in our case-. Classification systems developed over the past 20 years have identified three major morphological groups – “early”, polymorphic and monomorphic. The majority are of B-cell origin* -* as in our case-¹.

Tab. II. Pathological evaluation of specimens for diagnosis of PTLD, taken from WHO 2008 [6]

PTLD may involve the transplanted or native lung in isolation* or as a part of disseminated disease -* as in our case-[7]. When confined to the lung, it may be asymptomatic or associated with cough*, fever* and malaise* -*as in our case-.

Method of evaluation	Necessity	Purpose
Histopathology	Essential	Evaluate architecture and cytologic features, required for classification
Immunophenotype	Essential	Assess possible light chain class restriction* and basic lymphoid subsets, require for classification
EBER <i>in situ</i> hybridization	Essential**	Most sensitive method for assessing if PTLD is EBV-positive, aids in diagnosis and possibly prognostication, useful in differential diagnosis with rejection in allograft (if positive)
Genetic/cytogenetic studies	Variable	Determine clonality, lineage of clonal population(s), chromosomal and oncogene abnormalities, may be needed for classification
EBV clonality	Not required	Identification of minor clones

* Paraffin section immunohistochemistry will often fail to demonstrate monotypic B-cell populations, even if present, unless there is plasmacytic differentiation.
** If the less sensitive EBV-LMP1 stain is positive EBER *in situ* hybridization is not required

Tab. III. Criteria used in the categorization of PTLD, taken from WHO 2008 [6]

Difficulties encountered in diagnosis, especially on core biopsies, stem from the small size of the sample, the risk of crush artefact and the tendency for pulmonary PTLD to show extensive necrosis because of angioinvasion. The differential diagnosis of PTLD includes infections such as cytomegalovirus or *Pneumocystis carinii*, inflammation related to previous biopsy sites, other pulmonary lymphoid disorder such as lymphoid interstitial pneumonia, bronchus-associated lymphoid tissue and in transplanted lung, acute cellular graft rejection and, rarely, graft versus host disease. Thus the diagnosis of PTLD is a possible pitfall on TBB* -*as in our case-.

Pathologic category	Histopathology		Immunophenotype/ <i>In situ</i> hybridization	Genetics	
	Architectural effacement	Major findings		IgH@/TCR	Other abnormalities
Early lesions	Absent	Small lymphocytes, plasma cells, ± immunoblasts, ± hyperplastic follicles	Pcl B cells & admixed T cells; often EBV+	Pcl or very small mcl B-cell population(s)	None
Polymorphic PTLD	Present	Full spectrum of lymphoid maturation seen	Pcl or mcl B cells & admixed T cells; often EBV+	Mcl cells, non-clonal T cells	Some have BCL6 somatic hypermutations
Monomorphic PTLD Usually present Fulfills criteria for a NHL (other than one of the indolent B-cell neoplasm) or plasma cell neoplasm			Varies based on type of neoplasm they resemble. EBV more variable than in other categories.	Clonal B cell and/or T cells (except for rare NK cases)	Usually present
Hodgkin lymphoma type PTLD	Present	Fulfills criteria for CHL			

Pcl, polyclonal; Mcl, monoclonal; NK, natural killer cell; TCR, T-cell antigen receptor.

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Aula Gialla 3 – 15.00-18-00

Patologia endocrina: Innovazioni e controversie in Patologia Endocrina (GIPE)

MODERATORI: F. Basolo (Pisa), G. Pelosi (Milano)

Carcinomi neuroendocrini (NEC) gastro-entero-pancreatici: diagnosi e implicazioni cliniche

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²Department of Surgical and Morphological Sciences, University of Insubria, Varese, Italy

General concepts

The gastrointestinal tract and the pancreas are, taken together, the most frequent primary site of human neuroendocrine neoplasms. The term neuroendocrine neoplasm (NEN) is used to define neoplastic proliferations characterized by a neuroendocrine phenotype, identified by mean of morphology alone or with the application of ancillary techniques, mainly immunohistochemistry, and, more rarely, electron microscopy. However, although they share a number of morphological and immunohistochemical features, NENs encompass a very heterogeneous spectrum of neoplasms, ranging from well dif-

ferentiated, low proliferating (0-20 mitoses x10 HPF or Ki67 index <20%) and clinically indolent tumors (well differentiated neuroendocrine tumors – NETs) to poorly differentiated, highly proliferating (>20 mitoses x10 HPF or Ki67 index >20%), clinically aggressive carcinomas (poorly differentiated neuroendocrine carcinomas – NECs). In addition, gastroenteropancreatic (GEP) NENs include a group of mixed neoplasms, composed of a neuroendocrine (mainly NEC) and a non-neuroendocrine (more often adenocarcinoma, signet ring cell carcinoma, or rarely, squamous cell carcinoma) components. If each of these components represents more than 30% of the neoplastic mass, the tumor is called mixed adenoneuroendocrine carcinoma (MANEC), according to the WHO classification criteria. The histopathological differential diagnosis between NETs and NECs is generally straightforward and can often be performed just on routine hematoxylin and eosin-stained slides, with the help of Ki67 immunostaining. By contrast, immunohistochemistry for neuroendocrine markers is often mandatory to differentiate NECs from other non-neuroendocrine high-grade

carcinomas. Interestingly, several molecular studies have been published in the last few years demonstrating that NECs show a biological profile more similar to that of non-neuroendocrine epithelial carcinomas arising in the same site, than to that of NETs. These data shed light on the different clinical behavior, both in terms of patients' survival and in terms of response to therapy, between NETs and NECs.

Morphological and immunohistochemical bases for the diagnosis

GEP NECs show morphological and clinical features similar to small cell and large cell neuroendocrine carcinomas of the lung. Small cell carcinomas are characterized by a diffuse proliferation with large areas of necrosis, of round or oval small to intermediate cells (2-4 times the size of a small lymphocyte), with indistinct cell borders, a narrow rime of cytoplasm and a hyperchromatic nucleus with an inconspicuous nucleolus. Mitotic and apoptotic index are very high. In the large cell subtype, the neoplastic proliferation may be diffuse or vaguely organoid and is composed by large cells, with abundant eosinophilic cytoplasm, vesicular nuclei with prominent nucleoli. Vascular and perineural invasion are common in both subtypes and these neoplasms have a high tendency to deeply infiltrate the gastrointestinal wall and to extend into the soft tissues outside the pancreas.

Immunohistochemical stains for general neuroendocrine markers are a cornerstone for the diagnosis of GEP NECs and for their distinction from other poorly differentiated neoplasms. The most sensitive and specific general neuroendocrine markers are synaptophysin and chromogranin A. However, this latter marker may be absent or faintly and focally expressed in NEC. In addition, the transcription factor achaete-scute homolog 1 (ASH1), which is expressed in pulmonary and extra-pulmonary NECs and almost absent in NETs, has been proposed as an additional marker for NECs. The identification of the primary site of an occult NEC presenting with metastatic disease may be a challenging issue both for the oncologist and pathologist. In the last few years, several new immunohistochemical markers, mostly belonging to the homeobox family of transcription factors including CDX2, TTF1, PDX1, and ISL1 have been proposed. However, while these markers have proven to be affordable in NETs, their use in NECs is biased by their frequent aberrant expression outside their physiological site and should be considered with caution.

Prognostic evaluation and clinical aspects.

Biological and clinical aggressiveness of GEP NECs is a well known matter of fact. Notwithstanding, it has recently been shown that a subset of patients with these neoplasms are unexpectedly long survivors. This observation has risen the need to identify new prognostic markers, which are capable of stratify patients with NECs in prognostic groups with a different therapeutic requirements.

Among immunohistochemical markers, CD117 expression is a prognosticator of worse prognosis. However, as CD117 overexpression in NECs seems not to be related to c-kit gene mutation, imatinib mesilate (Glivec) is not a therapeutic option in these patients. By contrast, microsatellite instability and the loss of function of mismatch repair (MMR) proteins have been related to a longer survival in GEP NECs. Thus, the immunohistochemical stains for MMR proteins may possibly be included in the diagnostic work up of GEP NECs. Anyway, further studies are needed to identify prognostic markers and to evaluate their accuracy.

Finally, an emerging issue in the classification and prognos-

tic evaluation of these tumors has risen in the last few years from the application of the latest WHO classification of GEP NECs. In fact, a subset of tumors has been identified, in which morphology and proliferation index are discordant. In particular, there are neoplasms with well to moderately differentiated morphology and a Ki67 index higher than 20%, the clinical behavior and response to medical therapy of which are still to be fully understood.

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Nuove linee guida ATA per i tumori tiroidei

G. Tallini

Paper not received

Next generation sequencing: implicazioni in patologia endocrina

A. Scarpa

Paper not received

NET polmonari: Problematiche aperte

M. Papotti, L. Righi, M. Volante

Università di Torino

According to the new WHO classification appeared in March 2015 [Travis 2015], neuroendocrine tumors (NET) of the lung have been incorporated in a separate new category and classified into low grade typical (TC), intermediate grade atypical carcinoids (AC), and high grade poorly differentiated neuroendocrine carcinomas of the large and small cell types. Morphological criteria were confirmed as the only appropriate ones for identifying the different types, combining architecture (organoid versus diffuse growth) and cytological features (cell size, atypia, mitotic count, necrosis). The most relevant change is the reclassification of the large cell NE carcinoma (LCNEC) type from the group of large cell carcinomas (indeed a vanishing category) to a new group including all the lung NETs. “Combined neuroendocrine carcinomas” are also rarely observed in the lung and are characterized by the coexistence of a NE component of the small or large cell type and area of adenocarcinoma (more rarely squamous cell carcinoma). Such tumors usually bear a similar genetic profile in the two components, indicating that a single neoplasia developed different phenotypes as a result of divergent differentiation. This latter may be spontaneous or can follow chemotherapy, which may favor the selective growth of a NE differentiated tumor cell population.

Most diagnostic difficulties derive from the analysis of small biopsies or cytological samples, in which the scant amount of tumor cells may hamper a correct interpretation. While the definition of the NE nature may not be difficult, thanks to the demonstration of specific NE markers NE (including

lack of high molecular weight cytokeratins in all NE tumors from any location), less reliable in such materials is the definition of the histotype, especially the separation of low grade carcinoids from high grade NE carcinomas. In this respect, surprisingly, the most useful diagnostic marker turned out to be Ki67, which maintains its nuclear distribution even in tumor areas with crushing artifacts, as commonly observed in biopsies, thus allowing to take carcinoids apart from small cell lung cancer (SCLC) [Pelosi 2005]. The proposed classification scheme is strongly predictive of patients’ prognosis but relies on few and sometimes scarcely reproducible pathological parameters (in case of low mitotic count and/or focal/spotty necrosis), which have been demonstrated to affect the inter-observer agreement of the classification. Moreover, tumor and nodal staging schemes are not specific for lung carcinoids, at variance with neuroendocrine tumors of the gastro-entero-pancreatic system, despite these tumors have specific features that strongly differ from conventional lung cancer. Finally, there is no grading for lung neuroendocrine neoplasms and prognostication. The literature data indicate that although the evaluation of Ki-67 proliferation index has not been officially incorporated in the new WHO criteria, it is in any case a reliable and useful tool to predict the behavior. In a meta-analysis of the over 1800 lung NETs analysed for their Ki67 index, it was observed that mean ki67 values overlapped those of similar NETs in other organs, with approximately 1.5% for TC, 7% for AC, 50% for LCNEC and 60% for SCLC. In a recent study [Rindi et al 2014], the efficacy of the combined use of ki67 with the two “official” diagnostic parameters (mitotic count and necrosis) for the classification of lung NET was investigated: resetting the Ki67 cutoff values at 4% and 25% (compared to the values used for intestinal & pancreatic NETs), a three-tier grading system was developed, based on the presence of at least two of the three parameters (mitoses, necrosis & ki67), with a more homogeneous prognosis and significant survival differences. Therefore, although the current classification is accepted world-wide, familiar to clinicians and prognostically relevant, an improved homogenization of lung, gastrointestinal and pancreatic NET classifications would be highly welcome.

Nuovi e rivisitati marcatori prognostici in patologia surrenalica

G. Nesi

Paper not received

Giovedì 24 settembre 2015

Aula Blu 1 – 10.00-12.00

Ematopatologia: Infection related lymphoproliferative disorders and lymphoma I (GIE)

MODERATORI: M. Paulli (Pavia), S. Pileri (Bologna)

Castleman disease and HHV8-related lymphomas

K. N. Naresh

Paper not received

EBV-related lymphomas

L. Leoncini

Paper not received

HIV related lymphoma

M. Raphael

Hematology, University Paris Sud, France

HIV infection has been associated with an increased risk of lymphomas including non Hodgkin Lymphomas (NHL) and classical Hodgkin lymphoma (cHL). NHL, one of the criteria defining AIDS include mostly aggressive B-cell lymphomas usually diagnosed in immunocompetent (IC) patients as well as those more specifically described in HIV infection according to the description of WHO classification¹. Low grade lymphomas, are rarely observed and are not included in the AIDS definition, however, in HIV/HCV co-infected patients, marginal zone lymphomas are described suggesting the role of chronic antigenic stimulation². The frequency of cHL, the second more common non AIDS defining cancer (after lung cancer) already reported before highly active antiretroviral therapy (HAART) and the combination Antiretroviral Therapy (cART) is expected to increase the next years³.

The incidence of high grade B-cell lymphoma such as primary brain lymphoma (PBL), Burkitt Lymphoma (BL) and Diffuse large B-cell lymphoma (DLBCL) which has been reported since the emergence of HIV infection has changed since the era of HAART and cART with a dramatically fall of PBL. However increased risk of NHL remains strongly correlated to the severity of the underlying immunodeficiency and the rate of CD4 cells count. Since the HAART and cART, HIV infected patients has experienced improvement in their morbidity and survival. In fact, individuals having lymphoma continue to improve and their survival tends to be similar to non HIV patients especially in cHL patients⁴. At clinical level, HIV related lymphomas display a marked propensity to involve extra nodal sites such as gastro-intestinal tract, liver, bone marrow, but all extra nodal sites could be infiltrated by lymphomatous cells. Some sites are more specific as oral cavity in patients who develop plasmoblastic lymphoma. Advanced clinical stage and marked elevated LDH are frequent. DLBCL with immunoblastic features associated with EBV are more frequently associated with low count of CD4 cells than BL¹. We will describe the different categories of NHL and cHL according to the WHO description, their viral association and

hypothesis of their pathogenesis.

HIV related lymphomas are heterogeneous in terms of etiology and pathogenesis related to the severity of immunodeficiency and the role of herpes viruses: Epstein –Barr virus (EBV), and KS-associated virus or human herpes virus8 (KSHV/HHV8), the chronic antigenic stimulation, the altered cytokine expression, the genetic abnormalities including mostly MYC, BCL6, tumour suppressor genes and genomic complexity by interstitial deletions and aberrant methylation of regulative regions and finally the possibility of a direct role of HIV via the HIV Tat protein expression^{5,6,7}.

The histological subcategories concern essentially the aggressive B-cell lymphomas histologically close to those described in IC patients: DLBCL and BL. DLBCL contain numerous centroblasts admixed with immunoblasts with some plasmacytic differentiation¹. Looking at the subclassification into germinal center (GC) or non-Germinal Center (Non-GC), they display predominantly Non-GC phenotype expressing Mum1/IRF4 marker without expression of CD10 or BCL6, however, these subcategories GC or Non-GC do not affect survival. Some cases having a high proliferative index (Ki67>60%) are associated with improved survival⁸. Except in PBL associated with EBV in almost all cases, EBV is present in about 30 to 40% of DLBCL but less frequent in GC subcategory without any impact on clinical response. Most of these systemic DLBCL characterized by mutations of immunoglobulins genes are associated with BCL6 genes rearrangement and/or mutations and the recent genome wide DNA-profiling displayed a distinct genomic profile of HIV-DLBCL compared to IC-DLBCL^{5,6}. BL in HIV infected patients share the criteria of BL described in IC patients composed of monomorphic medium sized lymphomatous cells, however some cases display plasmacytic features with more abundant and basophilic cytoplasm. EBV is present in about 30% of classical BL. HIV-BL display on going somatic mutations and GC subtype without expression of BCL2 in most cases, the proliferative index determined by the expression of Ki67 is close to 100%. The common genetic abnormalities involve c-MYC alterations by rearrangement and mutations and inactivation of p53^{1,5}.

Primary effusion lymphoma (PEL), a large B-cell neoplasm usually presenting as serious effusion without detectable tumour masses is characteristically associated with KSHV/HHV8 mostly co-infected with EBV. This is one of the specific lymphomas arising in the setting of profound immunosuppression of HIV infected patients. These lymphomas showing a large spectrum from immunoblastic to plasmoblastic and anaplastic features lack of B-cell markers, but express plasma cell-related antigens such as CD138. All the lymphomatous cells expressed the KSHV/HHV8 LANA and the non coding RNA EBER for EBV. The survival is extremely low^{1,9}. Plasmablastic lymphoma, another distinct entity of HIV associated NHL, involving the oral cavity is related to chronic immune stimulation and EBV. The large B-immunoblasts with plasmacytic differentiation expressing cytoplasmic immuno-

globulins in two third of cases display plasma cell phenotype (CD138, CD38, MUM1/IRF4). EBV is present in almost all cases with without KSHV/HHV8¹⁹.

The large B-cell lymphoma in KSHV/HHV8 associated Multicentric Castleman Disease (MCD) is characterized by large plasmablasts expressing IgM arising in the setting of MCD in HIV infected patients. The presence of KSHV/HHV8 in plasmablasts as well as viral chain of IL6 demonstrate the role of this virus in the pathogenesis of this very unfavorable lymphoma¹⁹.

Some polymorphic lymphoid proliferations resembling post-transplant lymphoproliferative disorders were also described since the emergence of HIV infection, but their frequency was low without any change of the frequency since the HAART and cART

Rare T-cell lymphomas are also described. They are developed from CD4 or CD8 T-cell as well as from natural killer (NK) cells¹.

Beside NHL, cHL which has been described since the emergence of HIV infection but which is not included in the definition of AIDS, shows interesting characteristics. In the setting of HIV infection, cHL present high risk characteristics with frequent advanced stages, however, the outcome of HIV-related cHL have recently shown a more favorable outcome. The histological subcategory is mainly of mixed cellularity with high frequency of EBV association expressing the oncogenic Latent Membrane Protein (LMP1). The most recently striking feature is the higher incidence of cHL while the incidence of NHL is decreasing. These risks are closely related to CD4 cells count.

In conclusion, HIV related lymphomas remain a major complication of HIV infection with remarkable changes since the era of HAART and cART: dramatically decrease of PBL, higher frequency of cHL correlated with intermediate CD4 cells count³. Several features must be pointed out: the persistence of EBV positive BL and DLBCL, the very high association of cHL with EBV expressing the oncoprotein LMP1 despite a moderate or low underlying immunodeficiency¹⁰, the role of chronic antigenic stimulation especially in HIV/HCV co-infected patients and the direct role of HIV via its

proteins, especially Tat⁷. Moreover, the aging of HIV infected patients population must be taken account in the appraisal these lymphomas.

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- ⁸ Chadburn A, Chiu A, Lee JY et al. *Immunophenotypic analysis of AIDS-Related Diffuse Large B-Cell Lymphoma and clinical implications in patients from AIDS malignancies consortium clinical trials 010 and 034*. J Clin Oncol 2009;27:5039-48.
- ⁹ Gloghini A, Dolcetti R, Carbone A. *Lymphomas occurring specifically in HIV-infected patients: from pathogenesis to pathology*. Semin Cancer Biol 2013;23:457-67.
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Detection of infectious agents in lymph node biopsies

C. Parravicini

Paper not received

Aula Blu 1 – 13.00-13.45

Lettura Magistrale Cutaneous eruptions associated with alimentation and nutritional deficiencies

C. Martin Mihm Jr

Paper not received

Aula Blu 1 – 15.00-18.00

Ematopatologia: Infection related lymphoproliferative disorders and lymphoma II

MODERATORI: V. Franco (Palermo), L. Ruco (Roma)

HP and Chlamydia related extranodal marginal zone lymphoma

M. Ponzoni

Paper not received

HCV-related lymphomas

M. Lucioni¹, L. Arcaini², D. Rossi³, G. Croci², R. Riboni¹, V. V. Ferretti⁴, M. Rosa Arra¹, M. Bonfichi⁴, A. Ramponi⁵, G. Gaidano³, M. Cazzola², M. Paulli²¹Department of Pathology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy;²Department of Molecular Medicine, University of Pavia, Pavia, Italy;³Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy;⁴Department of Hematology Oncology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy;⁵Division of Pathology, Department of Health Science, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

Hepatitis C virus (HCV) is a positive single stranded RNA virus belonging to the family of Flaviviruses. It affects millions of patients worldwide and represents a major burden in terms of morbidity, mortality and social costs. Estimated prevalence of HCV infection in Italy is up to 10%, representing one of the highest rates among western countries. It is now well recognized that, in addition to hepatic manifestations, HCV infection is linked to a spectrum of cryoglobulinemic and non-cryoglobulinemic B-cell lymphoproliferative disorders. Many epidemiological studies have shown that hepatitis HCV infection is associated with B-cell non-Hodgkin's lymphomas (B-NHL). The prevalence of HCV infection in B-NHL patients is 15% in comparison with 1,5% in the general population. The association between HCV chronic infection and Lymphoma development is more evident in countries with high HCV seroprevalence, such as Italy, Japan, south of United States. Beside epidemiological data, the most convincing evidence for a direct relationship between HCV infection and lymphoma development is the observation of B-NHL regression after HCV eradication by antiviral treatments. In fact for HCV-positive indolent B-cell NHL, first line antiviral therapy instead of standard immune chemotherapy might be considered. Molecular mechanisms of HCV-related B-NHL development are still poorly understood. Although HCV lymphotropism has been demonstrated in vitro and in some cases in vivo, many evidences rather support the hypothesis of an indirect transformation mechanism based on chronic antigen-driven stimulation, that may induce a multistep development of NHL, evolving from mixed type-II cryoglobulinemia (MC) to overt low-grade B-NHL and eventually to high-grade B-NH. This model mirrors the well-defined *Helicobacter Pylori* related lymphomagenic pathway reported in gastric MALT lymphoma and DLBCL development.

In spite of some discrepancies in the incidence of different lymphoproliferative disorders reported by various studies,

B-NHL most frequently associated with HCV infection are marginal zone lymphoma (MZL), in particular splenic marginal zone lymphoma (SMZL) and diffuse large B cell lymphoma (DLBCL). The association between HCV infection and SMZL has been proposed as a paradigmatic example of a HCV-driven lymphoproliferative disorder. Recently, unbiased genomic studies have unravelled the typical coding genome of SMZL, which is characterized by mutations of genes involved in the physiological differentiation and homeostasis of marginal zone (MZ) B-cell. Indeed, the commitment and retention of mature B-cells to the MZ compartment requires NOTCH2 signaling activation. Consistently, the NOTCH pathway is affected in ~30-40% of SMZL, including mutations of NOTCH2, the master regulator of MZ B-cell differentiation, in ~25% of cases. In addition to NOTCH2, other modulators or members of the NOTCH pathway are recurrently targeted by genetic lesions in SMZL, including NOTCH1, a paralog of NOTCH2, and SPEN, the main negative regulator of NOTCH signaling in MZ B-cells. Disruption of these molecular patterns were found both in HCV positive and HCV negative SMZL. On the contrary, a recent study based on miRNA expression analyses, showed that SMZL is characterized by a specific dysregulation pattern of miRNA expression, with a possible implication in its molecular tumorigenesis, and that miR-26b, a miRNA known to have tumor suppressive properties, is significantly downregulated in HVC+ SMZL.

Also other MZL subtypes arising in HCV+ patients seem to have distinctive clinicopathologic features including a predilection for some extranodal localizations, such as liver, and non-gastric mucosa-associated lymphoid tissues (MALT) sites (i.e. ocular adnexa, skin and major salivary glands). In the past we reported a series of HCV-positive patients who developed unusual primary subcutaneous MZL, with lipoma-like presentation and a relatively indolent course. Extensive molecular and genetic investigations of HCV-related MZL seem to indicate that HCV infection play a causative role also in this lymphoma subset. Some cases of HCV-related MZL of MALT-type resulted to be associated with translocations specific for MALT-type MZL, including the t(11;18)(q21;q21)/(API2-MALT1), the t(14;18)(q32;q21)/(IGH-MALT1), the t(1;14)(p22;q32)/(IGH-BCL10) and the t(3;14)(p14;q32)/(IGH-FOXP1).

In spite of the high frequency of DLBCL in the setting of HCV chronic infection, only few studies have been published on this issue, mainly focusing on clinical aspects and therapeutic problems due to the interaction between chemotherapeutic regimens and liver toxicity. Only one study investigated clinico-pathological correlations in HCV+ DLBCLs, showing that HCV-related DLBCL are characterized by preferential involvement of the spleen and, histologically, by the presence of a residual component of low-grade lymphoma, usually with MZL features. These clinical and pathologic features suggested that at least a fraction of HCV-positive DLBCL may represents the transformation of a pre-existent, though unrecognized MZL clone. However these observations were not supported by molecular and biologic data. Although the

genetics of DLBCL arising in HCV negative patients has been extensively investigated, few data are currently available about the molecular mechanisms involved in the development of DLBCL in HCV subjects.

In order to clarify the molecular pathogenesis of HCV-related DLBCLs, and their possible relationship with SMZL and MZL of MALT-type, recently we investigated a series of 46 de novo DLBCL arising in HCV+ patients, for the presence of mutations of NOTCH pathway signalling and/or MALT1 translocations. FISH analyses to detect MALT1 translocations were performed using MALT1 DNA Probe, Split Signal (Dako), IGH/MALT1 and BIRC3 (API2)/MALT1 Dual Color Dual Fusion probes (Vysis). Three molecular pathways usually involved in MZL and/or DLBCL were analyzed including NOTCH pathway (NOTCH1, NOTCH2, SPEN), NF- κ B pathway (MYD88, BIRC3, IKKKB, TNFAIP3, TRAF3) and BCR pathway (CARD11, CD79A, CD79B). The mutation hotspots were analyzed by PCR amplification and direct sequencing of genomic DNA. For comparative purposes, 64 HCV-negative DLBCL were also included in the study.

Careful histological examination documented that in 12/46 of cases (36%) a minor area of the diagnostic biopsy was composed of small to medium sized monocytoid B-cells. FISH investigations demonstrated MALT1 translocations in 6/45 (13%) cases of HCV+ DLBCL, the partner gene being IGH in 3 cases and API2/BIRC3 gene in 3 cases. MALT1 translocations were more frequently detected in the subgroup with a residual marginal zone-like component (3/12, 25%) than in cases without MZL component (3/33, 9%). Mutational analyses documented that the NOTCH pathway is recurrently mutated in HCV+ DLBCL, NOTCH2 mutations being detected in 9/46 (20%) patients, and NOTCH1 mutations in 2/46 (4%). By contrast, only 1/64 HCV-negative patients had a NOTCH1 or NOTCH2 mutation. Comparison of NOTCH2 mutation prevalence between HCV+ and HCV- DLBCL resulted statistically significant ($P=0.002$). NOTCH pathway mutations were enriched in cases with a component reminiscent of MZL in diagnostic biopsy. NOTCH pathway was mutated in 6/12 (50%) cases with MZL component and in 6/34 (18%) cases without MZL component. NOTCH mutations and MALT1 translocations were mutually exclusive.

The 5-year overall survival was 27% (95% CI, 5%-56%) for HCV-positive DLBCL patients carrying a NOTCH pathway mutation versus 62% (95%CI: 42%-77%) for those without these genetic lesions. By univariate analysis, age >60 years, NOTCH2 mutation, and any mutation of the NOTCH pathway (NOTCH2, NOTCH1, SPEN) were associated with shorter overall survival. In multivariate analysis (multivariate

Cox regression model), only the the adverse prognostic impact of NOTCH mutation was confirmed (HR=2.5; 95% CI: 1.0-5.8; $p=0.041$).

In conclusions our study documented that about 25% of HCV+ DLBCL harbour a minor area of the diagnostic biopsy consistent with MZL. SMZL-associated NOTCH pathway mutations are a molecular clue associated to 25% of HCV+ DLBCL, whereas MALT1 translocation were detected in 13% of HCV+ DLBCL. NOTCH pathway disruption associate with the coexistence of a MZL component in the diagnostic biopsy and poor outcome. Overall these data suggest that at least a fraction of DLBCL arising in HCV+ patients may represent the transformation of a clinically unrecognized, indolent MZL clone.

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Giovedì 24 settembre 2015

Aula Blu 2 – 10.00-12.30

Patologia mammaria: l'Anatomia Patologica e le Breast Unit

MODERATORI: S. Bianchi (Firenze), A. Sapino (Torino)

Il documento ministeriale

L. Catallioti

Paper not received

La formazione del Patologo "Esperto" in patologia mammaria: revisione delle normative europee

F. Pulcini

Paper not received

La discussione multidisciplinare: il ruolo del patologo

I. Castellano

Paper not received

Implicazioni medico legali alla condivisione del documento di indirizzo terapeutico nella discussione medico legale

G. Divella

Sezione di Medicina Legale, DSSPP, Università degli Studi di Torino

Medical liability has become increasingly important in the Italian legal system during the last four decades. This appears to be related to the growth of the National Health Care System (NHCS) and is characterized by a new generation of hospitals staffed by personnel with varied levels of competencies. The transition from the individual medical providers to the collective partnerships has complicated care delivery. The medical field has been impacted by the rapid expansion of jurisprudence in the realm of medical malpractice and increasing complexity of the justice system, and health care consumers are better informed about their legal rights. The relationship between the physician and the citizen has changed and patients have become more proactive, contacting the physician, the hospital, insurance providers or a lawyer when they believe that something went wrong during their treatment. Definitive statistical data are not available about the incidence of medical-legal cases, and little relevant data can be derived from informatics sources. Nevertheless the large number of pronounced judgments that are available about medical malpractice cases illustrates the transformation of the evidence-based medicine into the sentence-based medicine or jurisprudence-based medicine. This phenomenon has produced a negative effect on the budgets of the NHCS and the Regional Health Services, and has caused many insurance companies to withdraw from the medical liability market due to this climate of so-called defensive medicine. In the Italian legal system, physicians must demonstrate responsibility for the consequences resulting from unlawful conduct that may include care omissions or the violation of a

specific standard. In order to satisfy the Penal responsibility, leaving aside the intentional offense, article 43 of the Penal Code disciplines the culpable crime that is distinguished as generic fault, (negligence by omission of the minimum qualified diligence required by practice of medicine; imprudence by absence of prevision of possible harmful consequences of interventions; inexperience by ignorance in the managing what another physician of the same professional level would properly do in the same case) and a specific fault due to the violation or non-application of specific rules ("regulations, orders and disciplines"). Moreover, the Italian Court of Cassation have defined (as "obiter dicta"), other rules that specify duties and behavior for all the health care providers. Among these, it is important to point out the so-called warranty obligation by which all professionals, involved in the treatment process (diagnostic and therapeutic), have to cooperate in safeguarding the patient's health at different levels of respective work. It means that the common goal is the protection of the patient from damages derived from a violation of *leges artis*, guidelines or official protocols that are concerning specific discipline or from mistakes that may be done inside of the synergy of different competencies (Cass. Pen. Sez. VI n. 9638, 2.3.2000). So, every professional must assure standards of diligence, prudence and experience with regard to their personal duty. At the same time, when the common scientific knowledge or one's own specific competency allow the professional to perceive a mistake arising from the actions of a colleague, especially when the mistake is predictable and avoidable (risk of mistake), he/she must do anything to correct the mistake or to remove the risk by which the patient may suffer a damage. This duty becomes particularly relevant in the sharing of the diagnostic and therapeutic decision-making processes. This also appears in similar wording within the Civil contest (Cass. Civ – Sez III - n.8826, 13.4.07). By Civil law, the physician and patient are united by a contractual relationship originating from a social contact and the assumption of liability is the existence of a compensable damage (L. 98/2013); the damage is a consequence of the failure and the damages recoverable are those expected at the time when the debt was incurred. In defining an error and mistake, it may include cognitive versus operational error, clinically significant versus academic error (differences in classification, grading, etc.), and prospective versus retrospective review. It is often related to the degree of the knowledge of the reviewer or of the consultant for the specific case. The error may occur in the diagnostic, prognostic or therapeutic phases. The diagnostic error means that the physician fails to reach a correct diagnosis of the disease that afflicts the patient (wrong collection of anamnestic data, misidentification or underestimation of a symptom, an objective examination performed in a wrong way, or an error in the execution or interpretation of imaging and/or laboratory studies). A diagnostic delay results in a delay of treatment. If the physician reaches a conclusion that is then proved to be unfounded and this affects further therapeutic interventions causing harm, the mistake is detectable

in the prognostic phase. A mistake in the therapeutic phase is when the physician makes a mistake either during the choice of the therapy or at the time of its execution. These mistakes frequently depend upon previous diagnostic mistakes and they can be distinguished either as a medical mistake or as a surgical therapy mistake. Many corrective factors and various methods may be cited in preventing medical mistakes or in the reducing the risk of its incidence. Improving communication with the patient and recording information in the medical records, along with assistance by risk managers may be of benefit to medical staff. The use of medical guidelines that conform to the best evidence-based medicine, or the use of clinical protocols or hospital checklists may improve quality and safety in the health care system (Zarbo RJ et al, 2005; Meier et al., 2008). However, these do not save the health professional from the risk of criminal or civil proceedings if the consequences of their application do not meet the best interests of the patient (Cass. Sez. IV pen., 23/11/10, dep. 2/3/11; L. 189/2012). A decision-making process with the patient, a so-called therapeutic alliance, plays the most important role in the prevention or reduction of failures in the relationships between the health care system and its consumers.

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Giovedì 24 settembre 2015

Aula Gialla 1 – 10.00-12.00

Dermatopatologia

MODERATORI: C. Clemente (Milano), M. Santucci (Firenze)

Stato dell'arte delle patologie bollose dermatologiche

R. Gianotti

Paper not received

Linfomi cutanei: cosa c'è di nuovo?

M. Santucci

Paper not received

I sarcomi della cute

A. Parafioriti, E. Armiraglio, A. Di Bernardo

Anatomia Patologica, Azienda Ospedaliera Istituto Ortopedico Gaetano Pini, Milano

Mesenchymal tumors of the skin represent a wide and heterogeneous group of lesions, including newly described entities, that poses a diagnostic challenge given their rarity, in comparison to epithelial and melanocytic tumors, and the dermatopathologist reluctance to deal with them. Primary cutaneous sarcomas are relatively rare in comparison to carcinomas and superficial benign mesenchymal tumors of soft tissue such as lipoma, fibrous histiocytoma, vascular and smooth muscle neoplasms, including angioleiomyoma, and peripheral nerve sheath tumors. The aetiology of sarcomas is still widely unknown with the exception of the small num-

ber of tumors occurring in the context of hereditary cancer syndromes associated with well-known genetic mutations and specific molecular pathways alterations. There are also cutaneous virus-driven sarcomas such as Kaposi's Sarcoma associated with Human Herpes Virus 8 (HHV8) infection and multiple leiomyosarcomas associated with Epstein-Barr Virus (EBV) which usually develop in immunosuppressed patient and in predisposing conditions like chronic lymphoedema, radiation therapy etc.. Cutaneous sarcomas usually present as a palpable mass with or without ulceration. When a patient presents with a superficial/sofrafascial tumor of at least 5 cm of diameter or a deep/subfascial tumor of any size, that should give rise to the suspicion of malignancy, a proper multidisciplinary work-up in a soft-tissue tumor centre is recommended. Punch, shave and fine needle biopsy of superficial skin tumors must be avoided if the diameter is less than 5 cm. and, under those circumstances, surgical excision would be the most advisable approach. Complete removal of the lesion and negative margins on histological examination are factors of primary importance in the prevention of local recurrence. Late development of recurrence and metastasis, even several years after the excision of the primary tumor, are associated with some specific histotypes, e.g. classic epithelioid sarcoma. Every histotype of sarcoma, either of superficial or deep soft tissue, may be found in the skin as well in accordance with the concept that mesenchymal and neuroectodermal progenitor cells may give rise in the cutis to a wide range of tumors with different biological behaviour including locally aggressive le-

sions with recurrence potential (e.g. dermatofibrosarcoma protuberans, inflammatory myofibroblastic sarcoma etc), malignant rarely metastasizing lesions (e.g. low-grade fibromyxoid sarcoma, fibrosarcomatous dermatofibrosarcoma protuberans, pseudomyogenic hemangioendothelioma), and high-grade malignant neoplasms (e.g. angiosarcoma, epithelioid sarcoma, extraskeletal myxoid chondrosarcoma, Ewing sarcoma etc..). In general, sarcomas of the skin compared to their deep soft tissue counterpart show a more favourable prognosis. For example, radical excision of leiomyosarcoma confined to the skin is considered curative. Tumor location may influence the prognosis as well, for example cases of low-grade fibromyxoid sarcoma, which frequently arises in the skin and has a high rate of local recurrence, involving the extremities have a better prognosis compared to those involving head-and-neck region where a complete surgical excision can be difficult to achieve. However histological grading remains the most important prognostic and predictive factor.

2013 WHO Classification of Soft tissue and Bone Tumors is the reference system currently used for the diagnosis of sarcomas which identifies different lineage of differentiation: fibrohistiocytic, fibroblastic/myofibroblastic, adipocytic, smooth muscle, skeletal muscle, pericytic, etc.. An integrated multidisciplinary approach is mandatory for the proper management of soft tissue sarcomas: patients should be directed to dedicated institutions with high expertise in the field and specific programs tailored to deal with this kind of rare challenging tumors and the related continuously evolving diagnostic-therapeutic and care processes.

Il melanoma nella popolazione nera

L. Viberti¹⁻⁶, D. Fenocchio²⁻⁶, M.L. Fibbi³⁻⁶, R. Tumino⁴⁻⁶, A. Sidoni², M. Ogowang⁵

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² S.C. Anatomia Patologica, Azienda Ospedaliera Perugia.

³ Policlinico di Monza, Monza, Italy

⁴ Anatomia Patologica e Registro Tumori Azienda Sanitaria Provinciale di Ragusa (Italy)

⁵ Institutional Director of St. Mary's Lacor Hospital, Lacor, Uganda

⁶ Associazione Patologi Oltre Frontiera (APOF), NGO

Introduction: We have collected 157 cases of malignant melanoma from three Departments of Pathology in Sub Saharan Hospitals, where the NGO "Associazione Patologi Oltre Frontiera (APOF or "Pathologist Beyond Borders Association") develops cooperation projects with the local health staff for the implementation of histological and cytological activity.

It is known that there is a considerably lower incidence of cutaneous melanoma in pigmented skin populations than in Caucasians, therefore the aim of this study is to evaluate the incidence in populations living in Sub-Saharan Africa, with particular regard to the anatomical localization.

Materials and Methods: We have retrospectively analyzed three sets of cases collected from three different Sub-Saharan hospitals: Lacor Hospital in Uganda; Bugando Medical Center (BMC) in Mwanza, Tanzania; and Mtendere Mission Hospital (MMH) in Chirundu, Zambia.

Results: We have collected 157 diagnoses of melanoma: 83 cases from 2004 to 2007 at BMC out of a total of 5,570 histological cases (1.5%); 60 cases from 2009 to 2014, diagnosed at Lacor Hospital in Uganda, 14 cases out of 1,208 overall histological cases (1.2%) diagnosed at MMH during the years 2007-2013.

The total number of skin-located melanomas (excluding lymph node metastasis and mucosal sites) was 141. In 21 cases the anatomical localization was not specified. Of the remaining cases with a proven primary skin location, 89 were located on feet (74%) and 17 on legs (14%) with a total ratio of 88% of cases localized on lower limbs.

Conclusions: Malignant melanomas of the skin in black populations living in sub saharan areas show a prevalent acral distribution, with higher prevalence on the feet. This finding may suggest a lack of correlation between ultraviolet exposure and the potential protective effects of melanin.

Foot melanoma (together with hand melanoma) could represent a specific subgroup of melanomas, possibly linked to genetic or environmental factors or both. Therefore the prevalence of melanoma in acral sites in pigmented skin populations needs to be further studied with molecular and genetic studies.

Aula Gialla 2 – 8.00-11.00

Biobanche

MODERATORI: M. Barbareschi (Trento), M. Truini (Milano)

Presentazione del Gruppo di Studio

M. Barbareschi, M. Truini

Paper not received

Presentazione BBMRI.IT

M. Lavitrano

Paper not received

Organizzazione del gruppo NIPAB (materiali di archivio)

G. Stanta

University of Trieste

Some years ago a group of pathologists of SIAPEC-IAP (Società Italiana di Anatomia Patologica e Citodiagnostica – International Academy of Pathology) started to discuss the possibility to develop a network for clinical research using the fixed and paraffin-embedded tissues stored in the pathology archives of hospitals. These tissues are usually called archive tissues. They come from biopsy and surgical activities, and after only few sections are used for histopathological diagnosis, they are stored for decades in pathology archives. Those collections are the widest biorepositories of human tissues available, with any even rare lesions. We can estimate that in Italy in all hospitals about one billion specimens have been stored in the past 20 years. Today clinical research on

this type of tissues is especially important because of the new therapies based on target driven molecules and the subsequent acquired resistances to the therapy that developed after some time. This phenomenon is related to clonal heterogeneity of tumours, which means that cancer is not homogeneous at the genetic level, but has different subclones. Therapy usually fights against the major clones, but other clones are not killed by therapy and develop recurrences. This fact has taken to a new medicine that is strictly related to clinical research as a unique activity, and this activity is directly performed on archive tissues that are the only tissues available for any patient. The Italian network has been the first one to be organized in Europe and it is based on a voluntary collaboration of pathologists to specific projects. This is a specific requirement because these are not tissues specifically stored for research, but they are clinical tissues and the pathologist is responsible for them. So the biorepository starts to act as a biobank only after participating in a specific project with pseudo-anonymization of the case study through a specific algorithm that can be reversed only by the pathologist.

The use of these tissues also has a specific bioethical aspect, because the pathologist that can assess tissues and clinical information is involved as a doctor in the diagnostic process, and for this reason he or she is bound by professional secrecy, which is a lot stricter than any privacy rule. This represents an effective firewall for the protection of patients' data.

The network is going to be increasingly important in the future because the development of clinical research is strictly related to today's patients who are, at the same time, donors and beneficiaries of the research. This is quite different from basic and translational research that benefit patients on average after 10 years with a long clinical validation process.

Ipotesi di segreteria centralizzata del gruppo

V. Canzonieri

Paper not received

Problematiche relative alla fase preanalitica

M. Truini

Paper not received

Come dai tessuti in paraffina si può utilizzare il DNA e l'RNA

S. Bonin

Dept. Medical Sciences-University of Trieste-Italy

Background: In the last decade, the clinical specimens which are routinely used in histological examinations, have become a source of macromolecules for molecular diagnostics and molecular pathology studies. The accuracy and reproducibility of those investigations are highly dependent on the quantity and quality of DNA, RNA, and proteins isolated from those specimens, which vary according to tissue acquisition and processing¹. Fresh or snap-frozen tissue specimens are the appropriate material for molecular analysis, because they provide intact macromolecules. However, those samples are not routinely used in pathology laboratories because they do not provide accurate morphologic details, thus impairing histological diagnosis. Although in recent years several alternatives to formalin have been proposed for tissues fixation, at present the evidence is that formalin is still the preferred fixative, because it

is relatively cheap and easy to use. In appropriate conditions, it guarantees optimal and reproducible morphological preservation². Therefore it is manifest that substitution of formalin with alternative fixatives cannot be applied at present.

While protein-protein cross-links are the most frequent reaction, extensive intermolecular cross-linking of proteins with nucleic acids also occurs. Formaldehyde reacts with nucleic acid bases to form firstly an N-methylol group (-CH₂OH), and later, due to the reaction of this N-methylol group, a methylene bridges could be formed between two amino groups³. This reaction allows tissues to harden for later microscopic and immunohistochemistry analysis, but the methylene bridges between proteins and nucleic acids, as well as the addition of CH₂-OH to nucleic acids bases, are critical for extracting intact nucleic acids from formalin fixed and paraffin embedded tissues (FFPE)².

Nucleic acids isolation: Because of the abovementioned reactions, extraction of nucleic acids from FFPE tissues requires special protocols based on a comprehensive proteolytic step to remove the proteins' cross-links and allow the extraction of nucleic acids². In most cases proteolysis is achieved by proteinase K, with variable concentration and time/temperature. Time and incubation temperature have an impact on the extraction procedure, since they are directly related to the input of thermal energy to remove the cross-links⁴.

The DNA isolated from FFPE is degraded, however the extent of degradation is not so critic as for RNA. Several methods can be used to extract DNA from FFPE after the proteolytic step, they range from salting out, phenol/chloroform extraction and silica based filtering methods. The performance of the different methods in DNA extractions is quite similar and there are no specific indications to prefer one to the others. Diagnostic applications in molecular pathology are mainly based on the use of commercial kits, because they can increase the reproducibility by decreasing also the variability in the reagent preparations⁵.

As it is the case with DNA, different protocols and commercial solutions are available to extract RNA from FFPE: they range from the use of digestion in homemade or commercial solution, followed by phenol chloroform extraction or mono-phase solution to the use of commercial kits, which provide purification of the extracts, mainly by the use of spin columns and with magnetic tools². For RNA extraction most protocols and commercial kits recommend a digestion step at 55-60°C, from few hours to overnight. To implement the reversal of CH₂-OH moieties in RNA extracts, it is also possible to perform a de-modification step after extraction by heating the RNA solution for about 20 min at a temperature from 70° to 94°C in different buffers².

Nevertheless, degradation of RNA can occur at other steps during the tissue processing and even during the storage, because RNA molecule is labile. The fragmentation of the extracted RNA reflects both the storage and the conditions and also the treatment of the tissue prior to embedding. Besides, the low yield and quality of RNA could also be due to poor extraction/isolation processing for thermal or enzymatic degradation by exogenous RNAses.

Conclusions: Considering that there is no general consensus among scientists in preferring one kit or one specific method in the extraction of nucleic acids from FFPE to obtain an accurate molecular analysis from those specimens, a proper test to check the quality of extracts is mandatory and should be taken into consideration for data comparison among different laboratories.

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lecular analysis of non-tumoral liver, spleen, and brain from autopsy samples: The effect of formalin fixation and paraffin embedding. Pathology research and practice 2012;208:584-91.

Regole di utilizzo dei biomateriali, MTA, cost-recovery

M. Macilotti

Paper not received

Aula Gialla 2 – 11.00-13.00

Grading nella neoplasia del tratto genitourinario(il punto sulle consensus dell'ISUP)

MODERATORI: M. Colecchia (Milano), M. Scarpelli (Ancona)

Il consenso sul grading delle neoplasie della prostata 2015

R. Montironi

Polytechnic University of the Marche Region, Ancona, School of Medicine United Hospitals, Ancona

There are deficiencies with the original Gleason system that have impacted patient care. Gleason score 7 can either represent 3+4=7 or 4+3=7, yet treatment decisions for prostate cancer have often relied on the simplified single Gleason score of 7. Another critical weakness is that the lowest grade assigned is currently 6 out of a scale from 2-10, where patients logically but incorrectly think that they have a cancer in the middle of the grading scale, compounding the fear when they are informed that they have cancer. In the literature and for therapeutic purposes, various scores with different prognoses have been incorrectly grouped together. First in a published study and then verified in a meta-analysis of over 19,000 men treated by radical prostatectomy, and multi-institutional data on thousands of patients analyzing biopsy and radiation data, grade groups were distilled down to the lowest number of grades where each has a unique prognosis. A new prostate cancer grading system has been proposed and accepted by 85 pathologists and clinicians from 17 different countries with

expertise in prostate cancer to be used initially in conjunction with Gleason grading. The new proposed system has the benefits of: 1) more accurate grade stratification than current systems; 2) simplified grading system of 5 as opposed to 12 grades; and 3) lowest grade is 1 as opposed to 6.

Gleason score < 6:	Prognostic Grade Group 1
Gleason score 3+4=7:	Prognostic Grade Group 2
Gleason score 4+3=7:	Prognostic Grade Group 3
Gleason score 8:	Prognostic Grade Group 4
Gleason score 9-10:	Prognostic Grade Group 5

Il grading WHO 1973 e 2004 del carcinoma della vescica

A. Lopez Beltran

Paper not received

Il grading del carcinoma renale secondo Fuhrman versus ISUP

G. Martignoni

Paper not received

Aula Gialla 2 – 15.00-16.30

Urologia

MODERATORI: M. P. Gardiman (Padova), R. Montironi (Ancona)

TNM del testicolo e del pene: attualità e controversie

A. Lopez Beltran

Paper not received

I tumori del testicolo in età prepuberale

P. Collini

Soft Tissue and Bone Pathology, Histopathology, and Pediatric Pathology Unit

Department of Diagnostic Pathology and Laboratory Medicine Foundation IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Testicular tumors have a different frequency, pathogenesis and in some instances behavior in different ages. In the first decade the only germ cell tumors that are seen are the yolk sac tumor (YST) and teratoma. The juvenile granulosa cell tumor is seen only in this decade. Leukemias are frequent, lymphomas are occasional. Metastases are very rare, most frequently represented by neuroblastoma and nephroblastoma. Puberty is the watershed time for the increase of germ cell

tumors, and all the types are seen in the second decade apart from spermatocytic seminoma. YST represents about 50% of prepubertal testicular tumors. It occurs at a median age of 16-20 months. Relatively few pure cases occur after 4 years of age. Elevated AFP serum levels are seen in 95% of cases. The value of elevated AFP is limited under 6 months of age given its physiologic elevation. The morphological features are the same as in adults. Sarcomatoid features are exceptionally described, if ever. Solid pattern can resemble seminoma. There is no association with IGCNU. Gain of 12p is exceptional. Virtually all cases are positive for keratins, GPC3, and GATA. EMA is typically negative. At the time of diagnosis, about 80% are stage I. Of untreated cases, only 6% develop subsequent metastases (retroperitoneum and lung).

In these cases, chemotherapy is very effective, with an overall survival of 100%. Teratoma is the second commonest tumor. The behavior is uniformly benign even in presence of immature elements. It is virtually never associated with IGCNU. Secondary malignancy is exceptional in these ages. Other germ cells tumors are very rare and virtually confined to patients with gonadal dysgenesis and Down syndrome.

Nuove acquisizioni dalla conferenza USCAP Boston 2015 sui tumori del testicolo e del pene

M. Colecchia

Paper not received

Giovedì 24 settembre 2015

Aula Gialla 3 – 9.00-11.00

Patologia ginecologica (1a parte)

MODERATORI: G. Negri (Bolzano), G. L. Taddei (Firenze)

Tumori Borderline dell'ovaio

G. Zannoni

Paper not received

Leiomioma uterino Morcellato. Problemi diagnostici

L. Resta

Paper not received

Ovarian tumors: new classificative approach

S. Losito

Istituto Nazionale Tumori "G. Pascale", Napoli

Introduction

Over the past quarter of a century, several scientific developments have changed traditional concepts in ovarian cancer, especially in epithelial tumors. Overall, it has been recognized that the ovarian cancer is not a homogeneous disease but, rather, a group of diseases with different morphology and biologic behavior¹. Based on microscopic features, immunohistochemistry and molecular genetic analysis, 5 main histotypes are recognized: High-grade Serous Carcinoma (HGSC- 70%); Endometrioid Carcinoma (EC-10%); Clear Cell Carcinoma (CCC-10%); Mucinous Carcinoma (MC-3%) and Low-Grade Serous Carcinoma LGSC (<5%). Each histotype has different precursor lesions, pattern of spread, molecular events during oncogenesis, response to CT and prognosis.

Results

Many news are introduced in the new WHO Classification 2014². In particular, the new Classification 2014 definitively accepts, based on the molecular alterations involved in serous carcinogenesis, two distinct types of serous carcinoma. LGSCs are often associated with SBTs/APTs and include tumors historically classified as grade 1 serous carcinoma; HGSCs include the majority of malignant non-uterine serous carcinomas of the female genital tract historically classified as grade 2 or grade 3 serous cancers.

Since serous carcinomas, independently if they arise from fallopian tube, ovary or, rarely, from peritoneum, share common epidemiologic features and clinical behavior³, some authors have proposed that HGSC might be considered as an amalgamation of primary (extra-uterine) "Pelvic HGSC"⁴.

Last FIGO classification has proposed a new staging system to include cancers arising in these three sites, recommending to delineate primary site or, if it is not possible, to list it as "Undesignated".

A still controversial issue is represented by borderline tumors. The serous histotype is biologically distinct from borderline tumors of other histological types. SBTs can present at high stage, sometimes with lymph nodes involvement and can rarely evolve to LGSC. Other borderline tumors of non-serous histology are generally confined to the ovary (Stage I) and there are no cases of malignant evolution. Some authors consider SBTs as essentially benign and favor the term atypical proliferative tumor (APT), while others still prefer the term borderline because it better reflects the biologic behaviour of these tumors; in the new classification, the terms borderline and atypical proliferative tumor are considered synonymous. In the new classification, moreover, the category SBT-Micropapillary variant/Non-Invasive LGSC has been introduced, equivalent to in-situ carcinoma, characterized by micropapillary architecture while the traditionally defined invasive implants of the SBTs are now best defined metastases in a LGSC, because of a similar behaviour to that of LGSC. Among mucinous tumors, a definitive distinction has been made between the two categories endocervical and gastrointestinal-like, based on morphological, immunohistochemical and genetic differences, introducing the new histotype called "seromucinous". Moreover, the term "Transitional Cell Tumors" has been replaced by "Brenner Tumors".

At last, also the sex-cord-stromal tumors have been separated in three groups: pure stromal tumors, pure sex-cord tumors and mixed sex-cord-stromal tumors.

Conclusions

Anyway, new classifications are based on understanding of the disease processes at a single point in time, setting the stage for the next one².

References

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Citologia endometriale

A. M. Buccoliero

Anatomia Patologica, AOU Meyer, Firenze

Several diagnostic procedures are available to investigate the endometrium, i.e. sonography, hysteroscopy, biopsy, endometrial curettage and cytology. Among these, endometrial cytology is less commonly utilized. Although the use of cytology in the diagnosis of endometrial adenocarcinoma has already been proposed due to its low cost and simple execution, a general consensus has not been

reached. The improvement of the diagnostic capacity of endometrial cytology following the introduction of a liquid-based method suggests that this test should be routinely used in endometrial diagnosis. The main advantages of this method are the reduction in confounding factors, the distribution of cells on a thin layer and the possibility to obtain more slides from the same sample. Endometrial cytology may be an efficient diagnostic method. It could be applied to selected patients solely or in association with ultrasonography. The combination of these two noninvasive procedures may improve their diagnostic accuracy and reduce unnecessary hysteroscopies, thereby producing benefits for women and society.

Studio e grading del carcinoma endometriale

R. Carinelli

Paper not received

Carcinoma a cellule chiare

M. L. Carcangiu

Paper not received

Aula Gialla 3 – 15.00-16.00

Patologia ginecologica (2a parte)

MODERATORI: G. Negri (Bolzano), G. L. Taddei (Firenze)

Precancerosi vulvare

G. Negri

Paper not received

Carcinoma vulvare

B. Ghiringhello, L. Micheletti¹

AO Città della Salute e della Scienza di Torino. Presidio Ospedaliero S. Anna.

SC Anatomia Patologica. ISC Universitaria Ginecologia e Ostetricia I.

Squamous Cell Carcinoma (SCC) of the vulva is a rare disease with an incidence of about 2x100.000 cases. It is now recognized that two different types of vulvar SCC exist: 1) HPV-related SCC, usually warty-basaloid in type, associated with usual-type or undifferentiated VIN, generally occurring in women under the age of 60, and 2) HPV-independent SCC, usually keratinizing type, associated with chronic dermatoses like lichen sclerosus or lichen planus, occurring in women aged 70 years or older. The possible explanation for low prevalence, in the latter, of differentiated-VIN, accounting for only 2-10% of all reported VIN, is that the intraepithelial lesion is a transient lesion that rapidly progresses to invasion and/or it is an underdiagnosed and underreported lesion. Actually in our series of 342 vulvar SCC, diagnosed and surgically treated between 1982 and 2014, the HPV-independent keratinizing SCC type accounts for approximately 87% versus the HPV-related type, while among our 192 VIN the differentiated or HPV-independent keratinizing type accounted for only 15%. Regardless of histological type, vulvar SCC has a tendency to spread locally to the vagina, the distal urethra, and the anus.

Lymphatic spread is initially to the ipsilateral inguinofemoral lymph nodes, but central tumors can spread simultaneously to both groins, then from the groins the tumor cells spread to the pelvic nodes; no pelvic metastases are found in absence of positive inguinofemoral nodes.

The treatment of vulvar SCC still remains predominantly surgical, but the mutilating approach of en bloc butterfly excision, regardless of the site or size of the primary lesion, known as the Way-Taussig radical vulvectomy, has been replaced by the unanimously accepted conservative and individualized approach. Current safe surgical conservative modifications regarding vulvar lesion are separate skin vulvar-groin incisions, drawn according to the lesion diameter, and wide radical excision or partial radical vulvectomy with 1-2 cm of clinically clear surgical margins. Regarding groin lymph-node management, surgical conservative modifications that do not compromise patient survival are: omission of groin lymphadenectomy only when tumor stromal invasion is ≤ 1 mm; unilateral groin lymphadenectomy only in clearly lateralized early lesions; and total or radical inguinofemoral lymphadenectomy with preservation of femoral fascia when full groin resection is needed. Sentinel lymph-node dissection is a promising technique but it should not be routinely employed outside referral centres. Pelvic nodes are generally better managed by radiation. Locally advanced vulvar lesions can be managed by ultraradical surgery, exclusive radiotherapy or chemioradiation.

Novità in tema di Lichen Scleroso vulvare

G. L. Taddei

Paper not received

Venerdì 25 settembre 2015

Aula Blu 1 – 8.30-12.30

SESSIONE GIOVANI

"Innovation and Improvement in Pathology"

PRESIDENTE: M. Barberis (Milano)

Chairman: F. Pagni (Monza)

Experience with a new device for pathological assessment of the colonic endoscopic submucosal dissection

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Goal

Endoscopic submucosal dissection (ESD) is gaining popularity worldwide in the treatment of neoplastic lesions of the gastrointestinal tract. However, the experience in Western countries is quite limited and restricted to large or academic centers. Besides, this approach requires an optimal pathological assessment. The aim of this study was to report our experience with colonic ESD using a new device that allows complete handling of the resected specimens and especially of lateral margins, for pathological analysis.

Methods and Materials

In a 1-year period, 14 patients (6 men, 8 women, age range

Fig. 1. A: Picture of the fenestrated biocassette. B: The specimen is put in the cassette and covered with a sponge (C). Once fixed, pictures are taken (D) and multiple sections made (E, F) for pathologic analysis.

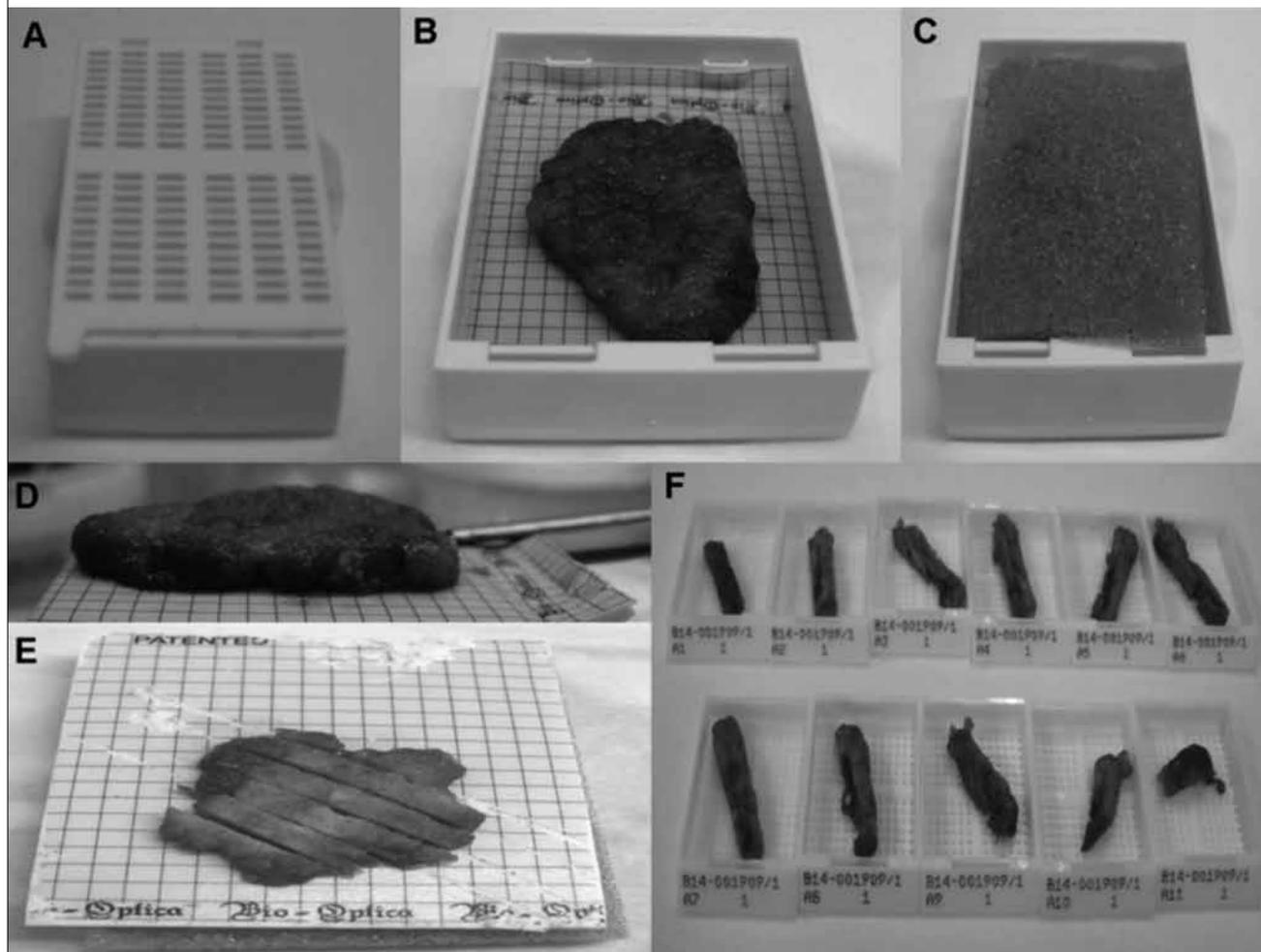
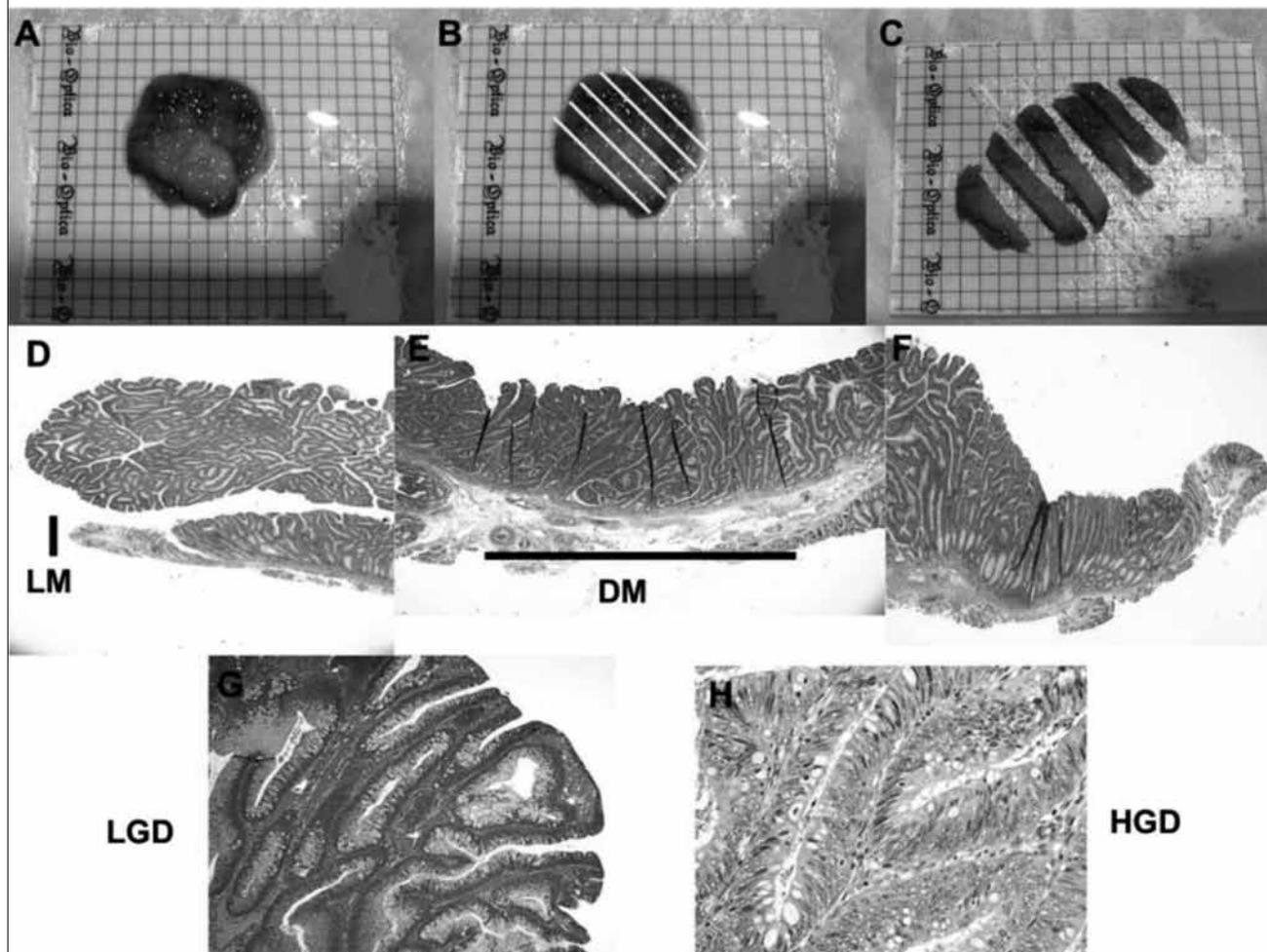


Fig. 2. Representative results as provided from the pathology report; DM deep margin, HGD high-grade dysplasia, LGD low-grade dysplasia, LM lateral margin.



50–82 years) underwent colonic ESD in a non-academic hospital. The endoscopic procedure was carried out successfully en bloc in more than 90 % of cases. Perforation requiring surgery occurred in one patient (7 %).

Results

Pathological assessment with the new device allowed entire and complete examination of both the deep and lateral margins of the excised specimens.

Conclusion

Colonic ESD is a viable option for non-surgical treatment of large bowel lesions even in relatively small centers and in non-academic settings. The new device allows good handling of the specimens, and it seems to be useful for the entire examination of the resection margins. (see figures below)

Tungiasis In Italy: an imported case of *Tunga Penetrans* and review of the literature

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Tungiasis is an animal and human parasitic disease caused by fleas of the genus *Tunga* (Siphonaptera, Tungidae), endemic in equatorial and subtropical regions and rarely described in European countries, where clinicians and general pathologists are not aware of this parasitic disease.

To our knowledge, only 75 cases of tungiasis (not all described in details) were previously reported in Italy.

We describe a new case in a 34 year-old Italian flight attendant who developed a granuloma-like, ulcerated nodule in the subungual region of his left 5th toe, partially detaching the nail, about 20-30 days after his return from Brazil.

We performed a detailed review of the literature of the Italian cases, suggesting the use of histochemical stains (especially Trichrome stain) in order to underline parasitic details.

Tourism in endemic regions and globalization may result in new cases in developed countries and the spread to previously unaffected regions, therefore pathologists should consider this parasitic disease.

Morphologic and genetic heterogeneity in mixed endometrial carcinomas

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Introduction

Mixed endometrial carcinomas (MECs) comprise a heterogeneous group of tumors characterized by an admixture of two or more distinct histologic subtypes of endometrial carcinoma. The genetic underpinning of MECs has yet to be fully established.

Aims

We sought to define the repertoire of somatic genetic alterations in four MECs by targeted capture sequencing of 341 cancer-related genes in each of their distinct histologic components.

Methods

Six gynecologic pathologists reviewed slides from 13 tumors originally diagnosed as MECs; 4 tumors were unanimously diagnosed as bona fide MECs. Representative sections from each case were subjected to p53, PTEN, and Ki-67 immunohistochemical analysis and to microdissection, either laser-assisted or with a needle under a stereomicroscope. DNA extracted from each tumor component and matched normal tissue was subjected to massively parallel sequencing using the MSK-IMPACT™ platform that targets the coding regions of 341 actionable cancer-related genes. Median coverage obtained was 445x (range 53x-702x). Somatic single nucleotide variants were detected by MuTect; insertions and deletions were detected by VarScan2 and Strelka; copy number alterations were defined using VarScan2, as previously described. The functional effect of each mutation was investigated using a combination of MutationTaster, FATHMM and CHASM (uterus). Private mutations of each component were confirmed by Sanger sequencing.

Results

Figure 1. Representative micrographs and results of targeted massively parallel sequencing analysis of case 1. H&E stained sections and p53 immunohistochemical analysis of the low-grade and high-grade endometrioid components of MEC case 1 (left). The mutations identified in each of the components are shown on the right.

Figure 2. Representative micrographs and results of targeted massively parallel sequencing analysis of case 2. H&E stained sections and p53 immunohistochemical analysis of the low-grade endometrioid and high-grade ambiguous components of MEC case 2 (left). This case harbored an extraordinary high number of mutations (top right), including a POLE P286R hotspot mutation (i.e. ultramutator phenotype). The driver mutations identified in each of the components are shown on the right.

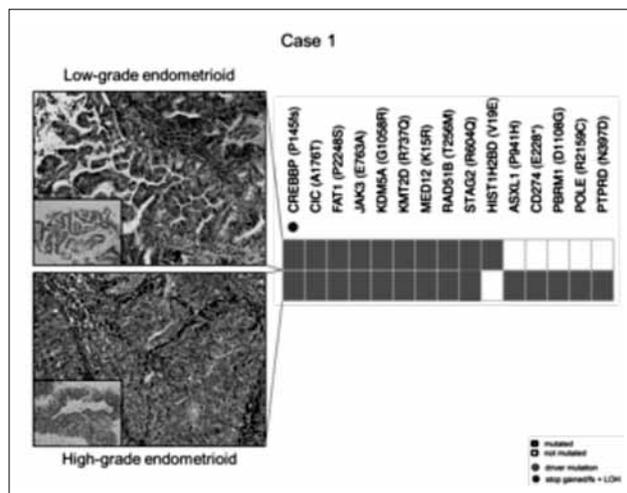


Fig. 1

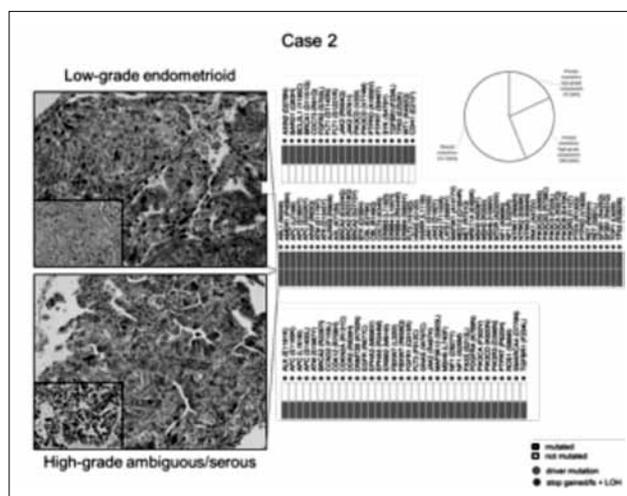


Fig. 2

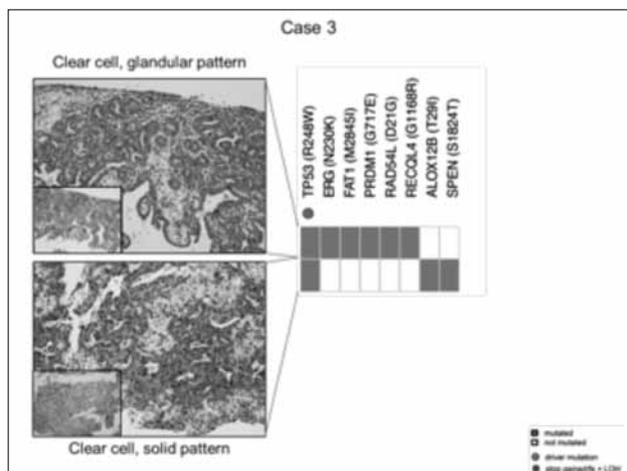


Fig. 3

Figure 3. Representative micrographs and results of targeted massively parallel sequencing analysis of case 3. H&E stained sections, and PTEN immunohistochemical analysis of the glandular and solid components of the clear cell MEC case 3 (left). Both components were diffusely and strongly positive for p53. The mutations identified in each of the components are shown on the right.

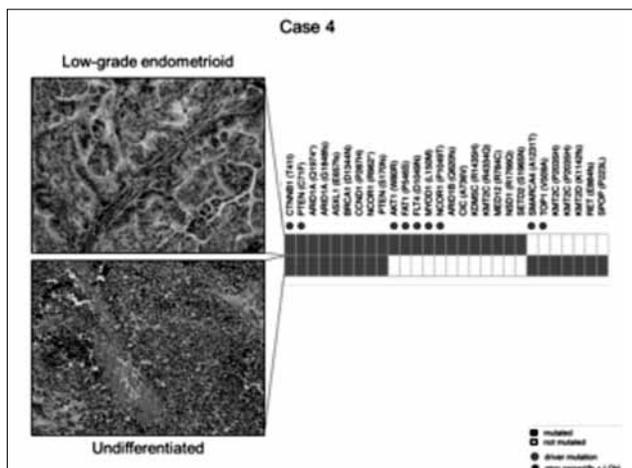


Fig. 4

Figure 4. Representative micrographs and results of targeted massively parallel sequencing analysis of case 4. H&E stained sections of the low-grade endometrioid and undifferentiated components of MEC case 4 (left). The mutations identified in each of the components are shown on the right. The undifferentiated component harbored a private somatic SMARCA4 missense mutation.

Conclusions

The histologically distinct components of MECs share the same initiating genetic events (i.e. founder somatic mutations). However, each component harbors private somatic genetic events, including potentially pathogenic genetic alterations such as mutations affecting NCOR1 or AKT1. Our findings have potential therapeutic implications, as mutations affecting targetable cancer genes may vary according to the histologic component of MECs.

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Specific expression patterns of epithelial to mesenchymal transition factors in gestational molar disease

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Goal

The epithelial to mesenchymal transition, a well-known and re-emerging model in pathology, has not been completely investigated in the field of gestational pathology. This study aims at improving the comprehension of this process in molar disease, even looking for new possible immunohistochemical markers.

Methods and materials

We have analyzed the immunohistochemical expression of Twist1 and Snai2, two of the most important transcription factors involved in epithelial to mesenchymal transition, in formalin-fixed paraffin-embedded samples of 23 spontaneous abortive pregnancies, 22 molar pregnancies (10 partial and 12 complete) and 7 term placentas.

Results

Twist1 and Snai2 were highly expressed in stromal villi cells of molar disease. Particularly, Twist1 was highly expressed in complete moles compared to both abortive pregnancies (p < 0.001) and partial moles (p < 0.05). Also Snai2 was more expressed by complete moles, differentiating them from non-molar abortions (p < 0.05).

Conclusion

On the basis of the known cadherins and claudins expression in these pathologies, our new findings reinforce the hypothesis of the involvement of epithelial to mesenchymal transition in early molar pregnancies and above all in complete moles. Furthermore, we highlighted that in molar disease not only the trophoblast, but even the villi stromal cells are involved. Thanks to their specificity, furthermore, these Twist1 and Snai2 could be used as additional immunohistochemical tool in the diagnosis of complete molar disease, with Twist1 as the first choice.

Pediatric renal cell carcinoma: 2012 Vancouver classification of 18 cases

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Background and goal

Renal cell carcinoma (RCC) is a very rare tumor in pediatric ages (up to 18 years). The collection of many cases in national and international Renal Tumor Study Groups allowed the discovery of distinctive morphologic, immunophenotypic and molecular characteristics. In the 2004 ‘WHO Classification of Tumours of the Urinary System and Male Genital Organs’ a new category was introduced, ‘the renal cell carcinoma associated with Xp11.2 translocations/ TFE3 gene fusions’, showing nuclear expression of TFE3 correlated with TFE3 gene translocations. There are still some controversies about the impact of this histotype on behavior and prognosis respect to adult cases. Now it is also known that TFE3 immunohistochemical expression can be due to mechanisms other than translocation. Our goal is to study the morphology, immunophenotype and molecular characteristics of a series of pediatric renal cell carcinomas retrieved in Italy from 1993 to 2015, possibly correlating these characteristics with a particular clinical behavior.

Methods and materials

From 1993 to 2015 in the AIEOP (Associazione Italiana di Ematologia e Oncologia Pediatrica) TW2003 Italian Protocol, TREP (Tumori Rari in Età Pediatrica) Project and SIOF (Société Internationale d'Oncologie Pédiatrique) Protocol, 18 cases of RCCs were collected in patients up to 18 years of age, with available representative histological material. Clinicopathologic data at diagnosis were available in all cases. FISH analysis for TFE3 gene (break apart probe) was applied to a selected sample of each case, along with a panel of antibodies (PAX8, WT1 (180 and C19), cytokeratins AE1/AE3, cytokeratins CAM 5.2, vimentin, carbonic anhydrase IX, racemase, cytokeratin 7, cytokeratin 34betaE12, cytokeratin 19, CD117, p63, INI1, TFE3, HMB45, Mart1, Alk(5A4) and succinate dehydrogenase complex, subunit B (SDHB)). Immunohistochemical results were scored as negative (no reactive neoplastic cells), focally positive ($1\% < x < 50\%$ reactive cells) and diffusely positive ($\geq 50\%$ reactive cells). Appropriate positive and negative controls were used.

Results

The male /female ratio was 5/13, with a median age of 11 years (range 9 mos-16 years). The left kidney was involved in 13/18 cases, being the tumor bilateral and multifocal in a case. All 18 cases were reactive for PAX8 and negative for WT1 (both 180 and C19). Cytokeratins AE1/AE3 and cytokeratins CAM 5.2 expression was poorly represented. All cases were INI1 positive and CD117 negative.

FISH analysis showed a rearrangement of TFE3 in 11/18 cases, classified as translocation RCC (TRCC). In these cases, TFE3 immunohistochemistry showed focal nuclear positivity in 4 cases and diffuse nuclear positivity in 7 cases. The majority of these cases showed a characteristic morphology, with clear to oxyphilic cells with a granular cytoplasm, and a large nucleus with a prominent nucleolus. The histological pattern was papillary, trabecular or solid, with psammomatous bodies. A very scarce expression of epithelial markers was found in particular in this group. Four TRCCs were p63 positive (2 focally positive), and 2 cases showed HMB45 and MelanA focal positivity. The seven cases without TFE3 rearrangement were reclassified as papillary renal cell carcinoma, type 1 (1 case), papillary renal cell carcinoma, type 2 (1 case), clear cell renal cell carcinoma (1 case) and renal cell carcinoma, unclassified (four cases, one associated with Wilms' tumor). Two cases showed reactivity for TFE3 and one case ALK positivity.

	Percentage of positive cases	
	TRCC (#11)	Others (#7)
TFE3	100%	29%
Vimentin	45%	100%
Carbonic Anhydrase IX	36%	42%
Racemase	100%	86%
Cytokeratin 7	55%	43%
CD117	0%	0%
Cytokeratin 34betaE12	10%	0%
Cytokeratin 19	50%	71%
p63	40%	0%
INI1	100%	100%
HMB45	18%	0%
MART1	9%	0%
ALK	0%	14%
SDHB	91%	29%

Conclusions

We confirmed the distinctive morphology of TRCCs, together with the poor expression of epithelial markers. TFE3 is a valuable tool if FISH analysis cannot be applied, having in mind

that also non translocated RCCs can seldom be TFE3 positive. TRCC occasionally expressed melanocytic markers.

In the group of non translocated RCCs, the application of classificative criteria as for adults was not so easy, and unclassified RCCs represented 57% of pediatric cases (respect to 4-5% in adults).

We are aimed at studying a larger number of cases, thank to the collaboration of all Italian pediatric pathologists. A virtual archive of e-slides is being created. The collection of clinical data is in progress.

The Authors gratefully thank the "Associazione Bianca Gravaglia" for their unstinting support in the studies of pediatric pathology.

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Evaluation of a consecutive series of follicular variant papillary thyroid carcinomas according to nuclear features, invasion and molecular status

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Background

The diagnostic controversies behind the follicular variant of papillary thyroid carcinomas (FVPTC) are debated topic. This tumor is a common subset of papillary thyroid carcinoma (PTC) found in 9% to 22.5% of PTC patients. It has less than 1% papillary formations and is composed predominantly of follicles lined by cells having the nuclear features of PTCs. For the encapsulated tumor without invasion of surrounding thyroid tissue, the diagnosis of malignancy relies solely on the presence of the nuclear features of PTC, which can often be borderline. Therefore, the diagnosis of non invasive, encapsulated FVPTC (EFVPTC) is subject of considerable interobserver variability. The BRAF V600E mutation is the most common genetic alteration in PTC and it is associated with aggressive behavior. Although many studies have found that the molecular profile of EFVPTC is characterized by an higher frequency of RAS mutations and no BRAF mutations. Telomerase reverse transcriptase (TERT) promoter mutations have been recently described in poorly differentiated and anaplastic thyroid carcinomas and have been observed to be of very low impact in differentiated PTCs.

Methods

We evaluated 75 FVPTCs underwent total thyroidectomy at our department. 47 (63%) cases were EFVPTCs, completely surrounded by a fibrous capsule, 21 (28%) were EFVPTCs with capsular invasion, defined as complete penetration of the capsule by tumor and 7 (9%) invasive FVPTCs, as infiltrating tumor through the neoplastic capsule into the surrounding thyroid tissue. In all cases we applied a quantitative score of the nuclear features (irregular nuclear membrane, clearing and grooves) from 1 to 10. Molecular analysis was conducted investigating BRAF, H, N, KRAS and TERT status.

Results

9 (12%) of FVPTCs were BRAF mutated, 23 (31%) were RAS mutated and only 3 (4%) were TERT C228T mutated. We observed a statistical correlation (p<0.05) between the presence of BRAF mutations and an higher nuclear grade (8 to 10). On the other hand nuclear grade was not associated nor with RAS mutations neither with TERT mutations. TERT mutations were correlated both with male sex and larger tumor (>2 cm). We did not find any association between the clinicopathologic features (sex, age and tumor dimension) and nuclear grade and invasion.

Conclusion

We confirmed the low frequency of TERT promoter mutations in FVPTCs, supporting their mildly aggressive behavior. On the other hand, a higher nuclear grade correlates with the presence of BRAF mutation, suggesting a more aggressive behavior for these FVPTCs.

Analysis of HER2 truncated fragments in gastric cancer

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Introduction

HER2 has been proven to represent a new therapeutic target for 9.5%–18% of patients affected by gastric cancer. HER2 overexpression has an important predictive role for response to trastuzumab (Herceptin®), a humanized monoclonal antibody that, by binding to the extracellular domain (ECD) of HER2, leads to downregulation of the signalling pathway and stimulation of the immune system by antibody-dependent cytotoxic activity. In breast cancer, experimental and clinical studies have suggested that the integrity of the ECD of HER2 may influence the trastuzumab binding capacity and therefore response to treatment¹. Interestingly, BT474 breast cancer cells challenged in vitro with a cocktail of proteases, i.e. pronase, can acquire truncated forms of HER2 (such as for instance p95HER2) together with the full length HER2 (p185HER2)². In addition, pronase-based antigen retrieval can enhance HER2 positivity in tissue sections, in particular when an antibody directed to the trastuzumab binding site is employed (Biotinylated trastuzumab, i.e. BioHER)^{2,3}. At present, little is known on truncated forms of HER2 in gastric cancer.

Goal

The aims of this study were two-fold: i) to investigate by western blot (WB) the pattern of positivity of full length and fragmented forms of HER2 in gastric carcinomas; ii) to assess whether the trastuzumab binding capacity in gastric cancer cells and tissue samples may be influenced by protease-based antigen retrieval.

Methods and Materials

Tissue samples

A series of 99 gastric carcinomas were retrieved, 53 of which had availability for paired formalin fixed paraffin embedded (FFPE) tissue blocks and snap frozen specimens. Pronase in vitro treatment of N87 gastric cells and WB analysis N87 gastric cancer cells were treated with 1 ml of 0.02% pronase for 10 minutes at 37°C. Control cells were incubated in PBS in the same conditions. Cells were then collected for cell lysates and for cell block preparation. HER2 was considered overexpressed

and scored (+++) if the WB the intensity of the band was comparable to that observed in control cells. Lower expression levels were classified as HER2 (++) or (+). Immunohistochemistry (IHC) Cell and tissue sections were immunostained using Herceptest™ (DAKO) to assess the expression of HER2 intracellular domain. Expression of the trastuzumab binding epitope was assessed using BioHER. To evaluate the effects of pronase on the accessibility of the trastuzumab binding epitope FFPE sections were subjected to pre-treatment with 0.05% pronase. Fluorescence in situ hybridization (FISH) FISH test was performed using probes for HER2 (17q12) and CEP17 (Abbott Molecular Diagnostics). Gene and CEP17 signal counting was performed using the FDA approved PathVysion V2 software of the Metafer Scanning System (Carl Zeiss MetaSystems).

Results

HER2 expression in N87 gastric cancer cells by WB and IHC By WB N87 cells showed a 185 KDa band whose intensity was almost twice as much as the one observed in BT474 breast cancer cells. In vitro pronase treatment of N87 cells induced two new bands, of 150 and 95 kDa respectively, that replaced the full length 185HER2. In FFPE sections of N87 cells the BioHER intensity was increased by pronase pre-treatment (score 1+ in control N87 sections versus score 3+ after pronase challenge). Herceptest showed a strong score 3+ immunostaining, regardless of pronase treatment. HER2 expression in tissue samples by WB and IHC By WB analysis, only p185HER2 was detectable in 8 out of 53 (15%) cases, the remaining cases were negative by both WB and BioHER. Only 1 case was HER2 (+++), 3 cases were HER2 (++) and 4 were HER2 (+). Here below in Table 1 the details of the relationship with IHC and FISH results. Of note, two cases showing a score 2+ positivity by BioHER achieved a score 3+ intensity after pronase challenging (red symbols). Among negative cases by WB, 6 were Herceptest score 2+ and 8 were score 1+. All of them were BioHER negative and HER2 not amplified.

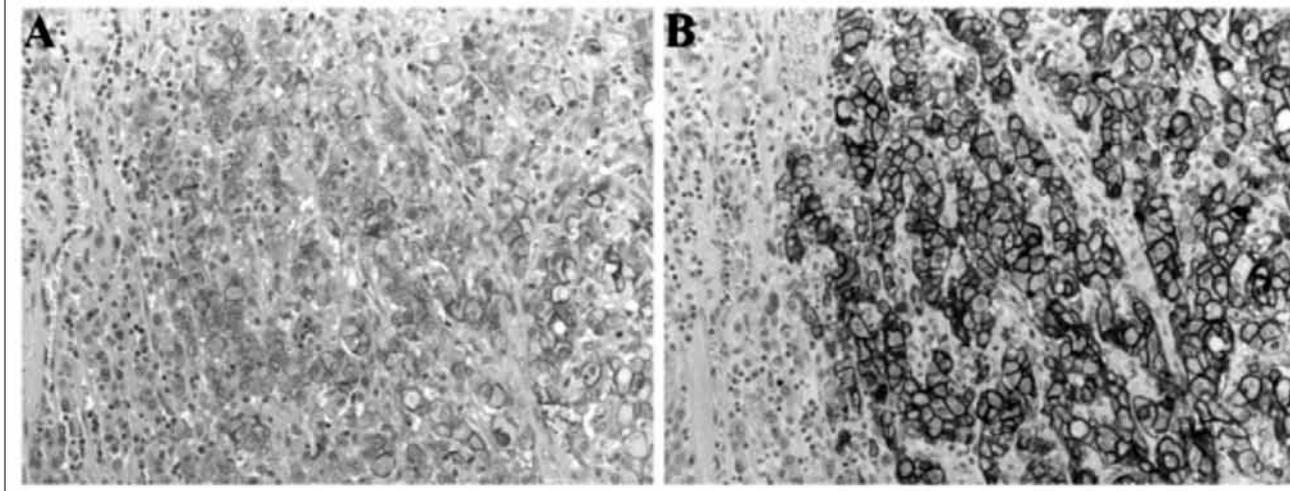
IHC staining in HER2 amplified gastric cancer

The 46 FFPE gastric tumours without frozen samples available were analyzed by IHC only. Of the 18 HER2 amplified cases, 17 were scored 3+ by Herceptest and, of these, 8 resulted positive with BioHER. After pronase treatment, BioHER staining intensity was increased in all positive cases and 3 negative cases resulted positive. The remaining 28 cases were HER2 not amplified and BioHER negative, however 8 of them were Herceptest score 2+ and 12 Herceptest score 1+.

Tab. I. Positivity for HER2 assessed with different techniques in the 8 cases positive for p185HER2 by WB. Each symbol represents a case with a different pattern of HER2 overexpression and gene amplification. n and v represent two cases whose staining intensity changed following pronase challenging as antigen retrieval (in red). Amp: amplified; Not Amp: not amplified; WB: western blot.

	WB (+++)	WB (++)	WB (+)
Amp	n		v
Not Amp		uu	ccc
BioHER 3+	n		v
BioHER 2+	n		v
BioHER 0/1+		uu	ccc
Herceptest 3+	n	☒	v
Herceptest 2+		uu	
Herceptest 0/1+			ccc

Fig. 1. Representative images of a gastric adenocarcinoma showing a score 2+ pattern in IHC by BioHER (A), which increases to score 3+ after pronase-based antigen retrieval challenging (B).



Conclusion

The analysis on frozen samples showed that HER2 is detectable in 15% of gastric carcinomas, confirming that the HER2 positivity rate is comparable between breast and gastric cancer, however the truncated forms of HER2 receptor (p95HER2 and p150HER2) that have been described in breast cancer were not identified in the cohort of gastric carcinomas here analyzed, even when the amount of p185HER2 was high. When FFPE sections of tissues and N87 cells were exposed to pronase treatment we observed an increased intensity in the IHC staining following pronase exposition in those cases that exhibited HER2 overexpression and this was observed in tissue specimens as well as in N87 cells. Western blot analysis of in vitro pronase treated N87 cells demonstrated the shedding of the full length HER2 receptor into 150 and 95 kDa fragments, thus showing that HER2 retains the susceptibility to protease action also in gastric carcinoma cells.

Our results on a cohort of gastric cancers using BioHER showed that the availability of the trastuzumab binding site is correlated with HER2 amplification: all BioHER positive cases were amplified and overexpressed (Herceptest 3+), and this finding was even clearer when a pronase-based antigen retrieval was employed. Conversely, in HER2 not amplified carcinomas, even though the HER2 protein is detectable by WB or by using an antibody against the intracellular domain in IHC, the trastuzumab binding site seems not to be accessible.

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Intratumoral Heterogeneity Of Pulmonary Adenocarcinoma

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Background

Adenocarcinoma of the lung is an extremely heterogeneous neoplasm in terms of morphology, with different patterns of growth and cytological variants, outlined in the 2015 WHO classification. It is well known that clinical treatments, especially those directed to molecular driver targets, may select cell clones responsible for secondary resistance development. Little is known, however, if there exists any relationship between morphologic and genetic intratumoral heterogeneity.

Aim of the study

This study was aimed at verifying the occurrence of mutation heterogeneity in pulmonary adenocarcinomas and its relationship with architectural changes inside tumors. **Methods and Materials:** We collected 20 lung adenocarcinomas, including 5 cases with mutation of EGFR gene, 5 with mutation of KRAS gene (both identified by Sanger sequencing), 5 cases with ALK translocation identified by FISH analysis and 5 cases “wild type” (WT) for the above mentioned molecular events.

For each case, we selected 3 to 6 tumor areas with different architectures or different areas with the same morphology, which were carefully laser microdissected to raise at least 500 tumor cells. These samples were analyzed by using the next-generation sequencer PGM™ IonTorreant according to Ion AmpliSeq™ Cancer Panel v2 Hotspot, which contains “hot spot” regions of 50 oncogenes and tumor-suppressor genes frequently mutated in human neoplasms.

Results

Hematoxylin and eosin-stained tumor sections demonstrated intratumoral morphologic heterogeneity in all 20 adenocarcinomas. The same gene mutations for EGFR and KRAS were confirmed by next-generation sequencing in all areas under assessment, regardless of morphology, indicating a driver effect on tumor development (ubiquitous mutation). ALK-translocated tumor cells ranged from 25 to 90%.

About 40% of tumors, showed a variety of recurring gene alterations, either heterogeneous/branched mutations (PIK3CA, EZH2 and TP53) present in only some regions or private mutations (MET, SMARCB1, BRAF, LKB1, EGFR, HER2, PTEN, ALK) present in one region only of tumors.

No relationship was found between mutation profile, in terms of type and number of mutations, and different tumor patterns/

variants. Interestingly, WT tumors did not exhibit any molecular alterations, except for one private PIK3CA mutation in a single neoplastic area.

Conclusions

We found less mutation intratumoral heterogeneity compared to morphological heterogeneity, without an apparent connection between them. Therefore, other molecular mechanisms, including epigenetic changes and microenvironmental interactions, could play a role to explain the morphologic intratumoral variability of pulmonary adenocarcinoma.

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TERT promoter mutations in early-stage (T1N0M0) non-small cell lung cancer (NSCLC) and in associated non-neoplastic lung diseases

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Background

The mutations detected in the promoter of the telomerase reverse transcriptase (TERT) gene, namely C228T and C250T, were first identified in sporadic and familial melanoma and subsequently in several cancer models, notably in glioblastoma, thyroid cancer and bladder cancer. Recent findings demonstrate that mutation of the TERT promoter may be one of the most common genetic mechanisms contributing to telomerase activation in malignant cells. Mutation of the TERT promoter seems to be an indicator of worse outcome and is correlated with tumor aggressiveness and patient survival. The mutation frequency varies considerably among cancer types. To date, only two studies examined primary lung cancers for mutations in TERT promoter gene. Ma X. et al ¹ demonstrated the presence of TERT promoter mutations in 2.57 % of non-small cell lung cancers (NSCLCs) while Li C. et al ² didn't find any mutated case out of 174 NSCLCs analysed. Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause associated with the histopathologic and/or radiologic pattern of usual interstitial pneumonia (UIP) ³. IPF is the most common manifestation of telomere-mediated disorders. Germline mutations in the essential telomerase genes, hTERT and hTR (human telomerase RNA), are the most commonly identified genetic risk factor in IPF. Recent reports have documented that genetic variants within hTERT or hTR are associated with familial pulmonary fibrosis and are present in some patients with sporadic IPF.

Goal

We would like to investigate the presence of TERT promoter mutations in NSCLC, in UIP and in diffuse pulmonary fibrosis. In patients with two synchronous lesions we studied the mutational status of TERT promoter gene both in the neoplastic lesion and in the surrounding parenchyma (with fibrosis or UIP).

Materials and methods

In this study, polymerase chain reaction and direct sequencing analysis of a TERT promoter region using formalin-fixed and paraffin-embedded tissues were performed to investigate the presence of TERT promoter mutations in a series of 95 early-stage (T1N0M0) non-small cell lung cancer (NSCLC) samples, including 48 adenocarcinomas (ADCs) and 47 squamous cell carcinomas (SCCs).

In the NSCLC samples that harbored the mutations we also analysed the surrounding parenchyma. Histological analysis of the parenchyma of mutated cases revealed diffuse fibrosis in 4 cases, emphysema in 5 cases and organising pneumonia in 1 case. Then, with the same methodology, we examined TERT promoter gene mutations in 24 cases of UIP. In two cases UIP was associated with adenocarcinoma, in these cases our analysis was extended to the neoplastic tissue.

Results: Sequencing analysis was performed in the TERT promoter region containing the two previously reported hot spot mutations. Our results revealed that TERT promoter mutation occurred in 10.52% of early-stage (T1N0M0) NSCLC patients, with higher frequency observed in ADCs (14.58%) compared to SCCs (6.38%). This result is of particular interest, although it does not reach statistical significance. Sequence analysis revealed that these mutations affected only one of the alleles of the TERT gene locus; their somatic nature was confirmed by their absence in normal tissues collected at a distance from the tumor in the 10 patients harboring TERT mutations. In eight mutated cases out of ten the DNA of the surrounding parenchyma was analyzable: none of them showed the hot spot mutations. Three samples carried mutations at other positions within the TERT promoter core, different from the hot spots (C206T/C205T, C212T/C211T and C205T).

For what concerns the UIP samples 20 of them were amplifiable: none harbored the hot spot mutations. Nine samples out of 20 showed the presence of mutations at other positions within the TERT promoter core, different from the hot spots (C209T in two cases, C211T in two cases, C217T in two cases, C231T, C236T, C237T/C240T).

Finally, the two adenocarcinomas samples associated with UIP showed a wild-type genotype of TERT promoter gene both in the neoplastic tissue and in the surrounding parenchyma with UIP.

Conclusions

In our study we showed that TERT promoter mutations are present even in non-small cell lung cancer, with particular incidence in adenocarcinomas. More detailed studies in larger cohorts of patients will be necessary to clarify the role of these mutations in NSCLC cancer progression with specific reference to the regulation of transcriptional mechanisms of TERT gene. To the best of our knowledge this is the first study that investigate on the presence of TERT promoter gene mutations in UIP and in non small cell lung carcinomas associated with UIP. None of the mutations found at other positions within the TERT promoter core generate de novo consensus binding sites for transcription factors. The molecular significance of these mutations, different from the hot spots, remain to be addressed.

Although hot spot mutations seem to be absent in UIP and in adenocarcinomas associated with UIP, further investigations are necessary to confirm these data and to understand the pathogenesis of UIP and the cancerogenesis in lung tissue with UIP.

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p16 Ink4A dichotomy in neuroendocrine and non-neuroendocrine lung tumors: a comprehensive analysis with clinicopathologic and molecular correlations

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Introduction

Lung cancer encompasses a constellation of malignancies with no sufficiently validated prognostic markers. The association between p16Ink4A overexpression and patients' survival have been studied in the lung: however its role remains controversial^{1, 2}.

Aims

We sought to define the significance of different patterns of p16Ink4A expression in a large series of neuroendocrine and non-neuroendocrine lung malignancies. Moreover, as a hypothesis-generating aim, we explored the correlation between p16Ink4A status and the most common molecular aberrations in EGFR and ALK.

Methods

502 cases, including 277 adenocarcinomas (ADC), 84 squamous cell carcinomas (SCC), 22 large cell carcinomas (LCC), 47 typical carcinoids (TC), 12 atypical carcinoids (AC), 28 large cell neuroendocrine carcinomas (LNEC), and 32 small cell carcinomas (SCLC) were reviewed and used to construct 15 tissue microarrays (TMAs). For each case, a mean of 4.7 tumor tissue cores (range 3 to 5 cores) was incorporated. Follow-up data were available for 444 patients until 2015 with a mean follow-up time of 31 months. Each TMA was subjected to immunohistochemical analysis of p16Ink4A and Ki-67. The pattern of p16Ink4A expression was recorded as negative, sporadic, focal or diffuse. The presence of somatic EGFR exons 19 and 21 alterations was detected by immunohistochemistry and validated by Sanger sequencing. The inversion event resulting in ALK-EML4 fusion was investigated by fluorescence in situ hybridization (FISH).

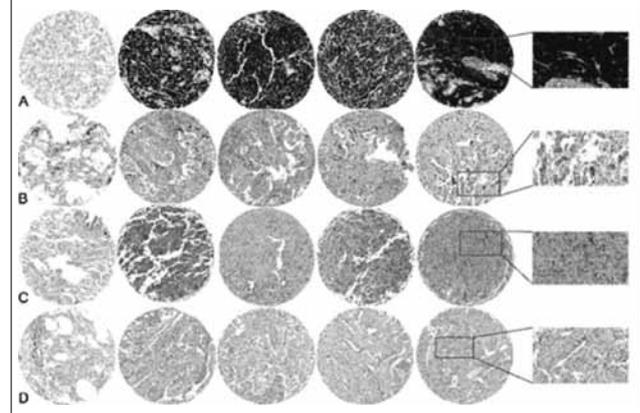
Results

Immunohistochemical p16Ink4A status of the primary lung carcinomas included in this study is summarized in Table 1. Homogeneous patterns of p16Ink4A expression were observed across the different topographic areas of each case (Fig. 1).

Tab. 1. p16Ink4A status in primary lung tumors.

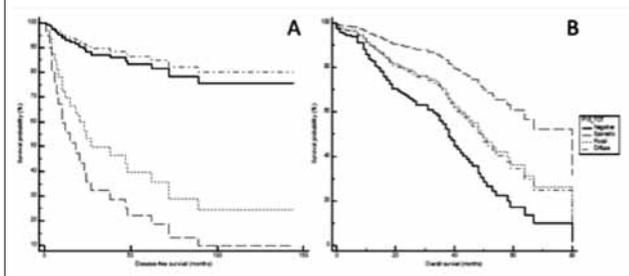
	negative (%)	p16 ^{Ink4A} overexpression		
		sporadic (%)	focal (%)	diffuse (%)
ADC	166 (59.9)	31 (11.2)	47 (17.0)	33 (11.9)
SCC	66 (78.6)	3 (3.6)	6 (7.1)	9 (10.7)
LCC	19 (86.4)	-	1 (4.6)	2 (10.0)
TC	40 (85.1)	7 (14.9)	-	-
AC	7 (58.3)	3 (25.0)	2 (16.7)	-
LCNEC	4 (14.3)	3 (10.7)	7 (25.0)	14 (50.0)
SCLC	-	2 (6.3)	-	30 (93.7)
	302	49	63	88

Fig. 1. Representative micrographs of the homogeneous p16Ink4A expression patterns in primary lung tumors. SCLC displaying diffuse expression (A); ADC, G2, showing focal expression (B); TC with sporadic expression (C); p16Ink4A-negative SCC, G3 (D). For each case, the first core on the left side is the matched normal lung tissue, whereas the following four cores represent different topographic areas of the tumor (5x). The histologic detail of the immunohistochemical analysis can be appreciated on the right side (20x).



Enrichments in cells expressing p16Ink4A were observed from lower- to higher-grade neuroendocrine malignancies, while a decrease was seen in poorly differentiated and undifferentiated non-neuroendocrine carcinomas. Ki-67 was higher in neuroendocrine tumors expressing p16Ink4A while non-neuroendocrine malignancies expressing p16Ink4A showed decreased proliferation indexes. Shorter times to relapse were seen in neuroendocrine malignancies harboring even a focal p16Ink4A positivity (Figure 2). Conversely, non-neuroendocrine carcinomas with diffuse p16Ink4A expression had a better outcome compared to p16Ink4A-negative cases (Figure 2). Multivariate analysis confirmed the independent prognostic roles of p16Ink4A pattern of expression. All cases expressing p16Ink4A and harboring EGFR or ALK alterations had excellent outcomes.

Fig. 2. Survival analysis of p16Ink4A status in lung neoplasms. A. Cox proportional-hazards regression of lung neuroendocrine tumors for disease-free survival. B. Cox proportional-hazards regression of lung non-neuroendocrine carcinomas for overall survival (tumor stage as a covariate).



Conclusions

We provided contingent evidence that in lung tumors the immunohistochemical assessment of p16Ink4A even in a small biopsy could be representative of the p16Ink4A status of the entire lesion. Since the presence of a submodal neoplastic population harboring p16Ink4A overexpression could lead to different outcomes, our data suggest that p16Ink4A represents a clinically meaningful biomarker in lung tumors. We also describe a subset of lung carcinomas harboring EGFR and ALK mutations and expressing p16Ink4A that shows good outcome. In conclusion, p16Ink4A plays a dichotomous role in lung tumors, being an independent poor prognostic factor in neuroendocrine tumors (“the bad”) and an independent good prognostic factor in non-neuroendocrine malignancies (“the good”). Our study opens new doors and specific goals to be chased in the controversial issue of p16Ink4A role in lung cancer.

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Sinonasal neuroendocrine carcinomas and olfactory neuroblastomas: clinico-pathologic analysis of 85 cases

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Goal

We performed a retrospective observational cohort study from a series of patients affected by sinonasal olfactory neuroblastoma (ONB) and neuroendocrine carcinoma (NEC) collected in two Italian tertiary care referral centers for the management of Skull Base Cancer. The aim of the study was to analyze survival outcomes and prognostic factors and to evaluate the role of immunohistochemical staining in differential diagnosis between the two entities.

Methods and Materials

The following data of patients with sinonasal ONB and NEC

diagnosed from February 1999 to April 2014 were retrieved: age, diagnosis, risks factors, symptoms at presentations, medical history including surgery, complications, chemotherapy and/or radiotherapy, pattern of relapse, and outcomes.

Patients who underwent endoscopic endonasal resection (EER) (with transnasal craniectomy or combined transnasal and transcranial approach, when necessary) were included and only N0 patients entered the study. The pre-operative diagnostic workup includes fine-cut computed tomography (CT) of the paranasal sinuses, head magnetic resonance imaging (MRI) scan with gadolinium and transnasal biopsy under endoscopic assistance in local anesthesia.

Post-operative radiotherapy (poRT) was planned for all patients. Elective irradiation of the neck was performed only for patient at high risk of regional failures. Chemotherapy was delivered preoperatively as neoadjuvant treatment in selected cases of sinonasal NEC with high risk of systemic dissemination or postoperatively concomitant to poRT (radiosensitizing chemotherapy) for the treatment of selected cases of high aggressive and locally advanced ONB and NEC.

All patients underwent endoscopic examinations and MRI every four months during the 1st year; every two and six months, during the 2nd year and, thereafter, at 6-month intervals, for a minimum of 12 months or until death, breaking off the observation only after 15 years.

All tissues were fixed in buffered formalin and routinely processed to paraffin wax. Five μ m-thick sections were stained with hematoxylin-eosin (H&E) for morphological evaluation and assessment of Hyams' grading.

For immunohistochemistry, three μ m-thick sections were mounted on poly-L-lysine coated slides, deparaffinized, hydrated, incubated with primary antibodies after endogenous peroxidase activity inhibition, followed by the avidin-biotin complex (ABC) procedure. Sections were counterstained with Harris' hematoxylin. The following antibodies were used: p53 (monoclonal, clone D07, 1:500, Dako), Ki67 (monoclonal, clone MIB1, 1:50, Dako), Synaptophysin (monoclonal, clone snp88, 1:100, BioGenex), Chromogranin A (monoclonal, clone LK2H10, 1:100, Ventana), S100 (polyclonal, 1:1, Ventana), cytokeratin (CK) 8/18 (monoclonal, clone 35BH11, 1:100, Dako) and CK AE1-AE3 (monoclonal 1:1, Ventana). The p53 positivity was scored as follows: score 1= positive cells $\geq 10\%$; score 0 = positive cells $< 10\%$. Ki67 index was scored as follows: score 1= positive cells $\leq 20\%$; score 2= positive cells between 20% and 55%; score 3= positive cells $\geq 55\%$.

The main endpoints measured were overall survival (OS), disease specific survival (DSS) and recurrence-free survival (RFS). The Kaplan–Meier method was used to estimate the probability of RFS and OS; values were compared using the log-rank test. A multivariable proportional hazard Cox-regression model was implemented for the RFS and OS. Results are shown in term of hazards ratios (HR), 95% confidence intervals (CIs), and p values. All statistical tests were two tail and p values were considered significant when ≤ 0.05 . All analyses were carried out using the SAS program, version 9.2 (Cary, North Carolina, USA).

Results

Clinicopathological features of population are reported in Table I.

Sinonasal ONBs and NECs showed statistically significant differences in survival (5-years OS, 92.6% versus 38.8%, $p=0.0001$).

Sinonasal NEC was found to be a highly aggressive tumor, usually presenting at advanced stages (62% of pT4b stage),

Tab. I. Clinicopathological features of the study population.

		ONB (64cases)	NEC (21cases)	TOTAL (85 cases)
Gender	Male	29/64 (45.3%)	16/21 (76%)	45/85 (53%)
	Female	35/64 (54.7%)	5/21 (24%)	40/85 (47%)
Age	Range	14-79 years	41-85 years	14-85 years
	Mean	51.2 years	59.8 years	52.9 years
pT classification	T1	7/64 (11%)	1/21 (4.7%)	8/85 (9.5%)
	T2	14/64 (21.8%)	1/21 (4.7%)	15/85 (17.6%)
	T3	14/64 (21.8%)	3/21 (14.3%)	17/85 (20%)
	T4a	7/64 (11%)	3/21 (14.3%)	10/85 (11.7%)
	T4b	22/64 (34.4%)	13/21 (62%)	35/85 (41.2%)
Previous therapy	No	53/64 (82.8%)	18/21 (85.7%)	71/85 (83.5%)
	Yes	11/64 (17.2%)	3/21 (14.3%)	14/85 (16.5%)

developing a broad range of systemic metastases (47.6% of patients) in a short interval of time (mean, 20 months), without significant possibilities to cure and dismal prognosis. Neoadjuvant chemotherapy improved survival (5-years OS, 80.1% versus 9.1%, $p=0.004$) in patients with NEC.

Conversely, ONB showed better survival outcomes even for locally advanced stage of disease, with 17% of local recurrences and 12.5% of neck failure after a mean interval of time of 69.5 months. Recurrences were generally localized, early detected during the follow-up and curable in 70% of cases. Dural infiltration and positive surgical margins were associated with poor prognosis. (5-years DSS, 100% versus 81.8%, $p=0.03$ and 5-years DSS, 95.6% versus 66.7%, $p=0.01$). Hyams grading accurately characterized the tumor's biology and was an independent predictor of loco-regionally aggressive disease and worse survival rates (Cox regression model: Overall Survival HR=0.18 with $p=0.05$ and Recurrences HR=0.25 with $p=0.02$ for Hyams grade I-II ONB versus Hyams grade III-IV ONB).

Concerning immunochemistry, NECs showed a strong and diffuse positivity for cytokeratin (CK) 8/18 and CK AE1-AE3, Synaptophysin (Syn), Chromogranin A (ChrA), while S100 staining was negative.

ONBs were characterized by a peculiar S100 staining in sustentacular cells, together with a strong and diffuse immunoreactivity for Syn and Chr A. Remarkably no significant stainings were observed for CK 8/18 and CKAE1-AE3, with only focal positivity in a few cases

The immunohistochemical evaluations suggested a possible misdiagnosis in three cases of ONB that showed immunophenotypic features of NEC.

Finally, we investigate the prognostic role of p53 and Ki67, evaluated in both tumor types. In our serie 17/46 ONBs (36,9%) and 6/13 NECs (46,2%) showed p53 expression. The average Ki 67 index value was 14.37% in ONB group and 60.8% in NEC group. None of them resulted statistically significant (ONB: p53 score $p=0.349$, MIB1 score $p=0.437$; NEC: p53 score $p=0.183$; Ki67 score $p=0.729$)

The Hyams grading system resulted to be a statistically significant prognostic factor independently associated with a lower risk of death for low grade tumors (Hyams I-II) (OS HR 0.18, $p=0.05$; DSS HR 0.02, $p=0.02$; RFS HR 0.25, $p=0.02$). Furthermore, in ONB patients, positive margins of resection were associated with increased risk of overall death (OS, HR19.09, $p=0.02$) and disease-related death (DSS, HR of 51.26, $p=0.01$). Patient's age at diagnosis and administration of postoperative treatments did not show any prognostic role.

For patients with NECs, statistically significant differences were present between patients that received neoadjuvant chemotherapy and patients that underwent surgery as first treatment (5year OS 80.1 vs 9.1%, $p=0.004$).

Conclusions

Given the distinct biological behaviors of these two neoplasms, the correct histopathological diagnosis to distinguish ONBs from NEC should be ensured before treatment begins, to provide the best treatment strategy and achieve better outcomes, sparing the patient from needless and potentially toxic treatments. For this reason, extensive immunohistochemical staining including, S100, Synaptophysin, Chromogranin A, cytokeratin 8/18 and AE1-AE3, P53, and Ki67 are mandatory to appropriately diagnose such tumors.

Prospective randomized trials on a larger cohort of patients are difficult because of the rarity of the disease but would be desirable in order to validate our findings. For this reason, multi-institutional and international collaborations will be necessary in collecting data and in reporting outcomes in a uniform manner

Bap1 (Brca1-Associated Protein 1) Is A Highly Specific Marker For Differentiated Mesothelioma From Reactive Mesothelial Proliferations

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Introduction

The distinction between malignant mesothelioma (MM) and reactive mesothelial proliferation (RMP) can be challenging both on histology and cytology. Recently, variants of the BRCA1-associated protein1 (BAP1) gene resulting in nuclear protein loss were reported in hereditary¹ and sporadic^{2,3} MM.

Goal, materials and methods

Using immunohistochemistry (anti-BAP1 monoclonal antibody, clone C-4, Santa Cruz, USA), we evaluated the utility of BAP1 expression in the differential diagnosis between

MM and other mesothelial proliferations on a large series of biopsies that included 212 MM, 12 benign mesothelial tumors and 42 RMP. A smaller cohort of MM was analyzed by fluorescence in situ hybridization (FISH; BAP1/CEN3q probe,

Fig. 1. Positive BAP1 immunoreactivity in normal mesothelium and lung alveolar epithelium (a) and in a case of adenomatoid tumor (b).

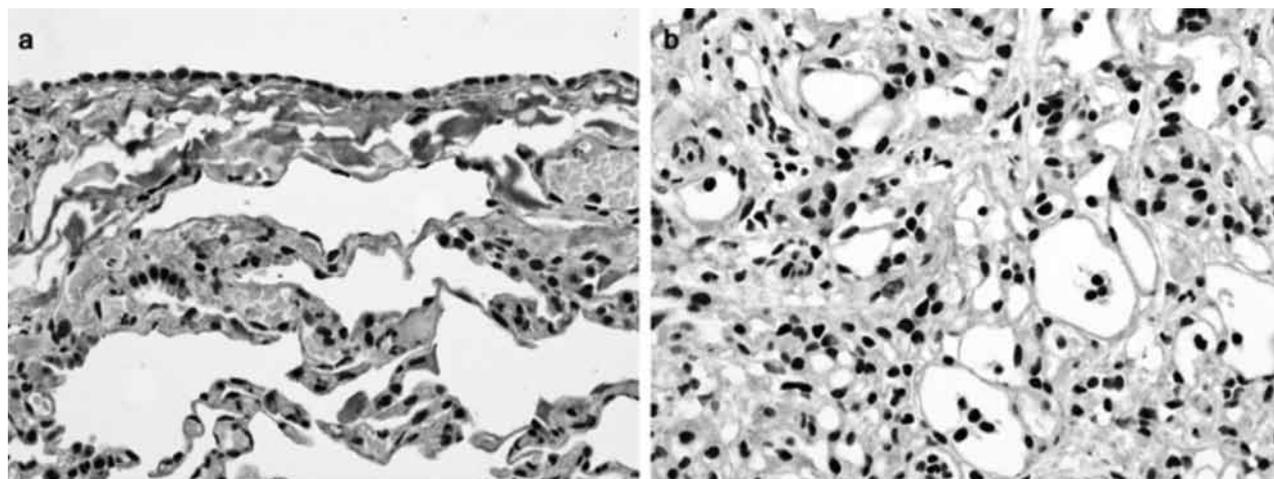
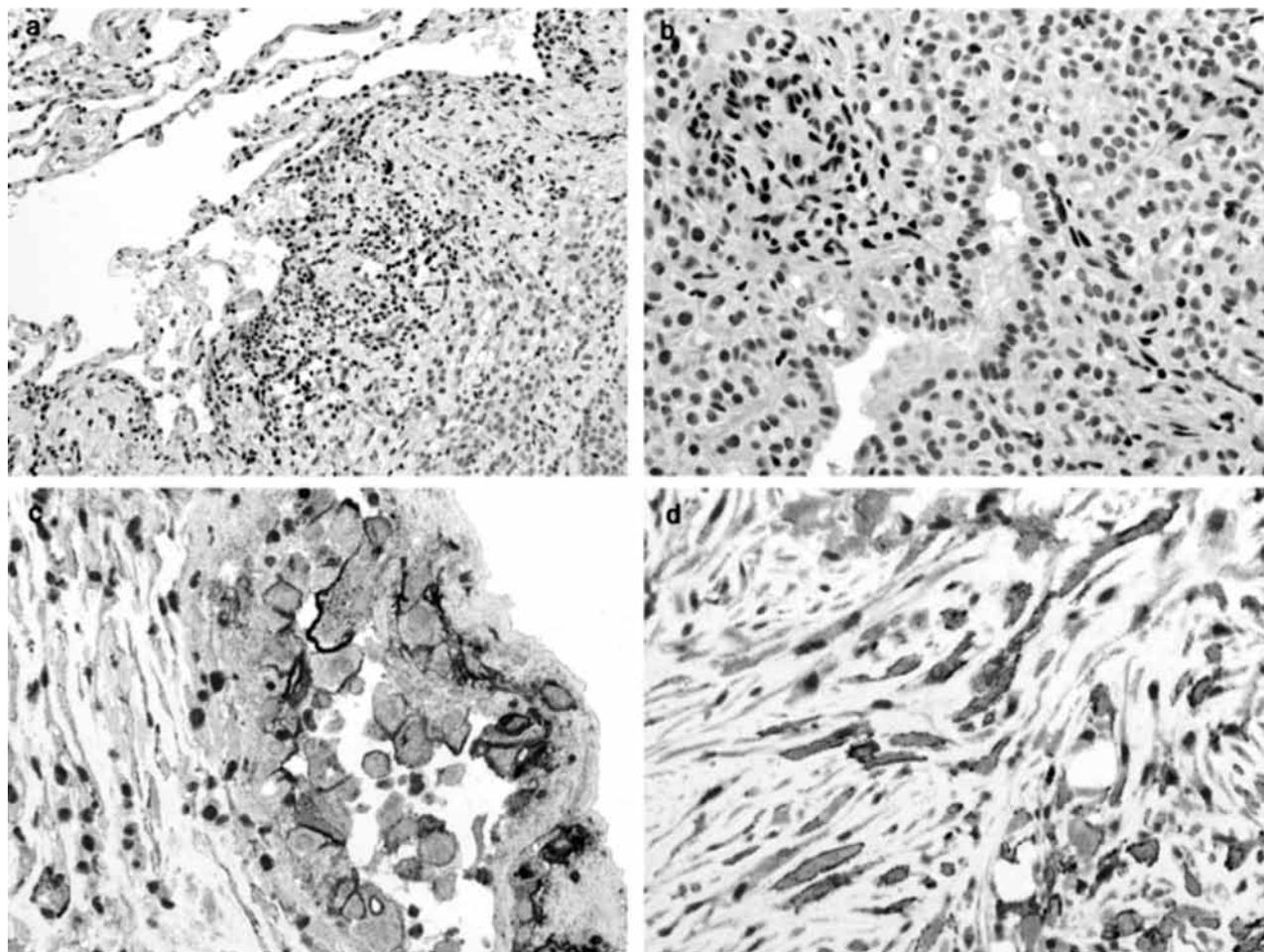


Fig. 2. a-d are examples of three BAP1- epithelioid and biphasic MM. Note in (a) the BAP1+ lung parenchyma (upper left) infiltrated by the tumor (lower right), in (b) BAP1 is expressed by inflammatory and vascular endothelial cells within the tumor. c-d are from a case of biphasic MM double stained for BAP1 (brown) and epithelial membrane antigen (blue), the latter being helpful to identify the BAP1- spindle cells in sarcomatoid areas.



Abnova, Taipei, Taiwan). BAP1 stain was also performed on 70 cytological samples (45 MM, 25 RMP).

Results

BAP1 was expressed in all benign mesothelial tumors (Fig. 1), while 139/212 (66%) MM were BAP1-negative, especially in epithelioid/biphasic (Fig. 2) compared to sarcomatoid/desmoplastic subtypes (69% vs 15%). BAP1 loss was homogeneous in neoplastic cells except for two epithelioid MM showing tumor heterogeneity. By FISH, BAP1 protein loss was paralleled by homozygous deletion of the BAP1 locus in the vast majority of BAP1-negative tumors (31/41, 76%), while 9/10 BAP1-positive MM were normal (Fig. 3).

Fig. 3. Fluorescence *in situ* hybridization using the *BAP1/CEN3q* probe in a case of BAP1 positive mesothelioma that shows normal copy numbers (a) and in a case of BAP1 negative mesothelioma with homozygous deletion of *BAP1* gene (b).

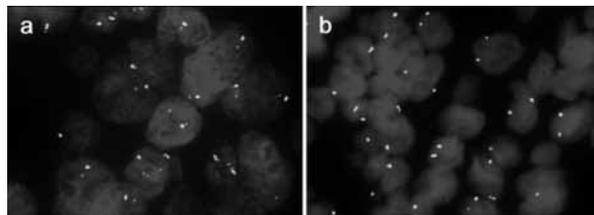
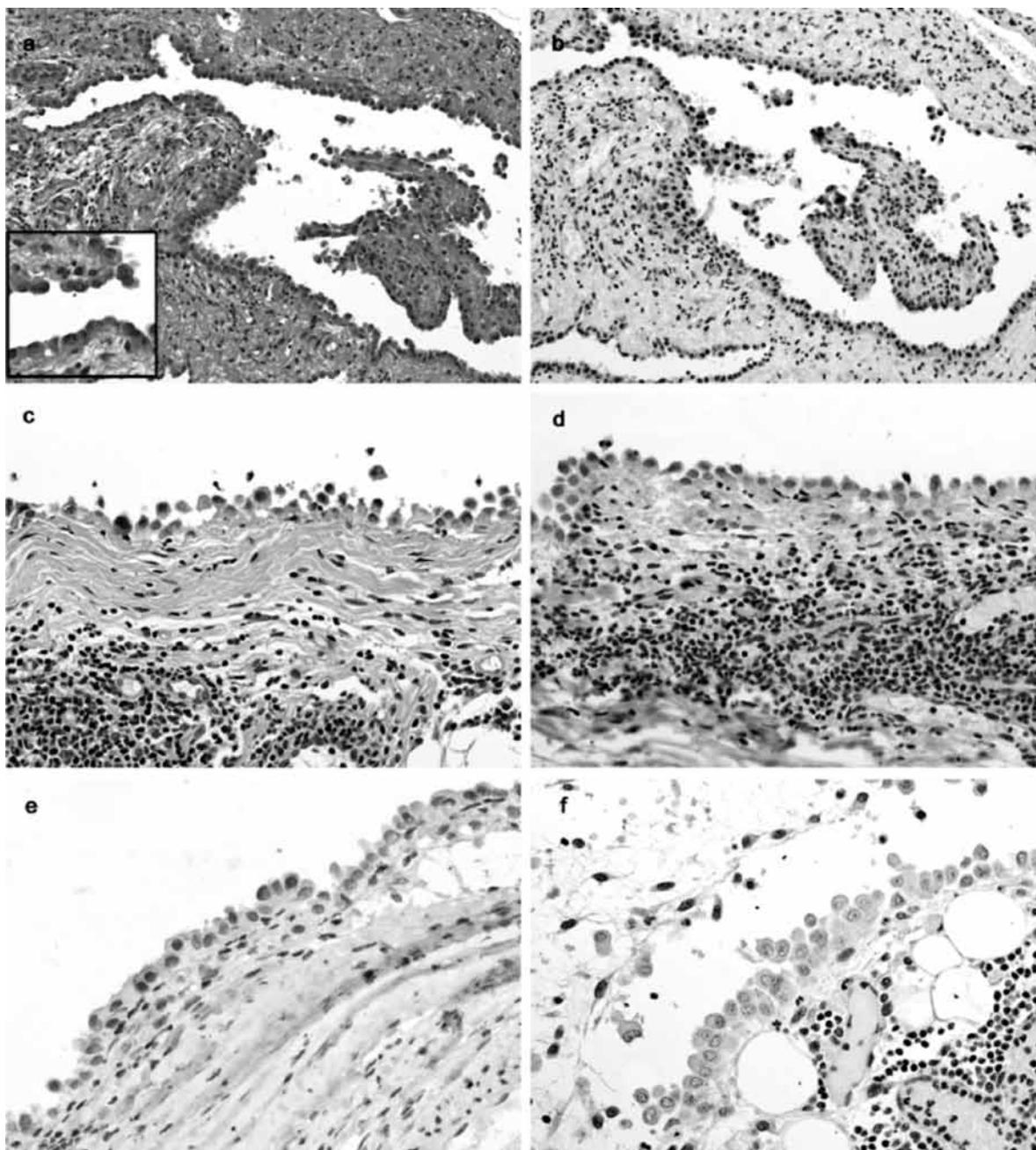


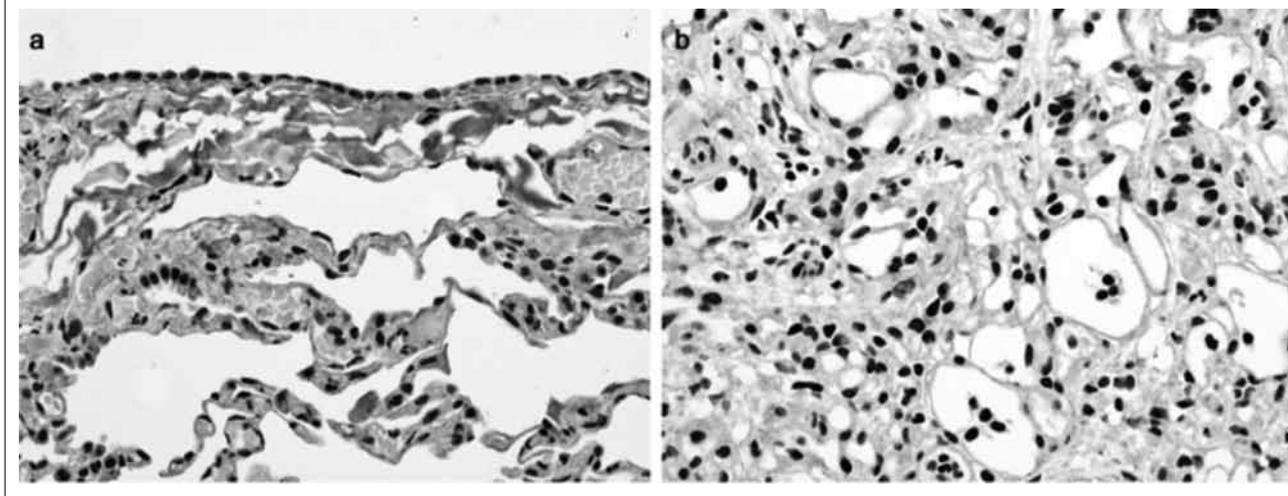
Fig. 4. BAP1 immunostain in cases of non-invasive mesothelial proliferations. H&E shows irregular mesothelial proliferation with papillae (a) and cytological atypical features (inset); BAP1 is strongly reactive in mesothelial and stromal-inflammatory cells (b). This case was associated with emphysema-tous bulla; no history of MM was recorded. c-f are from three different cases of mesothelial proliferation showing BAP1 negativity; all of them subsequently developed frankly invasive BAP1- MM



In biopsies originally interpreted as RMP, BAP1 loss was 100% predictive of malignancy, since all six cases subsequently developed BAP1-negative mesothelioma, whereas only 3/36 (8%) BAP1-positive cases progressed to mesothelioma (Fig. 4). On cytology/cell blocks, benign mesothelial cells were invariably positive for BAP1, while 64% of MM showed loss of protein;

all six cases showing BAP1 negativity were associated with histological diagnosis of BAP1-negative MM (Fig. 5). BAP1 stain showed utility also in the differential of mesothelioma from most common pleural and peritoneal mimickers, such as lung and ovary carcinomas, with specificity and sensitivity of 99%/70% and 100%/70%, respectively (not shown).

Fig. 5. BAP1 stain on cytological (a) and cell block (b) samples from two epithelioid MM, showing BAP1- tumor and BAP1+ inflammatory cells. In (b) double stain for BAP1 (brown) and CD11c (blue) reveals the histiocytic nature of the BAP1+ cells embedding the tumor papillae.



Conclusion

Our results show that BAP1 protein is frequently lost in mesothelioma, especially of epithelioid/biphasic subtype and is commonly associated with homozygous BAP1 deletion. BAP1 immunostain represents an excellent biomarker with an unprecedented specificity (100%) in the distinction between benign and malignant mesothelial proliferations. Finding BAP1 loss in mesothelial cells should prompt to immediately re-evaluate the patient; moreover, it might be useful in mapping tumor extent and planning surgical resection.

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BAP1 immunohistochemical cellular distribution in malignant pleural mesothelioma

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Goal

Malignant pleural mesothelioma (MPM) is a highly aggressive disease for which new prognostic biomarkers need to be identified. Currently, among other, histological type (i.e. epithelial, sarcomatoid or biphasic) is the most robust prognostic factor, since epithelial type is associated to a less aggressive course, compared to the pure sarcomatoid MPM type. Biphasic MPM subtype has an intermediate aggressive behavior quite similar to sarcomatoid type, but its histological recognition criteria are in some cases problematic, due to the

lack of clear cut evidences of sarcomatoid features in the stromal component. Recent data identified BRCA1-Associated Protein 1 (BAP1) among the most frequently mutated genes in MPM. BAP1 is a tumour suppressor gene, whose germline mutations have been identified in familial MPM and in MPM associated with cancer syndrome. Somatic BAP1 mutations were also reported at high frequency in sporadic mesotheliomas. Immunohistochemistry (IHC) for BAP1 appears to be a predictive and reliable technique for the detection of BAP1 mutation/inactivation in neoplastic cells and has the distinct advantage over molecular testing of low cost and widespread availability. We confirmed this finding in a series of sporadic MPM cases analyzed by NGS for several genes including BAP1 and in parallel stained for BAP1 with a significant concordance between BAP1 mutations and the loss of the nuclear protein localization (p=0.001). These data prompted an extensive analysis of the extent of BAP1 aberrant expression or nuclear loss in sporadic MPM, as well as a study of the cellular distribution patterns as detected by immunohistochemistry, in parallel with the gene mutational profile.

The aim of the present study was to define the BAP1 expression profiles in the different subtypes of MPM, in order to validate its potential role in the correct classification of MPM histological types.

Methods and Materials

A series of surgically resected MPMs (88 epithelial, 15 biphasic, and one of sarcomatous histotype) were collected at the Pathology Units at University of Turin (Orbassano, Turin) and Sapienza University (Rome) and investigated for BAP1 protein expression (clone-C4, Santa-Cruz Biotechnology, CA/USA) using an automated immunostainer (Ventana Benchmark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA). A pilot series of 10 cases were investigated for mutational BAP1 gene status with direct sequencing by Sanger.

Results

Negative staining for BAP1, defined as completely absent nuclear staining in the presence of positive internal controls in non-neoplastic endothelial cells and/or lymphocytes, occurred in 71% of MPMs either with complete protein loss or granular cytoplasmic delocalization. Upon revision of morphological variants, among BAP1 negative epithelial MPMs, 11 had reactive stromal cells with low-to-mild atypia, possibly leading to a mis-diagnosis of biphasic histotype. In these cases a positivity of BAP1 in stromal cells should confirm the epithelial variant with reactive stroma. On the other hand, in 5/15 cases diagnosed as biphasic based on morphology (14 sporadic and 1 familial MPM) a negativity of the epithelial and a positivity of the stromal component was found.

Molecular analyses showed 80% of concordance between BAP1 protein loss and gene mutation. In the two cases with BAP1 protein negativity and WT gene status, an intense inflammatory infiltrate was present interspersed with tumor cells thus probably leading to a dilution of mutated DNA under the method sensitivity.

Conclusion

We here show that BAP1 loss of protein or delocalization from the nucleus to the cytoplasm of neoplastic cells is present in approximately two thirds of MPM. Interestingly stromal cells maintain BAP1 expression and this may help in the correct distinction of true biphasic (sporadic or familial) MPM from epithelial subtypes having an atypical or activated reactive/desmoplastic stroma.

Nanostring's Prosigna™ breast cancer prognostic gene signature assay: concordance study between molecular subtypes and IHC4 classification

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Goal

The “intrinsic” molecular subtypes can be considered as a major classification framework of breast cancer. This approach has gained wide acceptance in preclinical and clinical research for in-depth exploration of the biology of breast cancer. In the last years, the intrinsic subtype classification has been established by a commercially available assay, named PAM50/PROSIGNA (Nanostring technology). To date, intrinsic subtyping can be approximated by immunohistochemistry (IHC), using a panel of four antibodies (ER, PR, HER2, Ki-67), collectively named “IHC4”. With IHC4, the numerical distribution of tumor types is similar to what would be expected from the distribution of tumor types seen by multigene array profiling. However, on an individual basis the concordance of intrinsic subtyping with conventional IHC is only moderate. Such a discrepancy may be due, among other factors, to the well-known variability of the immunostaining technique; indeed, the quality of immunohistochemistry relies heavily on the antibody clone used, the timing of formalin fixation, and the pathologist's evaluation. Moreover, quantitative evaluation of immunohistochemistry is not standardized among different laboratories, and there is a lack of criteria for the definition of IHC positivity¹. The aim of this report is to assess the IHC4 classification

effectiveness compared with PROSIGNA/PAM50 assay in patients with early breast cancer.

Methods and Materials

Forty-two early breast cancer patients, were selected from the tissue archives of the Pathology Unit at of the Campus Bio-Medico University Hospital on the basis of IHC ER positivity (luminal type). IHC4 subtypes were defined according to St. Gallen 2013 criteria (Luminal A-like: ER+, PGR<20%, Ki67<14%, Her2 negative; Luminal B-like: ER+, PGR>20% and/or Ki67>14% and/or HER2 positive)². Immunohistochemical data were obtained from the original pathology reports. All the selected cases have also been analyzed by Prosigna™ Breast Cancer Prognostic Gene Signature Assay. IHC4 data were compared with PAM50 intrinsic molecular subtypes.

Results

According to IHC4 score system, 9 patients resulted as Luminal A-like and 33 as Luminal B-like. However, by PAM50/PROSIGNA assay 23 patients resulted as Luminal A and 19 as Luminal B. Data showed that 16/42 (38%) cases were discordant according to IHC4 or PAM50 categorization.

Interestingly, when using Ki67 as the reference value for classification, breast cancer with Ki67 <10% (luminal A-like) or with Ki67>40% (Luminal B-like) showed 100% concordance with PAM50 classification (luminal A and B, respectively). On the other hand, in cases with Ki67 value between 11% and 40%, 16/28 (57%) resulted IHC4/PAM50 discordant. In particular, 15/16 breast cancers classified as Luminal B-like by IHC4, resulted Luminal A according to PAM50.

Conclusions

To date, the decision of using or not using chemotherapy in patients with breast cancer is based on the surrogate intrinsic phenotype determined by IHC4 (St. Gallen Consensus). However, the analytical validity of the Ki67 immunohistochemistry assay in diverse clinical laboratories remains poor¹. In this direction, the American Society of Clinical Oncology has refrained from recommending the use of Ki67 in clinical practice, largely due to lack of standardization in Ki67 assessment³. In line with these considerations, our preliminary data confirm that Ki67 value is often the crucial factor for IHC4/PAM50 discordance.

Our results also suggest that Ki67 value between 11% and 40% should be considered a critical “grey zone” with the higher rate of discordance among immunohistochemistry and PAM50/PROSIGNA assay. In such cases, Prosigna™ Breast Cancer Prognostic Gene Signature Assay could provide an accurate and reliable assessment of intrinsic molecular subtypes, therefore representing an effective support to establish the correct therapeutic option for breast cancer patients.

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The prognostic and predictive value of Endopredict® Test in luminal type breast carcinoma: preliminary results of a validation study in a single institution

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Goal

Carcinoma of the breast constitute a heterogeneous group of tumors with marked variation in biologic behavior and response to therapy. Morphological (tumor diameter, histological type, grading, nodal status and vascular invasion) and immune-phenotypical characteristics as estrogen (ER) and progesterone receptors (PR), human epidermal growth factor 2 (HER2) and Ki-67 proliferative indexes, have been the main parameters used in the management of breast carcinomas. However the increasing evidence of dissimilar clinical outcome despite apparently similar bio-pathological profile demonstrated the need of more reliable markers. Molecular classifications of breast carcinomas are able to capture the intrinsic biological variances among these tumors and stratify them into more clinically relevant groups respect to those possible by ER/PR/HER2 testing.

According to current guidelines, molecular tests predicting the outcome of breast cancer patients can be used to assist in making treatment decisions after consideration of conventional prognostic and predictive markers.

Endopredict® (EP) which has been specifically developed in ER+/HER2-/node negative/positive tumors (luminal type) is based on mRNA expression of 8 genes of interest and 3 reference genes measured using quantitative RT-qPCR on formalin-fixed-paraffin embedded (FFPE) samples ¹. In contrast with other commercially available multigene tests EP is suitable for decentralized testing instead of a single reference laboratory. The recent acquisition of EP by our Institution prompted us to start a validation study on a cohort of luminal breast cancer patients.

Methods and materials

The study is based on two groups of patients. The first one is represented by a retrospective analysis of 7 cases of luminal breast cancers with a mean follow-up of 74 months (range 22-98) and a complete therapeutic history available. The second group is constituted by 8 patients with a recently diagnosed luminal breast carcinomas showing peculiar clinic-pathological features. Representative samples of FFPE tumoral tissue were retrieved from our archive and reviewed in order to confirm the luminal typology. Ten µm thick section with at least 30% of invasive cancer were obtained for each sample and used for RNA extraction and gene analysis according to the manufacturer’s instructions. If the tumor content was <30%, the proportion of the tumor tissue had been increased by macro-dissection. After mixing the reagents and the sample RNA and dispensing the mixture onto the EP plate with the aid of pipettes, the RT-qPCR was performed in a VERSANT® kPCR System AD module. Using the previously obtained PCR measurement values, the Web-based EndoPredict® Report Generator (EPRG) software calculates the EP score from the relative expression of the informative genes. When the tumor size and number of positive lymph nodes were entered, the EPclin score and the 10-year-metastasis risk were calculated. Based on a “cut-off value”, classification of the risk of distant metastases as low or high is also performed. Moreover, the EPRG checks all data and compared the measured values of the controls and the sample to defined control limits ².

Results

In the first step our technical staff was subjected to a successfully training, as well as VERSANT® kPCR System has undergone implementation and validation. All the samples yield adequate mRNA for subsequent analysis.

In table 1 are summarized the main clinic-pathological features of the cases studied.

In all the 7 patients of the first group EPclin well fitted with the follow-up data. Cancer in patient n. 1 and n. 2 had morphologic and phenotypic features of not aggressiveness and we found low levels of EPclin and EP score; in opposite situation of aggressiveness, we had patients n. 5 and n. 6, where we saw high levels of two markers. Patient n. 3 was included in our study because she refused CHT at the time of diagnosis: EPclin calculated in our study resulted low in according to the actual state of the patient

Tab. 1. Patients clinic-pathological features of studied cases.

n.	Sex, Age	Histotype	pTNM	San Gallen 2013	RTH	CHT	ET	Metastasis	Prognosis
1	F, 64	IC, NST G1	pT1b, pN0(sn)	Luminal A-like	yes	no	yes	no	AW
2	F, 47	IC, NST G1	pT1c, pN0	Luminal A-like	yes	yes	yes	no	AW
3	F, 43	IC, NST G2	pT1c, pN0(sn)	Luminal A-like	no	no	yes	no	AW
4	F, 71	IC, NST G2	pT2, pN0	Luminal A-like	no	no	yes	Liver, bone	D
5	F, 57	IC, NST G3	pT2, pN1	Luminal B-like	yes	yes	yes	Lung	D
6	F, 50	IC, NST G2	pT2, pN1	Luminal B-like	yes	no	yes	Lung, liver	D
7	M, 50	IC, NST G3	pT2, pN1	Luminal A-like	no	yes	yes	Cerebellum	AW
8	F, 68	ILC	pT1b, pN2a	Luminal B-like	no	yes	yes	no	AW
9	F, 52	IC, NST G1	pT1b, pN1	Luminal A-like	no	yes	yes	no	AW
10	F, 60	ILC	pT1c, pN1(mi)	Luminal A-like	no	no	yes	no	AW
11	F, 55	ILC	pT1c, pN0	Luminal A-like	no	no	no	no	AW
12	F, 42	IC, NST G2	pT1b, pN0	Luminal B-like	no	no	yes	no	AW
13	F, 58	IC, NST G2	pT1b, pN0	Luminal B-like	NYP	NYP	NYP	no	AW
14	F, 68	IC, NST G3	pT1c, pN0(sn)	Luminal A-like	NYP	NYP	NYP	no	AW
15	F, 54	IC, NST G1	pT1b, pN1(mi)	Luminal A-like	NYP	NYP	NYP	no	AW

Legend. F: female, M: male, IC: infiltrative carcinoma, NST: no special type, ILC: invasive lobular carcinoma, RTH: radiotherapy, CHT: chemotherapy, ET: endocrine therapy, NYP: not yet planned, AW: alive and well, D: died

who is AW. Patient n. 4 was selected because she was treated with endocrine therapy alone and she died after 53 months; indeed EP score and EPclin are both high and the result of Endopredict® could have allowed a more aggressive therapy. In the second group we obtained interesting results also without follow-up data. In two patients with node metastasis we found low EP score with molecular profile demonstrating a strong endocrine sensibility: these results could favor endocrine therapy rather than chemotherapy. In other cases we controlled the utility of EP to help better therapeutic choices when there were intermediate features of risk, according to the current parameters or discrepancies between traditional prognostic and predictive markers. Finally we confronted EP score in primary cancer versus node metastasis, in patient n. 5, and primary cancer versus biopsy, in patient n. 11: in both cases we got comparable results.

Conclusion

In our study we assessed the new markers, EP score and EPclin, in a group of patients and we did not find discrepancies between results and prognosis. Moreover we demonstrated EP high prognostic and predictive value and that it can be used successfully in nodes, biopsies and in specimens with few cancer cells. Our data showed that EP is an easy-to-perform prognostic multigene expression test, which can easily be included in the routine workflow in a Pathology laboratory for the management of luminal breast cancer patients.

A larger number of cases is necessary to understand completely EP role in clinical practice.

However, to date, we can assert that it is very useful to predict the likelihood of distant metastasis and to improve therapeutic choices in selected patients, in addition to morphology and immunohistochemistry.

References

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- 2 Endopredict® instruction manual. 2014.

Microglandular adenosis: a clonal neoplastic lesion of triple-negative phenotype

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Microglandular adenosis (MGA) is a rare lesion of the breast characterized by non-lobulocentric proliferation of small round glands lined by S100-positive, estrogen receptor (ER)-negative epithelial cells and lacking myoepithelial cells. Although classified as a benign epithelial proliferation¹, evidence suggests that MGA may constitute a non-obligate

precursor of triple-negative breast cancer (TNBC)².

Goal: Here we sought to define the genomic landscape of MGA and of associated TNBCs, and to determine whether MGA may constitute the substrate from which TNBCs originate.

Methods and materials

Five cases of MGAs and three of atypical MGA (AMGA) associated with in situ or invasive TNBC were collected. DNA from distinct morphologic components and matched normal tissue was extracted from microdissected representative sections and subjected to massively parallel sequencing targeting all coding regions of 273 genes recurrently mutated in breast cancer or related to DNA repair mechanisms. Single nucleotide variants were detected by MuTect; insertions and deletions were identified by Strelka and VarScan 2. Copy number aberration were detected using Varscan 2.

Results

MGA (n=5) and AMGA (n=3) displayed at least one somatic mutation (range 5-17 and 1-10, respectively), whereas TNBCs (n=4) associated with MGA/AMGA harbored 6 to 11 somatic mutations. Four to seven mutations identified in MGA/AMGA were also detected in their associated invasive TNBCs, and in all cases identical TP53 mutations were found in the MGA and/or AMGA and matched TNBCs. Moreover, similar complex copy number profiles were identified in MGA/AMGA and associated TNBCs. In the MGA/AMGA lacking TP53 mutations, mutations affecting other known driver genes, including PIK3CA, ERBB3, PTEN, FGFR2 and INPP4B, were identified.

Conclusion

MGA is a clonal and neoplastic lesion, harboring recurrent mutations in TP53 and in other bona fide cancer genes and a complex pattern of copy number aberrations. Identical genetic events were identified in MGA/AMGA and matched invasive TNBCs, providing evidence to suggest that these lesions may constitute non-obligate precursors of TNBCs.

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Inflammatory breast carcinoma: high fidelity of morphological and molecular profiles before and after neoadjuvant treatment

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Goal

The aim of this study is to evaluate the histological response to neoadjuvant therapy, the pathological biomarkers predictive of recurrence and the variations of the morphological and molecular profiles in a series of inflammatory breast carcinoma before and after neoadjuvant therapy.

Methods and Materials: We retrospectively reviewed the records of patients affected by inflammatory breast carcinoma at Verona University Hospital. Pathological response according to different international systems and the morpho-phenotypic profiles pre and post-neoadjuvant therapy were compared.

Results

26 cases of inflammatory breast carcinomas were collected. The rate of pathological complete response was different among the methods used in the evaluation (range 3.8-11.5%). Lymphovascular invasion persisted in most cases post-neoadjuvant therapy (80%), and was still extensive in 30% of cases with less than 50% of residual tumor. Immunohistochemical profile was mostly conserved post-neoadjuvant therapy.

Conclusion

We concluded that i) inflammatory breast cancer has a variable spectrum of histological response to the neoadjuvant therapy depending on the classification, ii) the immunohistochemical profile resulted maintained and iii) there is a subset of IBC (30%), with a significant reduction of the neoplastic cellularity (more than 50%), that still demonstrates an extensive lymphovascular invasion. This latter category could benefit from emerging therapies against tumor emboli.

Gender related differences in polymorphism and miRNA targeting CLOCK genes in metastatic colorectal cancer patients

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Background

Experimental and clinical evidences support a role for the circadian timing system in metabolism and cancer. In patients treated with chronomodulated or conventional schedule, overall survival (OS) was influenced by both gender and delivery schedule (Levi 2006, Giacchetti 2014). Circadian rhythms are generated by a transcription-translation-based oscillatory loop and involves a set of clock genes including PERIOD (1 and 2), CLOCK, BMAL1, CRY (1 and 2). In this study we evaluated: the immunohistochemical (IHC) expression of PER-2 and proteins involved in proliferative activity (EGFR, ERβ1, ERβ2, Cyclin D1, β-catenin) the expression levels of miRNAs targeting CLOCK genes as well as polymorphism (SNPs) CLOCK genes related in a series of metastatic colorectal cancer (mCRC) patients, aimed to correlate phenotypic and molecular findings to clinico-pathological parameters and survival.

Methods

We retrospectively studied 83 mCRC patients treated as first line with chemotherapy (chronomodulated triplet combination of Irinotecan + Oxaliplatin + Folinic Acid + 5-Fluorouracil) plus cetuximab after RAS characterization. IHC phenotypic analysis, polymorphism (SNPs) (rs11133373C/G, rs11133391T/C, rs1801260T/C) and miRNAs expression level (miR-192-206-132-194-219) were performed on formalin fixed paraffin embedded tissues in 77 patients. In addition, using a custom panel, we studied the mutational status of 17 selected genes involved in CRC development and progression by next generation sequencing. OS was the primary study endpoint.

Our series of patients had a median follow-up of 25 months (range 1-132), a median progression-free survival (PFS) of 14 months (CI 95% 10-19) and a median OS of 35 months (CI 95% 22-47). Fifty patients were male and 33 female with a median age of 59 years (30-85). PFS and OS were significantly better in females vs males (PFS M/F=12/19 months (CI 95% 8-16/11-27) p=0.03; OS M/F=31/50 months (CI 95% 22-39/35-64) p=0.03).

Results

Higher percentages of EGFR (73% p>0.0001), ERβ1 (77%, p=0.07), ERβ2 (88.5%, p>0.0001), Cyclin D1 (69.2%, p=0.06), β-catenin (84.6%, p=0.02) IHC expression have been observed in the subset of mCRC presenting PER-2 downregulation. Multiple Correspondence Analysis (MCA) analysis evidenced that better response to therapy is associated to PER-2 IHC positivity and low expression of EGFR, ERβ1, ERβ2, β-catenin.

Increased OS in females is related to high expression level (H) of miR206 (p=0.003), miR219 (p=0.003) and miR194 (p=0.02), and low expression (L) of miR132 (p=0.06) considered as single factors. OS was also better in females vs males in the presence of genotype RS11133373C/C (p=0.01), RS1801260T/T (p=0.07) and RS11133391T/T (p=0.06). Subgroup analysis in females showed that a miRNA profile (≥2, 58 months vs 15 months, p=0.0002) together with the presence of RS11133391T/T polymorphism (median OS 87 months) additionally increased the reported beneficial effects. Of interest, OS significantly decreased in females when miR206 low expression level and miR132 high expression level were simultaneously present (11 months vs 56 months, p=0.02).

Conclusions

For the first time a set of SNPs and miRNAs related to CLOCK genes, principal regulator of the molecular clock, identify a subgroup of mCRC patients who benefits more for a gender related difference in survival. Furthermore the biological profile made up by high PER-2, low EGFR, ERβ1, ERβ2, β-catenin, miR-206 expression may identify a biological profile predictive of chemotherapy response. Correlation between these results and NGS mutational analysis will provide further information of clinical value.

Aula Blu 2 – 8.30-9.30

La patologia testa-collo nella diagnostica quotidiana

MODERATORI: M. P. Foschini (Bologna), G. Fraternali Orcioni (Genova)

Tipizzazione di HPV nel carcinoma testa-collo in Italia: mappa dei centri e delle tecnologie applicate

P. Morbini

Paper not received

Importanza prognostica di HPV nel carcinoma testa-collo

S. Staibano

Paper not received

Diagnostica istologica della necrosi da bifosfonati: cosa il patologo può e deve dire

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Jawbones alterations that are electively related to biphosphonates (BPs) therapy have been repeatedly reported over the last few years, due to increased administration, both parenterally and intra-orally, of different biphosphonate molecules in oncologic and osteoporotic patients. Though many of such lesions could be prevented by adequate intra-oral treatments before the start of therapies, prompt recognition of early lesions is mandatory to avoid massive bone destruction and to possibly adopt minimally invasive surgical treatments.

Recent studies have highlighted that biphosphonates reduce osteoclastic activity and bone resorption, and favour woven bone deposition, thus recovering bone losses due to osteoporosis and limiting bone invasion due to metastatic deposits. Nevertheless, such mechanisms of action also lead to the expansion of previously existing lamellar bone and accumulation of newly formed woven bone, thus inducing relative ischaemic conditions due to inadequate blood supply in involved areas. Therefore, bone lamellae progressively develop necrotic foci that are more prone to subsequent infection and inflammation. The latter are favoured by concomitant masticatory trauma, endodontic treatments and dental implant procedures which should be avoided in patients after the start of biphosphonates therapies.

Morphologically, the salient features of BP-related osteonecrosis of the jaws include enlargement of pre-existing lamellar bone, progressive reduction of Haversian canals, irregular deposition of woven (newly-formed) bone, extensive polymorphic (neutrophils, lymphocytes and plasma cells) acute and chronic inflammatory changes and accumulation of bacterial colonies. Opportunistic superinfection is rather frequent and is mainly sustained by *Actinomyces* species which deserve proper identification by PAS stains in view of specific antibiotic treatment, to be started as soon as possible. Also, patients harbouring long-standing and extensive necrotic foci may undergo additional superinfection by uncommon pathogens, such as *Mucormycetes* and *Aspergilli* that may be sparse and quite hard to identify in H&E stained slides, thus prompting for additional special stains (Giemsa, Grocott Silver Methenamine) to be performed. Proper identification of such pathogens is mandatory to promptly start specific treatments and avoid possible lethal complications, frequently due to massive extension of the infection to the paranasal sinuses and brain.

In conclusion, pathologists must be aware of the many subtle alterations that may be found in the jawbones of patients undergoing BP therapy to support clinicians in the choice of adequate treatments, which may include selective and high-dose antibiotic therapy in case resistant pathogens would be identified.

Anomalie vascolari nel distretto testa-collo: criteri diagnostici

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Vascular anomalies form a heterogeneous group of pathologies of the circulatory system that can affect every type of hematic and/or lymphatic vessel of any diameter or anatomic area.

The global incidence of vascular anomalies in the population is not known, but the incidence of vascular tumors is estimated between 6-8% and the one of vascular malformations about 1.5%.

The extreme variability of the type of tissue or organ involved may determine a wide heterogeneity of clinical conditions resulting in the need of different professionals and the need to undertake different diagnostic and therapeutic approaches. In addition, vascular abnormalities can be an expression of complex syndromes or malformations rare events; in these cases the clinical approach, diagnosis and treatment, which is already by definition multidisciplinary, will require more specific evaluations.

Precisely for these features vascular anomalies are lesions that require

- uniform classification criteria
- identification of specific diagnostic protocols in order to optimize the use of instrumental tests
- evaluation of different treatment methodologies in order to use the best therapeutic strategy in the various cases.

Specifically, head and neck is a district where the vascular anomalies are the most common benign lesion in infancy and childhood and are reported in 14-65% of cases of all vascular anomalies. The correct diagnosis is critical to provide the appropriate treatment because it is an area of particular sensitivity for the organs involved, both from the functional point of view as well as from that of social relations.

The authors reviewed the current literature on vascular anomalies looking for more innovative and credited diagnostic criteria and treatment protocols.

Referring to what is currently achieved by SISAV (Società Italiana per lo Studio delle Anomalie Vascolari) the adopted classification to diagnose vascular abnormalities was the ISSVA Classification (2014) that has its roots in the previous classification of Mulliken and Glowacky (1982)¹. This classification is very simple and schematic. It distinguishes the vascular anomalies in two main groups: vascular tumors and vascular malformations. Furthermore, vascular malformations are distinct in relation to the hemodynamic characteristics in two main subtypes (fast flow and slow flow).

Treatment protocols suggested after a full review of the literature and according to the experience of the authors were applied.

These protocols have been translated into national guidelines².

Even for the head and neck there is a multidisciplinary approach involving radiologists, maxillo-facial surgeons, plastic surgeons and pathologists as well as professionals such as speech therapists and unusual make-up artists. The review is divided in 2 principal sections: tumor and malformation and the last one in additional 4 sections (capillary, venous, arteriovenous, and lymphatic malformations). In each section, the clinical presentation, radiologic features, histological aspect and treatment options for each kind of vascular anomalies are described.

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Aula Gialla 1 – 9.30-12.30

Sicurezza e qualità in anatomia patologica: Una opportunità od uno svantaggio?

MODERATORI: P. Dalla Palma (Trento), D. Ientile (Palermo), F. M. Vecchio (Roma)

Eventi indesiderati in anatomia patologica

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The discussion on adverse events in anatomic pathology generally focuses on diagnostic error which involves problems related to diagnostic criteria, diagnostic threshold, competency, inadequate clinical correlation, and experience. Unfortunately, there is a small number of adverse events that occur at a very low rate and their impact on a patient's care may be dramatic, such as "misidentification of a patient or a specimen", "floaters" (or extraneous tissues, or contaminants), and the "loss of surgical specimens".

Misidentification of a patient or a specimen.

One of the most serious issues faced in Anatomic Pathology services is misidentification of a patient or a specimen, which includes mislabeled specimens, block identification problems, and tissue contaminants with the potential to cause patients harm¹.

The terms "patient identification error", "specimen identification error", "laboratory identification error", and simply "identification error" are used interchangeably. Any result that is reported for the wrong patient or specimen is considered an identification error. A mix-up of two specimens from the same patient collected from different sites is still considered an identification error, because the site can be important in interpreting results and planning subsequent care. Pathological examination of a surgical specimen is the mid-point of a complex, multi-step process, that begins with receipt of a labeled specimen and requisition, followed by accessioning of that specimen into the laboratory with assignment of an alphanumeric accession designation unique to that patient and case, and examination of the specimen with submission of tissue samples into labeled tissue cassettes, which are processed into paraffin blocks and subsequently cut to produce tissue sections adhered to labeled glass slides which are microscopically examined by the pathologist. The system in surgical pathology has little or no automation and is heavily dependent on numerous people with varying levels of training. In fact, the handling, processing and reporting of pathology specimens involves many members of the health-care team including physicians, nurses, pathologist, laboratory technologists and administrative personnel. Therefore, the system has a high potential for error. Identification errors can occur at any point in the process and involve a patient or a specimen. In a patient identification error (where the cause of the identification error was misidentification of the patient, i.e. a defective or missing patient identification number on cassette or paraffin block), a specimen is labeled with the incorrect patient name or identification number. In a specimen identification error (where the patient was correctly identified, but the specimen was not), a specimen is misidentified as to organ or site of origin, but the

specimen is correctly associated with patient name and identification number. Either error has the potential to cause patients harm such as inappropriate therapy or follow-up, wrong side treatment, withholding of therapy in patients with unrecognized malignancies, repeat diagnostic procedure, including tissue biopsy and delay in a necessary surgical procedure. In an anatomic pathology laboratory labeling errors for patient or sample can occur at several points in specimen processing involving case/patient (wrong accession number was applied to the entire case), specimen (wrong specimen labeling was due to mix-up of specimens within a case, e.g. right versus left specimens in a bilateral biopsy from the same patient), paraffin block (histologic block was labeled with the wrong patient/case identification or wrong sequence number or letter), and slide (histologic slide was labeled with the wrong specimen / patient identification, sequence number, or letter)²⁻⁵. Patient identification errors in surgical pathology are the most rapidly growing category of malpractice claims involving pathologists. In a study of medical malpractice cases against pathologists, Troxel⁶ describes four claims that resulted from misidentified pathology reports, blocks, or specimens leading to patients being told they had malignancies when they actually did not attributable to patient misidentifications at some point in the surgical pathology process, and in a follow-up study,⁷ he noted a sharp increase in histology errors, with 13 of 272 claims attributable to specimen mix-ups and 2 of 272 to mislabeled slides. In a study of 227 root cause analyses Dunn and Moga⁸ identified 8 cases in which mislabeling of anatomic pathology specimens, slides, or tissue cassettes led to significant patient harm, including unnecessary surgery (lung lobectomy, prostatectomy, hysterectomy), delays in diagnosis, and necessity for repeat procedures.

Few studies have addressed the frequency of labeling errors for anatomic pathology specimens. In a College of American Pathologists Q-Probes program, specimen identification and accessioning deficiencies were found in 60,042 of 1,004,115 cases accessioned (6.0%)³. Errors associated with specimen misidentification accounted for 9.6% of the total errors³. Labeling errors have been categorized as class 1 (typographical errors with no clinical consequences); class 2 (minor errors unlikely to have clinical consequences); and class 3 (errors that are significant and have the potential to detrimentally impact patient care).

In another study, Francis et al.⁹ documented the frequency of preanalytic errors in a high-volume endoscopy center. In a study of 8,231 specimen containers, they documented a 0.09% rate for class 3 errors in which specimen bottles were mislabeled for patient name or unique identification number. Makary et al.⁴ reviewed a total of 21,351 surgical specimens and detected 91 errors in specimen labeling. Of these errors, 11 (12%) involved specimens associated with an incorrect patient assignment, and specimens most commonly mislabeled were breast, skin, and colon.

Specimen labeling errors within the laboratory can occur at several points of specimen processing. Within the gross room, specimen containers can be paired with cassettes labeled

with an incorrect case number (wrong patient) or an incorrect part number (wrong site). In the histology laboratory, cassettes at the cutting station can be paired with incorrectly pencil-labeled slides (wrong patient or site), or a correctly pencil-labeled slide can have the wrong paper label applied (wrong patient or site). A surgical pathologist may pick up an incorrect slide and dictate a report with an incorrect diagnosis for the patient's laboratory data being reviewed. Errors may also occur during transcription when dictations are transcribed to the wrong report number and patient. Evaluations of labeling errors was performed through a Q-Probes study of the College of American Pathologists published in 2011². This study involving 136 institutions, reported a labeling error rate of 1.1 per 1000 cases, with mislabeling rates of 1.0, 1.7, and 1.1 per 1000 for specimens, blocks, and slides, respectively. Similar studies within a single institution have reported labeling error rates ranging from 0.03% to 0.21% of slides and 0.057% to 0.068% of blocks to 1.7 labeling errors per 1000 cases (5,10-14). The importance of avoiding labeling errors in the laboratory has received increased attention by regulatory and accrediting agencies. The Patient Safety Goals of the Joint Commission, the primary accrediting agency for hospitals in the United States, call for the use of at least 2 patient identifiers on every specimen when providing laboratory services¹⁵. "Floaters" (or extraneous tissues, or contaminants)

Pathologists and histotechnologists have long known that traditional methods of processing tissue for diagnosis have the potential to cross-contaminate human biopsy specimens. "Floaters", or extraneous tissues, or contaminants, are the part of the surgical pathology laboratory life and are undisputable processing errors. Surgical pathology floaters are different from specimen mix-up or misidentification. The term "floater" came in surgical pathology laboratory from the step in histology processing when tissue sections are floated on the surface of a water bath before being mounted on a slide. A small fragment of tissue can break free from the paraffin section and float in the water bath until it is picked up with a section from another case. If we exclude completely rare occasion of floating tissue debris in the tissue processors, there are only three areas of possible the floaters generation: grossing (sampling, cut-up), embedding, and microtomy. Grossing is the main area where diagnostically significant floaters are generated. Embedding is always blamed for a floater that is a many occasions true. Microtomy traditionally is considered as the first culprit of floaters although it is not true. The consequences of undetected "floater" can be serious. For example, a carryover from a lung tumor to a bronch biopsy which upon thoracotomy turned out to be histology only. No residual prostate cancer (or "vanishing cancer") in radical prostatectomy specimen, when prostate cancer cannot be found after histology examination of the entire removed gland, might be also a result of a contaminated prostate needle biopsy¹⁶. The College of American Pathologists (CAP) in 1994 conducted Q-Probes study in 275 laboratories on extraneous tissues in surgical pathology. The results were published in Archives Pathology and Laboratory Medicine in 1996¹⁷. The CAP Q-Probes showed that there was an overall extraneous tissue rate of 0.6% of slides in the prospective study and 2.9% of slides in the retrospective study; in 92, 3% the origin of extraneous tissue was in the surgical pathology laboratory¹⁷. Although in the retrospective study 15.9 % of extraneous tissue were on block only, there is not clear where the extraneous tissue was generated. Real floaters which stem from the waterbath are rare. Most extraneous tissues are contaminants. The Cap Q-probes article¹⁷ included a table with a list of 15 possible sources of extraneous tissue in following sequence (gloves, dis-

secting surface, instruments, strainer, saws, scales, container, microtome, tissue cassettes, ink, fixative solution, specimen container, staining solutions, water bath, and towels). In their study titled "Measurement of stainer bath contamination and evaluation of common mitigation strategies," Cahill and Pearson¹⁸ gathered data from 69 pathology laboratories. They found that each staining system used by the labs participating in the study produced extraneous tissue cross-contamination on the blank patient evaluation slides that were used in the study¹⁸. Most contaminants, often called "floaters" by laboratory staff, are easily recognized as such. However, depending on the tissue being evaluated and the clinical circumstances, contamination can be problematic for the pathologist. While misinterpretation due to slide contamination is rare, a contaminated slide usually requires additional time to fully evaluate. Preparation and examination of deeper sections of the tissue is often necessary, and more time is usually needed for careful consideration of the diagnostic possibilities suggested by the extraneous tissue. Recently molecular techniques have been detailed that can reliably differentiate tissues from different patients based upon the DNA fingerprinting¹⁹⁻²³. If the tissue is all suspected as being a mixed-up sample, less exact microdissection is needed. DNA is extracted from the tissue fragments and PCR is performed for a set of polymorphic markers.

Loss of surgical specimens

Another potentially tragic event in anatomic pathology service is loss of surgical specimens. The concept of "specimen loss" should apply to misplacement or willful destruction of a specimen. Surgical pathology specimens may be lost somewhere between specimen retrieval from the patient and processing in the laboratory. Some specimens can be repeated, such as a bowel biopsy to rule out inflammation or celiac disease. However, doing so places the patient at risk from the additional procedure and imposes a greater burden on the healthcare system through additional costs, time, and labor. Of greater concern are specimens that cannot be replaced, such as fully excised tumors, skin lesions, or organs. The loss of such specimens may result in inappropriate or unnecessary treatment²⁴. Furthermore, lost specimens may delay diagnosis, can preclude necessary studies, current and/or future, increase patient anxiety, or be a source of potential litigation²⁵.

In a study conducted by Sandbank et al. the incidence of specimen loss was 1 in 1,466 (0.068%) in biopsy specimens. Five specimens were reported as lost during the period. Two were retrieved, two were lost probably because of failure to insert the pathology specimen into the container, and one was lost in the pathology laboratory during processing²⁶.

Robert Jackson²⁷, in a review of 75 malignant melanoma malpractice cases, identifies two cases in which there was submission of multiple lesions in the same container, one of which was melanoma, resulting in wide resection of the wrong lesion. He also identifies two cases in which the specimen was lost. The true outcome of these types of errors is rarely described in the literature, but the potential for economic loss is easily conveyed in malpractice claims.

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Eventi indesiderati in anatomia patologica: aspetti medico-legali

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The medicolegal approach to such a complex issue as the one concerning the professional responsibility of the pathologist are substantially two: the normative one and the organizing one. The normative aspect of the problem fits in the present structure of the law 189/2012 that though has aroused interpretation problems and needs juridical interpretations of legitimacy, according to the writer, offers the jurists some useful instruments. If the Balduzzi Decree is the normative background of a greater picture where adverse events occur in Surgical Pathology, it is necessary to analyze one by one at least the most important aspects of such issues and the cases in point that may occur.

Diagnostic errors are always more frequently attributed to the histopathological exams and the incidence of lawsuits against pathologists continues to increase. This situation is understandable given that the histological diagnosis has a crucial role of in directing therapeutic choices with consequent repercussion in the prognostic perspective radically different if the diagnosis judgment regards a malign case or a benign case. Histopathology often presents difficult techniques of interpretation about where the sample is taken from, the scarcity of the sample or the ambiguity of the picture that the scientific literature lines and therefore should be considered in the legal medicine evaluation as justifications advanced in case of insufficient or mistaken diagnosis¹.

The nature of the damage that is charged to pathologists is obviously similar to the one noticed in various cases of professional guilt. But also from this point of view, the oncological pathology which mainly interest the histopathologist, shows more evident characteristics and sometimes even specific ones. In particular what is charged is the avoidable death respect to one or more possibilities of recovery or an earlier death respect to the foreseen length of life, technically possible in case of prompt and adequate cure of the tumor². The juridical consequences, and therefore medicolegal, of the diagnostic error are different depending on their criminal or civil relevance. In criminal ambit both hypothesis are considered as negligent homicide, but also cases of personal culpable lesions, that can be verified and considered "negligent" due to the worsening of the pathology or to late and inconsistent diagnosis or therapies. According to civil law the problems are far more complex. Actually, if one can demonstrate the negligent behavior of the pathologist, and the relation of fortuity that connects such behavior to the therapeutic delay and consequent damage of the patient, the need to discriminate between the consequences that, in any case, the pathology would have caused even after an adequate therapy and the consequences due to a professional guilt. But if, due to its same nature, it would reduce in any case the length of a life, the chronological anticipation of death implies probabilistic evaluations of the shortening of life and of the biological and property damage that eventually follows.

Consolidated jurisprudence both of merit and legitimacy, shared and unaltered in the run of time, has defined an item of damage autonomously refundable called loss of chances, singled out exactly to guarantee the refund of this type of

damage. It constitutes a patrimony entity on its own, juridically and economically susceptible of autonomous evaluation where its loss would constitute a diminished possibility to attain a useful result, in our case a total recovery or more survival³. In other terms, in a situation where it is certain and demonstrated that a diagnostic error has led to a wrong and inadequate therapy such deficiency aggravates the possibility that a negative result has been made. Therefore it is not possible to state whether the event would have happened but it is possible to state that the patient due to the dereliction of duty, has lost some chances that he statistically had, also bearing in mind the particular concrete situation⁴.

A further very important legal-medical issue which today is growing fast, is the omission of the communication of a medical report. As a matter of fact one of the duties of the referring physician in general and in particular of the pathologist, is to communicate the result of the histological exam. The formulation and communication of the diagnosis, within a process of cure, represents the basis of a physician-patient therapeutical alliance and they are specifically part of the duties of guarantee of the medical-health performance to protect public welfare. Until today, though, as far as communication of reports relative to diagnostic-instrumental tests a positive rule that regulates the duties of a physician or of the patient does not exist. However, just for the guarantor role of the physician, mainly for serious pathologies and severe prognosis moral and deontological duties are understood to exist in order to inform the patient urgently, should he delay the withdrawal of the report. Some authors, on the grounds of some judgment of Court of Criminal Cassation, have pointed out that the referring physician must be considered the most responsible person in the failed communication of a diagnostic result, being the only one to know the contents of the medical report.

From an organizing point of view the responsibility of the pathologist becomes part in the greater and complex system of the management of the health risk. Bearing firmly in mind that an error is an integral part of the human activity and that the more complicated the system, the higher is the level of risk⁵. According to the widest possible definition, the error can be defined as a failure of a planned action. However in medical literature the term "error" indicates a deviation of a process of cure that may or may not imply a damage to the patient. Instead, the adverse event represents the undesirable result of the process of cure that may or may not be the result of an error. The adverse event is considered as a damage ascribable to the health treatments that causes the protraction of the period in hospital, worsening of health conditions or death⁹.

Some psychologists, as Rasmussen and Reason, starting from the nineties have tried to classify and understand the behaviors that are the basis of human error. In particular Reason has elaborated a generic type of error, classifying it on the basis of cognitive process involved at the moment where it is made^{8,10,11,12}. According to this model the condition necessary for an error to occur lies in the presence of leaks and contributing factors along the clinical assistance route. The predisposing conditions constitute the latent errors that contribute to generate the adverse event that reveals itself only after an active error, located in a crucial point of the clinical assistance route. This concept is clearly illustrated by Reason in the "Swiss-Cheese model": the slices of cheese represent the defensive barriers of the organization, the holes in the slices, instead, represent the individual weaknesses in single parts of the system. The place and dimension in each slice vary continuously. The whole system fails when all the holes of all slices temporally line up, allowing the occurrence of an adverse event.

As it has already been mentioned, with the increasing of the complexity of the system, also the possibility of an error increases and consequently the occurrence of an adverse event. The surgical pathology can be taken as an example of complex system where errors may happen during each phase of a diagnostic process¹³. Luckily not all the errors made in a histopathology laboratory turn into adverse effects, i.e. undesirable.

It is possible to determine a classification of the seriousness of the error according to the effective clinical consequences. Some authors have proposed three categories of damages: minimum (degree 1), moderate (degree 2) and severe (degree 3)¹⁴. The adverse event determines a minimum damage (degree 1) when it is necessary to repeat further not necessary diagnostic procedures, when there is a diagnostic or therapeutical delay equal or less than six months, or when from a not necessary repetition of the diagnostic procedures springs a partial temporary incapacity. The moderate damage (degree 2) occurs when due to a diagnostic error a further invasive diagnostic procedure is necessary, when there is a diagnostic delay over six months or when from not necessary repetition of the diagnostic procedure has sprung a temporary absolute inability lasting six months or less. Lastly, the damage may be defined as severe (degree 3) when from a diagnostic error derives a temporary absolute inability lasting over six month, the loss of a limb or death.

The task of the pathologist in the complicated panorama constituted by the risks and the adverse events in the histopathological diagnostic process, is to ascertain whether there are profiles of guilt, understood as negligence, carelessness and unskillfulness due to the medical staff or the welfare structure intervened in the specific object of the medicolegal debate. The error of the medical staff according to the pathologist therefore represents the most important aspect of the behavior necessary to analyze in terms of predictability and forestalling of the error, after having demonstrated the connection of material causality between the culpable behavior and adverse event object of this research.

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Controversie diagnostiche in citostopatologica: casistica giudiziaria

O. Nappi

Paper not received

Il caso e la necessità (problematiche di errore casuale con effetti negativi)

F. Crivelli

Paper not received

Diagnosi errate indotte dall'immunoistochimica

M. Chilosi

Paper not received

La seconda opinione in anatomia patologica: chi, come e quando

G.L. Taddei

Paper not received

L'archivio e la richiesta dei preparati citostologici

A. Fabiano

Paper not received

La gestione del flusso di lavoro in anatomia patologica: opportunità e limiti dell'informatica e dell'automazione

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It is well known that 70% of clinical decisions require laboratory tests, compared with an expense that does not exceed 2% of the budget of any health system. In fact, this figure is difficult to prove in the case of the investigation of clinical biochemistry, given the heterogeneous nature of the disease and thus the different contribution of laboratory diagnostics against history, physical examination and other investigations, including imaging.

In the case of pathologic diagnosis, on the contrary, the role of the report of the pathologist is well measurable as, especially in recent years, it is more and more central in the diagnostic-therapeutic pathway. As defined in the recent guidelines issued in May 2015 by the Board of Health of the Ministry of Health, it “finalizes with a medical procedure, namely the diagnosis, a sequence of procedures of technical/cognitive type aimed to examination of organs or organ samples... which are the result of interpretation by the pathologist of morphological and...molecular features of the biological sample under examination”.

Accuracy, completeness and timeliness are the essential requirements of the quality of pathologic diagnosis. Although the cognitive process, which is the basis of cytological or histological diagnosis, is mainly based on the experiential knowledge of the physician, it is, however, of vital significance the

control of quantitative and qualitative data generated from analytical instruments used in a pathology laboratory.

In recent years has been added the belief that the correctness of the pathological report comes from the appropriateness of the request and has value if it improves clinical decision-making and health outcomes. The current state of the art shows that, compared to a significant reduction of the analytical error, the vulnerability and the risk of errors in the initial (pre-pre-analytical) and final (post-post-analytical) stages are increased. In fact, from the time the tissue sample is taken from the patient until the time in which it is treated with the fixation (or freezing), both the architectural characteristics and morphological and biological constituents can undergo degradation processes and alteration, which may limit or prevent the diagnosis. Similarly, to avoid identification errors or loss of samples, is essential to take advantage of computational systems that ensure traceability from the time of collection and throughout the working cycle. The automation of procedures must also provide a consistent and systematic collection of data deriving from the analysis of the entire working process, in order to measure the quality of procedures and making the necessary improvements.

The collection of data presupposes to know the types of the most common error: for example, mislabeling in the acceptance phase, exchange between the biological sample and cassette in sampling phase, exchange between inclusion and histological preparation during the cutting phase.

Data collection and statistical analysis will lead to appropriate improvements, which may include the introduction of new procedures, new equipment, or revision and the relocation of existing resources. Not to mention that the increasing numbers of requests of archival material for the determination of parametric biological features of prognostic and predictive value require to have an archive, whose management is a guarantee of proper storage, custody and handling of preparations and paraffin inclusions.

There are numerous products on the market that can provide automated and computerized “product quality”, with a number of opportunities of interest. In the selection of various operational solutions must still bear in mind the recommendations covered by existing guidelines, and in particular, the “Safety Manual in the operating room: Recommendations and Checklist” produced in 2009 by the Ministry of Labour, Health and Social Policy, and the “Guidelines Traceability, Collection, Transport, Storage and Archiving of cells and tissues for diagnostic tests” published by the Superior Council of Health in May 2015.

A second aspect to consider is that there may be, beyond the technology and the criteria used to implement it, some factors that may restrict, to varying degrees, the impact of these management systems on the quality of work, including: the welcome and acceptance by the staff involved (the worry: “it takes control”), the bulk of the components needed on available laboratory spaces, the impact on the dynamics of existing work, which must be rethought in a vision of laboratory efficiency and not uncritically imposed by the technology. The interaction with the organization of the hospital in which you operate is mandatory. The automation and the computerized management of a laboratory can be successful and then lead to an improved quality of work only if they are also built with respect to the working reality in which they should operate. Their implementation must be an opportunity to rethink the organization of our work and adapt it to the standard we want to achieve.

Aula Gialla 2 – 8.30-11.30

Patologia Molecolare

MODERATORI: A. Marchetti (Chieti), S. Bosari (Milano)

Update nella diagnostica molecolare del carcinoma polmonare

A. Marchetti

Paper not received

Ruolo di Alk nel carcinoma polmonare

G. Fontanini

Paper not received

Ruolo dei biomarkers in citologia

G. Troncone

Paper not received

Ruolo dei geni Hox in patologia

F. Castiglione

Paper not received

La biopsia liquida

F. Buttitta

Paper not received

Ruolo dei biomarcatori nell'epatocarcinoma

M. Roncalli

Anatomia Patologica, Humanitas Research Hospital, Milano

Hepatocellular carcinoma (HCC) is one of the commonest malignancies worldwide, with long-term poor survival. The issue of molecular biomarkers with diagnostic, prognostic and predictive significance is a particularly hot topic for the current management of HCC. Several biomarkers have been proposed as useful to clarify the stepwise pathogenesis of HCC but only specific mutations of the promoter of the telomerase gene (TERT) have been consistently documented, occurring either in dysplastic nodules and early HCC. Other biomarkers selected from gene expression studies, are today used in an immunocytochemical assay to diagnose early HCC, such as HSP70, GPC3 and GS. Other biomarkers have been extensively studied with the aim to demonstrate an independent prognostic impact from tumor staging, grading and vascular invasion such as CK19, but none of them has been consistently validated and translated into the clinical practice. Great efforts are currently dedicated to the selection and validation of druggable molecules useful to predict the individual sensitivity to biological compounds. C-met overexpression appears very promising to candidate patients to second line therapy in advanced HCC.

Ruolo futuro dei MI RNA

S. Bosari

Paper not received

Perché parlare ancora di RAS nel colon retto

G. Giuffrè

Department of Human Pathology "Gaetano Barresi", University of Messina

RAS family proteins, including KRAS and NRAS, are important downstream effectors within the mitogen-activated protein kinase (MAPK) pathway that couples the epidermal growth factor receptor (EGFR) with intracellular signalling cascades. In colorectal cancer (CRC) somatic mutations in RAS genes lead to constitutive activation of the MAPK pathway and consequently the dysregulation of cell proliferation, migration, differentiation and survival. The introduction of monoclonal antibody drugs targeting EGFR, such as cetuximab and panitumumab, in combination to chemotherapy regimens has widened treatment options in metastatic CRC (mCRC). However, not all patients can benefit from these agents; in fact, patients whose tumours carry an activating mutations in exon 2 of the gene KRAS are much less likely to respond to antibodies that block EGFR signalling. Moreover, prospective-retrospective studies underlined that additional mutations in RAS family genes, namely in exons 3 and 4 of KRAS and in exons 2, 3 and 4 of NRAS, predicted a lack of response to anti-EGFR therapy. Based on these data, regulatory authorities in North America and Europe updated their guidelines and in particular the European Medicines Agency currently requires extended RAS analysis for prescription of cetuximab and panitumumab, restricting their use to patients with RAS wild-type mCRC.

However this negative predictor role of RAS genes still leaves some questions open, making a current topic the discussion on EGFR signalling. In fact not all patients selected by RAS testing benefit from anti-EGFR therapies, suggesting a role for other genetic determinants of primary resistance. Among these latter the main candidate is the BRAF gene that plays a key regulatory role in the MAPK cascade, while other potential genes are those that act in the PTEN/PI3K/AKT pathway as well as in STAT pathway. Although there has been evaluation of these potential predictive biomarkers in patients with mCRC, RAS mutations represent the only clinically validated biomarkers. Moreover it is clear that a BRAF mutation is prognostic of a poor outcome irrespective of treatment. The American Society for Clinical Pathology, College of American Pathologists and Association for Molecular Pathology are currently developing revised guidelines regarding molecular markers for CRC. In particular, for patients who are being considered for targeted anti-EGFR therapy, the guideline still recommends mutational analysis of exons 2, 3, and 4 of both KRAS and NRAS. In addition, BRAF mutation status can be helpful to guide patient management, but information about tumour's mismatch repair and microsatellite instability status are also necessary in order to interpret the potential clinical implications of a BRAF mutation.

In the future, the comprehensive integrated analysis of whole EGFR signalling pathways will enable us to identify most of the mCRC patients who are unlikely to respond to anti-EGFR therapies. Prospectively the use of emerging technologies, such as next-generation sequencing allowing to screen simultaneous-

ly multiple somatic mutations in multiple genes in a single test run, might represent an optimal solution for rapid selection of mCRC patient to address to current and future target therapies.

Patologia molecolare target therapy nel melanoma

M. Barberis

Paper not received

Dieci motivi per un patologo in molecolare

A. Cavazzana

Paper not received

Aula Gialla 3 – 9.30-12.30

Sessione Tecnici sulla celiachia (AITIC)

MODERATORE: T. Zanin (Genova)

L'aspetto clinico ed endoscopico

P. Romagnoli

Paper not received

Il processo istopatologico, allestimento dei campioni e criticità

T. Zanin

Paper not received

Ipersensibilità al glutine non celiaca

P. Minale

Paper not received

La diagnostica istopatologica

M. Rutigliani

Paper not received

Ruolo dei marcatori anticorpali nella diagnosi della malattia celiaca

E. Cavanna

Paper not received

Giovedì, 24 Settembre 2015

Aula Blu 1 ore 9.00-10.00

Ematopatologia e Patologia Apparato Digerente

Moderatori: S. Ascani (Terni), E. Sabattini (Bologna)

Are myelodysplastic changes and increased CD34+ cells distinctive of accelerated phase of polycythemia vera?

M. Nicola, M. Onorati, I. Ricotti, F. Di Nuovo

Anatomia Patologica, "G. Salvini", Garbagnate Milanese, Italia

Introduction. Polycythemia vera (PV) is a rare hematologic disorder belonging to the group of chronic myeloproliferative neoplasm (MPN) and characterized by somatic mutations of JAK2, MPL or CALR genes. The most frequent lethal events are related to thrombosis or hemorrhage but anemia, post-PV myelofibrosis, myelodysplasia (MDS) and acute myelogenous leukemia (AML) may also develop. Herein we describe a case of a longstanding PV acquiring myelodysplastic features and an increased CD34+ cell count in an adult man.

Case report. In 2007 a 51 ys-old man was admitted at our Hematology department with polyglobulia (Hb: 18 g/L; Ht 52%). Other hematological, serological and radiological tests were within the range. He underwent a bone marrow aspirate coherent with MPN. Molecular biology analysis detected JAK2-V617F mutation. Therefore a clinical diagnosis of PV was achieved. From 2007 to 2009 the patient was treated with a combination of hydroxyurea and phlebotomies, achieving partial remission. From 2010 to 2014 he developed progressive splenomegaly (20 cm) and leukocytosis (14x10⁹L) without clinical symptoms.

A bone marrow biopsy was performed showing hypercellularity with panmyelosis. An architectural disturbance of erythroid and granulocytic precursors with decreased myelo/erythroid ratio and with left shifting erythropoiesis and granulopoiesis was detected. The megakaryopoietic series was composed by loose clusters of atypical megakaryocytes with variable nuclear aspects, from deeply lobulated to hypolobated.

The immunohistochemical analysis highlighted the presence of numerous CD34-positive cells (10-12%). Therefore, the diagnosis of chronic MPN, PV, with myelodysplastic changes and increased CD34+ cells was performed.

Discussion. PV is a chronic JAK2-positive MPN characterized clinically by increased production of red blood cells and histologically by increased cellularity and by panmyelosis. In our case the bone marrow biopsy was hypercellular for the patient's age and, on morphology, consistent with the previous clinical diagnosis of PV. However some features pointed to the acquisition of myelodysplastic aspects and this hypothesis was supported by the numerous positive CD34+ blasts.

The progression to MDS/AML is a well-known complication of PV but still retains some not fully understood issues as its incidence and its relationship with the use of cytoreductive therapy. Further studies are needed to achieve a deeper knowledge of these issues.

The role of the microenvironment in Non Hodgkin B-cell lymphoma

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Introduction. The tumor microenvironment is a very dynamic and heterogeneous tissue where the cancer cells are in continuous interaction with the stroma and the cellular components of the inflammatory response.

The tumor microenvironment undergoes numerous changes during tumor growth and influences the development and progression of cancer. The hypoxic environment of the tumor stimulates angiogenesis which can directly promote the survival of cancer cells and their invasion as well as the inflammatory infiltrate and stromal myofibroblasts, can modulate the behavior of the neoplastic cells, with anti and pro-tumoral effects¹. Moreover, some components of the tumor microenvironment are able to provide important predictive and prognostic informations. From here lies the importance of our work focused on a more detailed study of the cells involved in the microenvironment of Non Hodgkin B-cell lymphoma (NHL), in particular, given their epidemiological importance, follicular lymphoma (FL) prototype of indolent lymphoma and diffuse large B-cell lymphoma (DLBCL), which is the most common aggressive lymphoma.

The objectives of our study were then: 1) To assess the distribution and amount of CAFs (fibroblasts associated with cancer that differ from normal fibroblasts to the expression of α -SMA), TAM (tumor-associated macrophages) and mast cells in the lymph nodes of patients with NHL; 2) Evaluate these cell populations in angiogenesis, seen as microvessel density (MVD); 3) Correlate the presence of CAFs, TAM and mast cells with the response to therapy and the histological features of the disease.

Materials and methods. Our retrospective study included 38 cases of Non-Hodgkin B-cell lymphoma, selected on lymph node biopsies, diagnosed over a nine-year period from 2005 to 2014, of patients treated with R-CHOP later, including 16 cases of FL, of which 19% was grade 1, 56% grade 2 and 25% grade 3; and 22 cases of DLBCL. Patients then were divided into two groups, based on the response to first-line therapy 1) responding to therapy (58%) (R) those following treatment had achieved complete remission, and 2) not responding to therapy (42%) (NR), those who had never achieved complete remission, or who, after therapy, had relapsed within 6 months.

From tissue samples embedded in paraffin were cut, sections with a thickness of 3 μ m, transferred onto glass slides and subjected to immunohistochemical investigations. All immunohistochemical investigations were performed with

automated immunostainer DAKO Omnis, which uses the dynamic gap staining to reduce artifacts.

For classification of DLBCL cases we used antibodies MUM1, (clone MUM1P, Ready-to-Use, Dako, Glostrup, Denmark) Bcl6 (clone PG-B6p, Ready-to-Use, Dako, Glostrup, Denmark) and CD10 (clone 56C6, diluted 1:20, DAKO, Glostrup, Denmark).

For the evaluation of the tumor microenvironment, we used the Tryptase (clone AA1, Ready-to-Use, Dako, Glostrup, Denmark) to identify the mast cells, the CD163 (clone 10d6, dil. 1:200 Novocastra, Leica Biosystems, Nussloch, Germany) to identify TAM, the α -SMA (clone A2547, dil. 1:14000, Sigma Aldrich) for CAFs and the CD34 (QBEnd 10, Ready-to-Use, Dako, Glostrup, Denmark) to mark vascular structures. For each case, the four slides stained with the relevant antibodies, were scanned and acquired a 4x microscope with digital D-Sight Fluo (A. Menarini Diagnostics) and stored as digital images on the workstation associated with the tool.

For image analysis, sections were examined at 10x to outline and select four hot spot, which are 4 areas of 1mm² with the highest density for microvascular counts of CD34 (Fig. 1) or cells for counting of mast cells and TAM. The count was carried out at 40x with the help of software D-sight.

The expression of α -SMA was measured, however, by assigning a score from 0 to 3 according to the density of positive myofibroblasts to α -SMA, its distribution and to staining intensity. They were then assigned as negative the scores 0 and 1 and as positive the score 2 and 3.

Statistical analyses were processed with the SPSS 20.0 software.

The statistical significance among Tryptase, CD163 and CD34 and the different subtypes of tumor and patient outcomes was performed with the non parametric Mann Whitney U test, while for the assessment of the α -SMA was used Fisher's exact test. The correlation of α -SMA, Tryptase and CD163 with the MVD was performed with the non parametric Spearman correlation test.

Results. For classification of DLBCL cases was applied the algorithm of Hans, a model based on different phenotypic expression of the three markers in order to identify the two subgroups GCB (Germinal center B-like) (41%) and not-GCB (59%) corresponding to ABC-like subgroup (Activated B cell-like) identified by gene expression profiling (GEP) studies².

We observed, about Tryptase, a statistically significant increase of mast cells expression in FL of about twice than that of DLBCL (FL 164 vs DLBCL 80), where, a few mast cells are distributed randomly in the tumor especially around the vessels, while in the FL are concentrated in the interfollicular spaces. It has instead found an increase of Tryptase in more

Fig. 1. A) Selection of the areas counts in FL, 4x; B) detail of CD34+ cell counts by D-Sight, 40x.

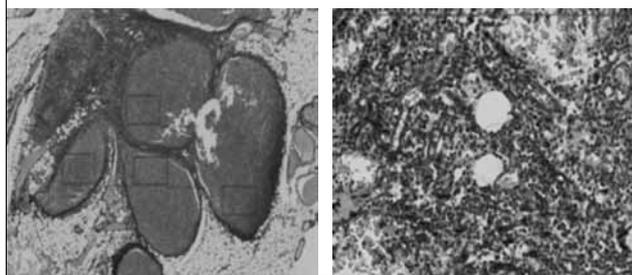
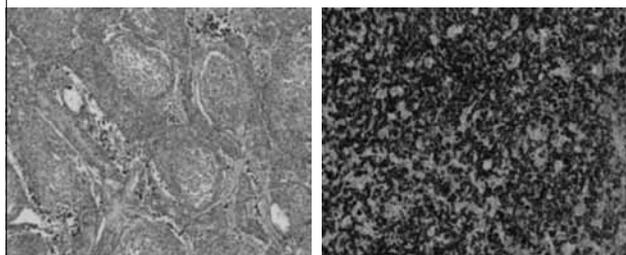


Fig. 2. CD163 expression A) FL 10x and B) DLBCL 40x.



aggressive subtypes, as follicular of grade 3 and in DLBCL of subtype ABC (FL G1/2 135 vs FL G3 249 and ABC 103 vs GCB 47).

Quantitative analysis of CD163 showed a marked increase of TAM in more aggressive DLBCL lymphomas, in which we observed an intense macrophage infiltration, compared to FL (DLBCL 1073 vs FL 360) (Fig. 2). Just as there has been a more remarkable increase in the FL grade 3 than those of low-grade (FL G3 663 vs FL G1/2 260) and a slight increase in ABC among DLBCL (ABC 1176 vs GCB 923).

As regards the expression of α -SMA, the stroma of DLBCL was largely cell-free α -SMA+, on the other hand, in indolent lymphomas, we noticed a progressive increase of the stromal cells α -SMA+ referable to myofibroblasts, to form, in low grade FL, an elaborate meshwork, encompassing vessels, spread in the lymph node parenchyma to define with an almost surgical precision neoplastic follicles (Fig. 3). The study of the vascular component recorded a slight increase of microvascular density in aggressive lymphomas, (FL 157 vs DLBCL 186) and also a growth in the ABC category compared to GCB (ABC 200 vs GCB165). Correlation studies, carried out on all cases of NHL belonging to our study, among the expression of CD163, the expression of Tryptase and α -SMA with the MVD, showed a positive correlation of MVD with CD163 ($\rho = 0.527$) and Tryptase ($\rho = 0.420$), which is generally maintained considering the different histological subtypes. Among the patient groups R and NR to first-line therapy R-CHOP, it was a significant difference in expression of all antibodies in the group of chemoresistant than R (Fig. 4).

From the results we can argue that the increase in the MVD, as a measure of tumor angiogenesis, in aggressive lymphomas than the indolent, which mainly MVD in the group of patients NR, in agreement with previous studies indicates that the angiogenesis plays a crucial role in tumor progression³.

Fig. 3. Myfibroblasts' meshwork in FL (grade 1), with digital double labeling of vessels

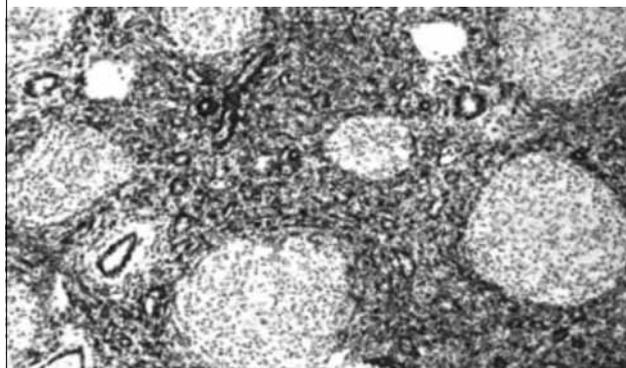
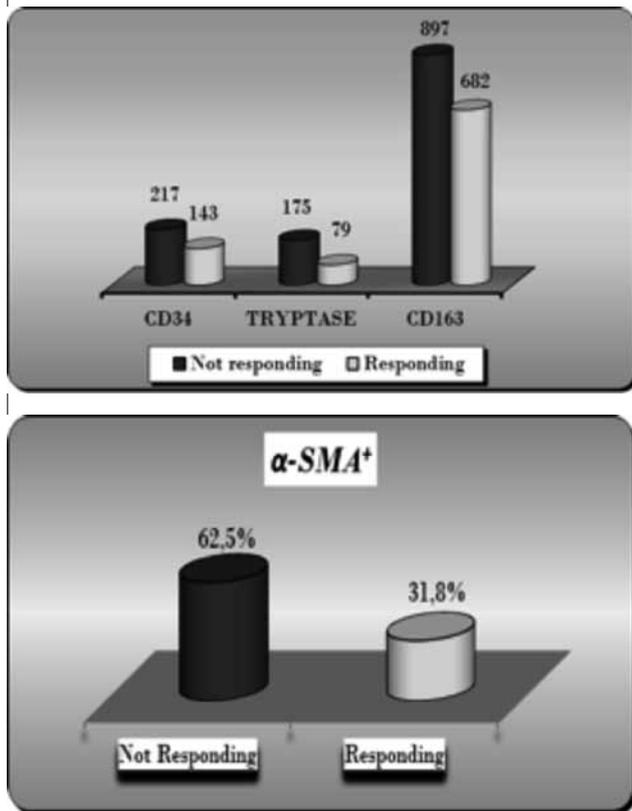


Fig. 4. Comparison of expression of A) CD34, Tryptase, CD163 and of B) α -SMA+ cells in the two groups R/NR to therapy



In addition, the statistically significant increase of mast cells in the group of patients chemoresistant indicates the angiogenic potential and the negative prognostic role of mast cells as also shown by previous studies⁴. However, the expression of Tryptase surprisingly lower in FL than DLBCL, although in agreement with other studies, requires further study⁵, but could suggest the intervention of inhibitors of degranulation mast cells.

These findings suggest the idea of the potential dual role of mast cells; on the one hand involved in the immune response to tumor accumulating in the tumor stroma; on the other, favour the spread of tumors by promoting the degradation of ECM (Extra-Cellular Matrix) and its vasculature, hence their name coined by Theoharides of "Jekyll and Hyde of tumor growth" (Theoharides TC, Trends Immunol. 2004).

The intense infiltration of CD163+ cells (subset M2 macrophage pro-tumor function) in aggressive lymphomas (DLBCL and grade 3 FL) obtained from us, demonstrates the critical role of TAM in the growth and progression of cancer. In agreement with the loss of the role of independent negative prognostic TAM in FL treated with immuno-chemotherapy containing rituximab⁶, we found no statistically significant differences in the number of macrophages in patients chemosensitive and chemoresistant. However, we have reported a sharp increase in population in the macrophage cell types ABC compared to GCB in the group of NR. Result supported by a recent study⁷. These data suggest that TAM are able, in aggressive lymphomas DLBCL, to modulate the effectiveness of anti-cancer therapies, facilitating the regrowth tumor revascularization and tumor spread after chemotherapy.

In addition, the positive correlation of Tryptase and CD163

with the MVD, realizes the cross-talk of the endothelial cells with the mast cells and TAM contributing to angiogenesis and tumor growth.

In this study we have shown that the stroma of aggressive lymphomas, in spite of an abundant macrophage infiltration tissue is largely devoid of α -SMA+ cells.

In contrast, the stroma of indolent lymphomas, mainly follicular of low grade, was found to be rich in myofibroblasts so as to form an elaborate and comprehensive meshwork along the peritumoral stroma (score 3), associated with a simultaneous absence of TAM, indicating that the population of stromal cells is dictated by a different cell recruitment CD163+, or of α -SMA+ cells, and that would be the basis of a resistance chemotherapy improving the stability vascular.

Conclusions. In this study, we demonstrated that tumor growth seems to be influenced in a determining way by the cells constituting the tumor microenvironment, which, co-evolves with the tumor and contributes to form a microenvironment unfavorable to therapies both from the structural point of view both by providing survival factors.

Although preliminary and perfectible, the observations made in the study of this amazing and dynamic microcosm of cellular actors existing within the tumor mass, could encourage the search for treatments in which the target is not the tumor cell, but the cells of the microenvironment with support functions. Moreover this study could represent a starting point for deepening the mechanisms by which the mast cells, despite the negative prognostic role that seem to assume, are reduced in number in aggressive lymphomas nodal and to understand the processes that are the basis of the switch of the population among TAM, CAFs and the various subtypes of NHL.

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Clonality analysis of immunoglobulin gene rearrangement by Next Generation Sequencing in endemic Burkitt lymphoma suggest antigen drive activation of BCR as opposed to sporadic Burkitt lymphoma

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Objectives. Recent studies using next generation sequencing (NGS) analysis disclosed the importance of the intrinsic activation of B-cell receptor (BCR) pathway in the pathogenesis of sporadic Burkitt lymphoma (sBL) due to mutations of TCF3/ID3 genes. Since no definitive data is available on the genetic landscape of endemic Burkitt (eBL), we first assessed the mutation frequency of TCF3/ID3 in eBL as compared to sBL, and subsequently the somatic hypermutation status of the BCR to answer the question whether an extrinsic activation of B-cell receptor signaling could also be demonstrated in BL.

Methods. We assessed the mutations of TCF3/ID3 by RNA-seq and the BCR status by NGS analysis of the immunoglobulin genes (IG).

Results. We detected mutations of TCF3/ID3 in about 30% of the eBL cases. This rate is significantly lower than that detected in sBL (64%). The NGS analysis of Immunoglobulin genes (IG) revealed intraclonal diversity, suggesting an active targeted somatic hypermutation process in eBL as compared to sBL.

Conclusions. These findings support the view that the antigenic pressure plays a key role in the pathogenetic pathways of eBL which may be partially distinct from those driving sBL development.

Localized IgG4-related lymphadenopathy, intra-GC plasmacytosis type. Case report and analysis of diagnostic criteria

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Background. IgG4-related sclerosing, autoimmune disease (IgG4-related disease) is a recently recognized syndrome characterized clinically by tumor like enlargement of one or more exocrine glands or extranodal tissues (most commonly pancreas, biliary tract, submandibular gland, and lacrimal gland), and raised serum IgG4 level, and pathologically by lymphoplasmacytic infiltration and sclerosis, as well as increased IgG4-secreting plasma cells.

The multiorgan involvement of IgG4-related disease was not recognized until 2003 but in the last 10 years substantial improvement has been made in understanding its pathophysiology. Nomenclature has been standardized, and a consensus has been achieved about the major and minor pathological manifestations. Effective treatments have been identified and important advances have been made in understanding of IgG4-RD but the epidemiology of the disease remains poorly understood, mainly because of challenges in recognition and differentiation from the many disorders it mimics.

Concomitant lymphadenopathy is common in IgG4-related disease but the morphologic features and diagnostic criteria have not been described in detail by the “Consensus statement on the pathology of IgG4-related disease” (2012).

In this study, we describe the pathologic features of a localized lymphadenopathy associated with IgG4 positive plasma cells. In literature there are some reports that share many clinicopathologic overlaps with this described case of lymphadenopathy.

Methods. A 60 years old, asymptomatic woman underwent a radiological check-up for an isolated axillary lymph node

enlargement (major axis: 6 cm), that was removed to confirm or rule out a lymphoma. No other superficial lymph nodes were detected by clinical and ultrasound inspection in this otherwise healthy patient. Lymph node was evaluated using both standard hematoxylin and eosin (H&E) staining and immunohistochemistry. A large panel of monoclonal Ab was tested using automated immunostainer with a polymer-based detection system. Results of IgG4 plasma cell staining were evaluated in the context of a concurrent IgG stain. Ig heavy-chain gene rearrangement was analysed by PCR. The reaction was performed according to the standard protocol.

Results. On microscopic examination, the lymph nodes showed reactive follicular hyperplasia. While only small to moderate numbers of mature plasma cells were present in the residual inter follicular areas, they were numerous within the germinal centre. Follicular hyperplasia was a common finding: the follicles showed germinal centers with atypical but benign features, including scarce polarization, tingible-body macrophages and poorly formed mantle zones. Progressive transformation of germinal centers was seen focally. Admixed mostly in the central portion were more irregular germinal center-type lymphocytes including small irregular lymphocytes and occasional larger, transformed lymphocytes, plasma- and plasmacytoid-cells, with a lot of irregular eosinophilic Russel bodies. In the inter follicular areas there was a mixture of activated lymphoid cells, plasma cells, eosinophils. The morpho-functional organization of the lymph node was completely compromised, with a Th2-switch (CD4/CD8 lymphocytes: 8/1, GATA3+, T-bet negative, FOXP3 negative).

Conclusions. These findings are consistent with IgG4-RD and rule out lymphoma or Castleman disease. The patient did not have systemic lesions; therefore, she has not undergone corticosteroid therapy. Pathological findings were consistent with IgG4-RD, intra GC plasmacytosis subtype as described by Sato et al. IgG4-RD should be considered as a differential diagnosis for localized superficial axillary and cervical lymphadenopathy by pathologists. We think that this case respects strict criteria for accepting newly proposed entities or sites as components of the IgG4-related disease spectrum.

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Histopathological findings in the oral mucosa of celiac patients

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Introduction. Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically

susceptible subjects. Although the small intestinal mucosa is the main site of the gut's involvement in CD, other mucosal surfaces belonging to the gastrointestinal tract and the gut-associated lymphoid tissue are known to be affected. Assuming that the oral mucosa could reflect the histopathological inflammatory alterations of the intestine in CD patients, this study wishes to assess the pattern of T-cell subsets in the oral mucosa of young adults with CD.

Methods. A group of 37 patients (age range 20-38 years; female: male ratio 28:9) with CD were enrolled. Out of 37 patients, 19 patients (group A) followed a gluten free diet (GFD) -2 patients from less than one year; 6 patients between 1 and 5 years; 11 patients more than 5 years- while 18 patients (group B) were still untreated. Fifteen healthy

volunteers (age range 18-35 years, female: male ratio 11:4) served as controls for the CD patients. Ethical approval for the research was granted by the Ethics Committee. Biopsy specimens were taken from normal looking oral mucosa. The immunohistochemical investigation was performed with monoclonal antibodies to CD3, CD4, CD8, and $\gamma\delta$ -chains T cell receptor (TCR).

Results. The T-lymphocytic inflammatory infiltrate was significantly ($p < 0.0001$) increased in group B (both compared with group A and with the control group).

Conclusion. This study confirms the oral cavity to be a site of involvement of CD and its possible diagnostic potentiality in this disease.

(see Fig.s below)

Fig. 1. Immunohistochemical stainings for CD3, CD4, CD8 and $\gamma\delta$ -TCR in the oral mucosa. Representative cases from group A (patients with CD and following GFD), group B (patients with CD and still untreated) and from control group. Counterstain with hematoxylin. Original magnification 200x.

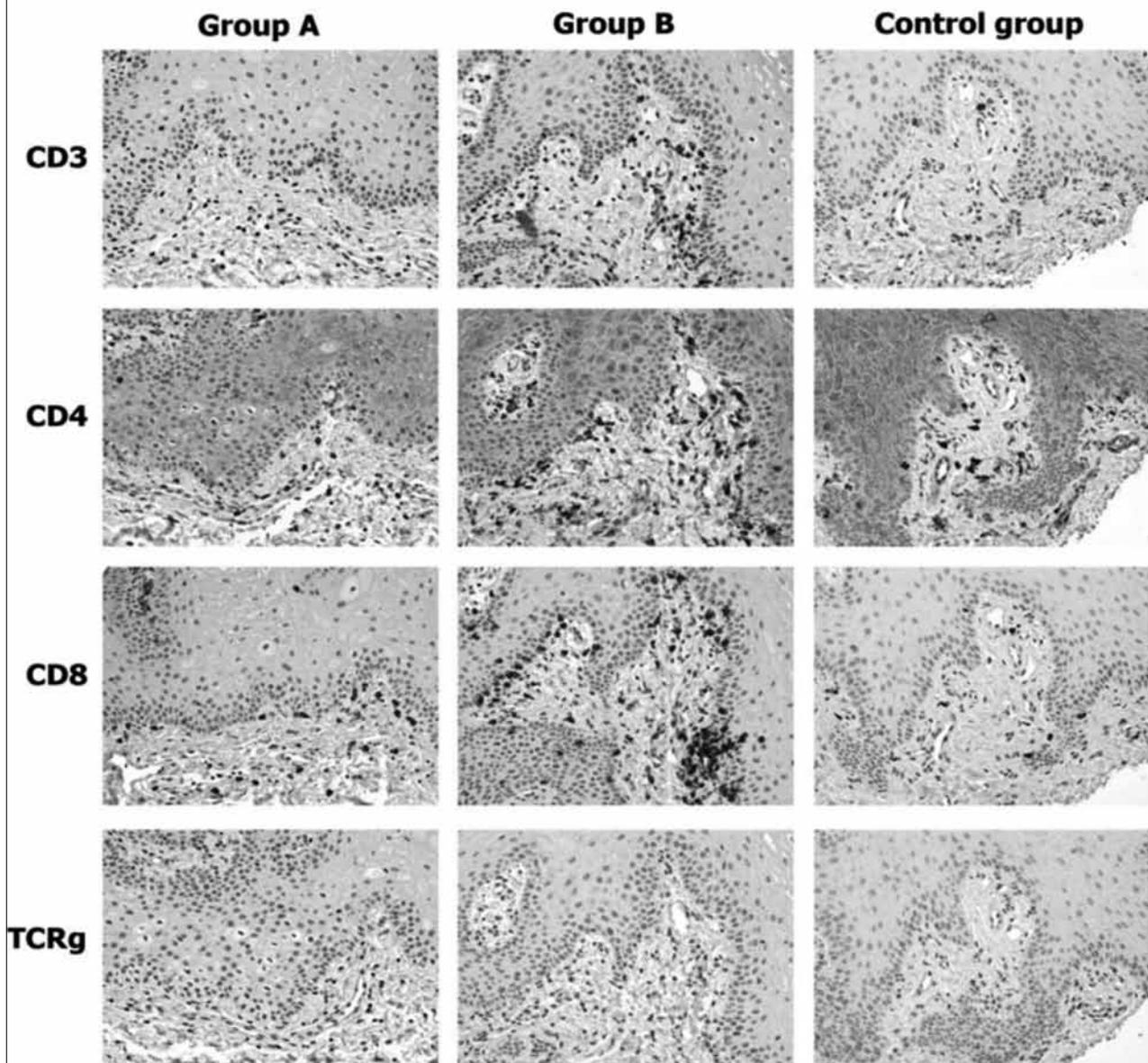
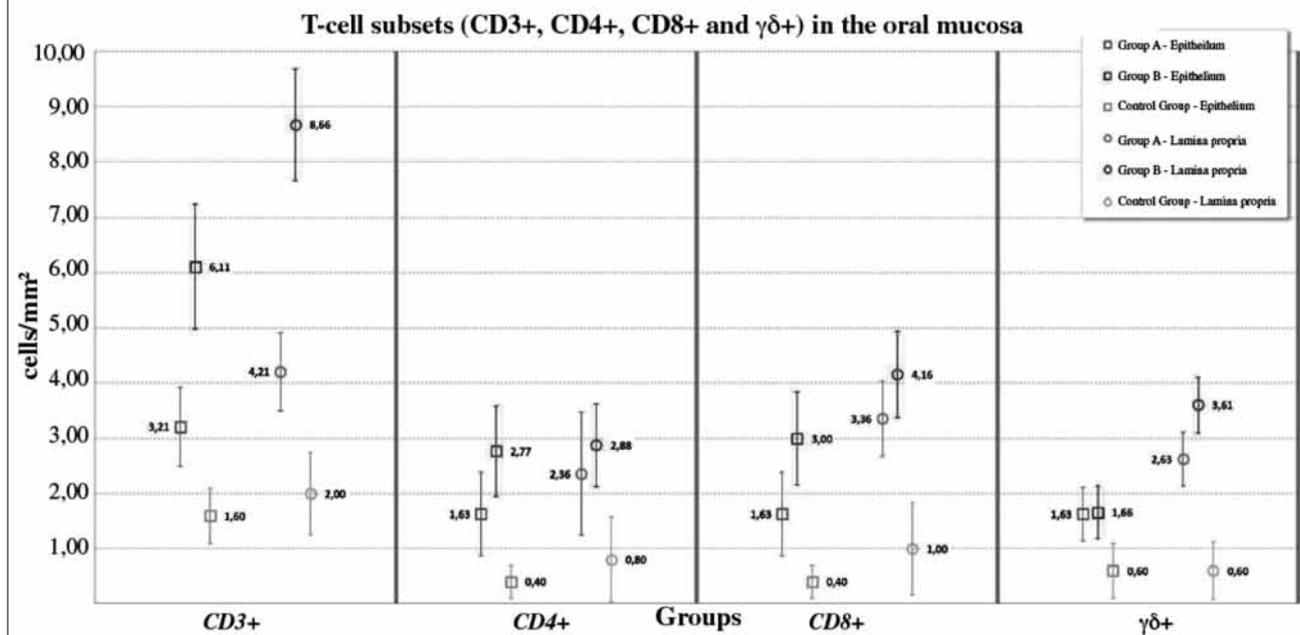


Figure 1

Fig. 2. T-cell subsets (CD3+, CD4+, CD8+ and $\gamma\delta$ +) in the oral mucosa (epithelium and lamina propria) of group A, group B and control group. The T-lymphocytic inflammatory infiltrate is significantly ($p < 0.0001$) increased in group B.



Fundic gland polyps associated with absorptive hypercalciuria. A key role for gastrin

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Introduction. Fundic gland polyps (FGPs) are tiny, often multiple sessile polyps of the acid-secreting gastric mucosa. They have been described as sporadic ^{1 2 3} associated with classic familial adenomatous polyposis (FAP) or attenuated variants (AFAP) ^{4 5}, associated with Zollinger-Ellison syndrome ^{6 7}, and with the rare Gastric Adenocarcinoma Proxymal Polyposis of the stomach (GAPPS) ⁸. Recently, we studied the gastric biopsies of 38 patients with absorptive hypercalciuria (AH)(9). AH is characterized by hypersensitivity of Calcium-sensing receptor (CaSR) of antral G-cells, with normal fasting gastrinemia and meal hypergastrinemia. G-Cell CaSR is the predominant chemosensor mediating Gastrin Secretion 10. Patients and methods. Criteria for patients' selection were published elsewhere ⁹ All 38 patients had calcium urinary excretion higher than 250 mg/d (6.25 mmol/, normal values, 2.5-6.25 mmol/d); they were on a free diet and were not taking calcium-sparing diuretics. After one month of dietician assisted calcium-free diet, they showed a reduction in daily calcium excretion (<100mg/d or 2.5 mmol/d), and a decrease of calcium/creatinine values in fasting 2-h urine (<0.35 mmol Ca/mmol creatinine, normal values 0.10-0.20 mmol Ca/mmol creatinine), so fulfilling conventional criteria for AH diagnosis. They were all free of severe dyspepsia, and none had taken anti-acid therapy. Whereas fasting gastrinemia was comparable to controls (60-70 ng/L), patients with AH responded to both calcium load test (1 g calcium gluconate Calcium Sandoz fortissimo) and pep-tone load test (10 mg Liebig meal extract diluted in 250 ml of 0.9% saline) with an abnormal rise in gastrinemia with almost doubling values when compared to controls. Endoscopically, all patients did not show significant lesions, except for sparse

antral microerosions or hyperemia; in five patients were seen small body-fundic polyps that were biopsied. Five antral and body normal controls and the biopsies, randomly taken from antrum (in all 38 patients) and body fundus (in 27 patients), plus the five body-fundus polyps were fixed in Bouin, embedded in paraffin, cut at 3 μ and stained with Hematoxylin-eosin and modified Giemsa. The Helicobacter pylori (H pylori) was extensively searched for on modified Giemsa section at 40X on both antral and body biopsies. Further sections were cut for the immunohistochemical study of endocrine population using anti-Gastrin polyclonal antiserum (Dako), diluted 1:300 for 30', room temperature.

We scored antral G-cells as follows) ⁹
 Normal: 1-2 G-cells for each gland, with uneven distribution
 Simple hyperplasia: 4-5 cells for each gland
 Linear hyperplasia: continuous chain-like distribution of G-cells (Fig. 1).

Results. The patients were mostly female (F/M: 4.4/1), with median age of 58.2 years. 12 patients were H pylori free, whereas 26 had a H pylori gastritis. All antral biopsies were stained with anti-Gastrin antiserum. In the patients with H pylori gastritis the post-eradication biopsies were taken at least after 8 weeks (20.6 months of mean interval) from the suspension of the PPI therapy. All 12 patients negative for H

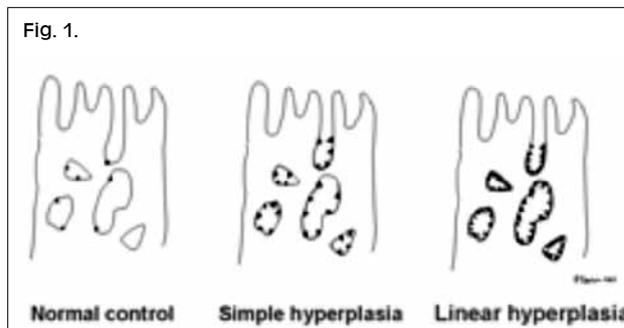


Fig. 2. Simple (2A) and linear hyperplasia (2B) highlighted by immunohistochemical stain for gastrin. (2A Gastrin antiserum, 10X original magnification; 2B Gastrin antiserum, 4X original magnification)

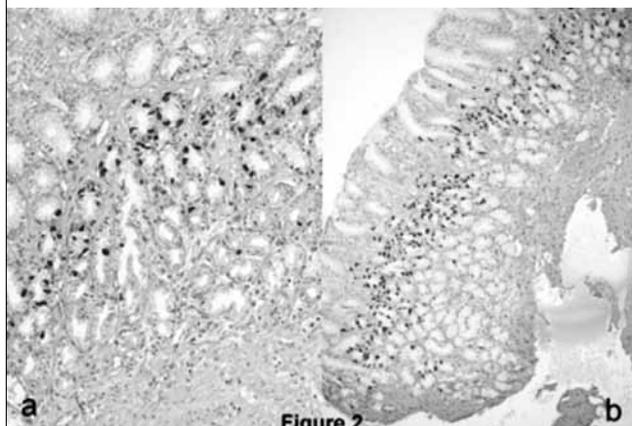
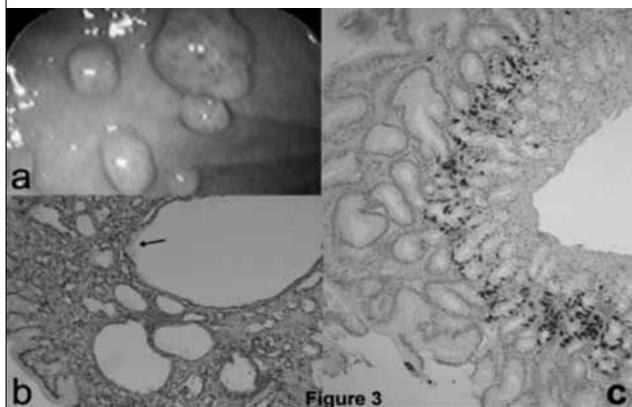


Fig. 3. Tiny polyps covered by pink glistening mucosa in the body-fundus were apparent at endoscopy (3A); histologically, they showed superficial and deep cystic dilation, covered by mucus (arrow), parietal and chief cells, diagnostic for FGPs (3B); anti-gastrin immunostaining evidence a persistent linear hyperplasia after the H. pylori eradication (3C). (3B Hematoxylin-eosin, 10X original magnification; 3C gastrin antiserum, 4X original magnification)



pylori showed G-cell hyperplasia (4 simple, 8 linear) as well as the 26 eradicated patients (11 simple, 15 linear)(Fig. 2).

In 2 of 12 H pylori patients and in 3 of 26 patients after H pylori eradication were biopsied 5 FGPs (Fig. 3). They showed shortened gastric pits, superficial and deep irregular cysts, bordered by mucous, parietal and chief cells. Neither signs of parietal cell changes nor dysplasia were apparent

Discussion. We studied morphologically and immunohistochemically the antral biopsies (and body-fundus biopsies in 27 cases) of 38 patients with Absorptive Hypercalciuria (AH). These patients show normal fasting gastrinemia and meal hypergastrinemia (Ca⁺⁺ and peptones) with median gastrin levels almost doubled compared to normal controls. It is now known that gastric CaSR and gastrin-secreting G-cells are both activated by Ca²⁺, amino acids and elevated pH. This association strongly suggest an important role of the calcium sensing receptors in the meal-stimulated gastrin secretion and regulation¹⁰. The first aim of our histological and immunohistochemical work was to define if patient with AH and meal hypergastrinemia had morphological antral G-cells alterations.

Twenty-six patients initially presented H pylori gastritis. As H pylori infection may induce a G-cells reactive hyperpla-

sia¹¹, immunohistochemical stainings were performed again after eradication therapy, with very long mean interval (20.6 months), with identical results in all cases. This very long interval seems to rule out a possible influence of both H pylori and PPI therapy. None of the patients had shown significant body atrophy, another possible confounding condition for G-cell hyperplasia. Moreover, comparing the percentages of simple and linear hyperplasia between the 12 patients originally H pylori negative and the 26 patients eradicated, there was not any statistically significant difference. This control between the two groups appears as a further proof of the primitivity of G-cell hyperplasia in patients with AH. So, we could conclude that the observed G-cells hyperplasia was primitive, and that represented the morphologic counterpart of meal hypergastrinemia in AH.

Unexpectedly, we found in five patients with AH an association with fundic gland polyps (FGPs). In two of our 12 patients, with gastric antrum H pylori negative, the polyps were already present at the first endoscopic exam. In the other three patients, FGPs appeared in the second endoscopic control, after H pylori eradication. So, even in patients with AH, we found an inverse relationship between polyps and H pylori infection^{12,13}.

The prevalence of FGPs associated with AH patients was more than ten-fold that of sporadic FGPs (13, 1% vs 0, 7% of sporadic FGPs)¹⁶, and this difference was extremely statistically significant. Aprile et al demonstrated in a multivariate analysis that among 106 patients with Zollinger-Ellison syndrome there was a statistically significant association between polyps' formation and levels of gastrinemia. Similarly, gastrin may have a role in the increased prevalence of FGPs in AH patients.

In a preliminary experiment, we stained with a Gastrin antiserum the gastric antrum biopsies of 79 patients with sporadic FGPs that had never been treated with PPI before; we found that 75.9% of these patients presented a G-cells hyperplasia morphologically similar to AH patients¹⁴. Furthermore, similar findings were shown by Worthley et al. in the gastric antrum of patients with GAPPS¹⁵. Apart this finding, our patients with AH had negative family history for gastric or colonic cancer, and none of the FGPs showed any dysplastic change.

Another important question to be addressed is to verify if among sporadic FGPs may exist a subset of patients with abnormal calcium excretion, stone formation or abnormal response to calcium or peptone load. At present, we have no data about this interesting question

In conclusion, we have seen that patients with AH, patients with sporadic FGPs, and patients with GAPPS share in common an antral G-cell hyperplasia. This fact may represent a promising field of future research.

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Left-sided early onset (≤ 50 years) colorectal carcinomas: a pathological and molecular study

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Introduction. The definition of "early onset colorectal carcinoma" (CRC) refers to cases diagnosed in early decades, usually before 50 years. Most cases of early onset colorectal carcinoma (CRC) shows proximal location and this is regarded as a marker of a hereditary syndrome. In fact they often harbor high microsatellite instability (H-MSI) due to germinal defects in mismatch repair (MMR) genes that cause the syndrome known as HNPCC (hereditary non polyposis colorectal carcinoma)/Lynch Syndrome (1). CRC of left colon represent mainly a neoplasm of 6th-7th decade (late onset) but epidemiologic evidences have been showing increasing incidence rate in patients ≤ 50 years (2-3). Data from literature seem to indicate that left-sided early onset CRC do not develop in the context of hereditary syndrome or cancer risk factors and it may imply different pathways in neoplastic transformation (4-5). The aim of our work was to investigate in a large series of early onset left-sided CRC molecular and pathological features.

Methods. We collected 62 patients ≤ 50 y (mean age 42,3, range 26y-50y) with left-sided CRC that underwent surgical resection and histological examination in Sant'Andrea Hospital in Rome from 2003 to 2014. Patients that received neoadjuvant chemo-radiotherapy, had association with inflammatory bowel disease or polyposis were not included in this study. Since a detailed oncological family history wasn't available for most cases it was not chosen as selection criterium. Patients were further divided in 3 subgroups according to age: A) 13/62 (20%) ≤ 40 y (mean age 34y); B) 16/62 (16%) ≥ 41 ≤ 45 y (mean age 43y); C) 33/62 (53%) ≥ 46 ≤ 50 y (mean age 48y). For all cases pathologic features as grading and staging were recorded. At molecular level all samples were evaluated for microsatellite instability (MSI) through PCR

amplification of quasimonomorphic mononucleotide loci and expression of mismatch repair (MMR) proteins hMLH1, PMS2, hMSH2 and hMSH6 through immunohistochemistry. BRAF mutation analysis and hMLH1 promoter hypermethylation were evaluated on cases showing MSI

Results. Male predominance was present especially in youngest groups (A and B). Most cases (41/62, 66%) presented at advanced pathological stage with lymph nodes or distant metastasis (III or IV) and showed low grade of differentiation (G1/G2). At molecular level five out 64 (8%) showed high-MSI (H-MSI). All MSI-H cases belonged to group A ≤ 40 y (5/13, 38%). H-MSI cases were respectively 26, 31, 36 38 and 39 years old respectively. Four out of 5 H-MSI presented at metastatic stages (IIIB or IIIC sec ajcc 2010) and 1/5 at stage IIA. No H-MSI case was observed in group B and C. Immunohistochemistry showed loss of hMSH2 and hMSH6 in 3 of 5 H-MSI cases and loss of hMLH1 and PMS2 in the other 2 (Tab 1). Since hMLH1 loss may be due to hereditary gene mutation or to promoter hypermethylation as in sporadic case, H-MSI CRC cases showing loss of hMLH1 protein were investigated for V600E BRAF mutation and hMLH1 promoter methylation in order to discriminate between HNPCC and sporadic H-MSI. Neither BRAF V600E mutation or hMLH1 methylation were observed.

Conclusions. Our results on a large, unselected, series of early onset CRC arising in the left colon show that early onset left sided CRCs are more likely to present at advanced stage. Although H-MSI cases represent only 8% of the all samples, interestingly all 5 belonged to the youngest subgroup (≤ 40 y). The 5 H-MSI cases may be regarded as HNPCC/Lynch syndrome since 3 showed loss of h-MSH2/hMSH6 and 2 of hMLH1/PMS2 without BRAF V600E mutation or hMLH1 promoter hypermethylation. Our finding implies that early onset left sided CRC, independently from history family, should be always screened for MSI especially the ≤ 40 y and that patients older than 40y are unlikely to represent Lynch syndrome.

Tab. I

	Group A (≤ 40 y) (13)	Group B (≥ 41 ≤ 45 y) (16)	Group C (≥ 46 ≤ 50 y) (33)	Tot 62
Age (years)				
Mean	34	43	48	
Sex n (%)				
M	9 (70)	10 (62)	17 (51)	36 (58)
F	4 (30)	6 (38)	16 (48)	26 (42)
pT n (%)				
pT1	0	0	2 (6)	2 (3)
pT2	2 (15)	2 (12)	7 (21)	11 (18)
pT3	7 (55)	11 (69)	17 (52)	35 (56)
pT4	4 (30)	3 (19)	7 (21)	14 (23)
pN n (%)				
N0	4 (30)	7 (44)	13 (40%)	24 (39%)
N+	9 (70)	9 (56)	20 (60%)	38 (61%)
pStage				
I-II	4 (30)	6 (38)	12 (36)	22 (35)
III-IV	9 (70)	10 (62)	21 (64)	40 (65)
G (grading)				
G1-G2	6 (46)	12 (75)	22 (67)	40 (65)
G3-G4	7 (54)	4 (25)	11 (33)	22 (35)
H-MSI n (%)	5 (38)	0/16	0/33	5 (8)
hMLH1-/PMS2-	2	-	-	
hMSH2-/hMSH6	3	-	-	

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Solitary fibrous tumor of the pancreas: a diagnosis made on fine needle cytology and core biopsy

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Introduction. In recent years, the use of ultrasound-guided fine-needle cytology (US-FNC) has reached excellent levels in making a diagnosis of pancreatic masses. Oncologic patients that obtain a positive diagnosis are often treated either surgically or pharmacologically on the sole basis of the cytological diagnosis. Pancreatic biopsies, either core biopsy or surgical biopsy, are more difficult to make and usually do not improve the overall quality of the diagnosis. Most patients are not operable, for them the quicker and less invasive diagnosis is represented by FNC.

Most pancreatic tumors are epithelial neoplasms, in the form of ductal adenocarcinoma. A considerable part of tumors, especially located in the body and tail of the pancreas, are neuroendocrine tumors. Very rarely, soft tissue pancreatic neoplasms have been described; among them few reports exist of primary pancreatic solitary fibrous tumor (SFT).

In this report we describe a diagnosis of SFT of the pancreas made by FNC and confirmed by a core biopsy (CB). Furthermore we discuss the available literature on this subject.

Case Report. The patient is a 86 year old male who has complained in the last 3 months of abdominal pain. Nothing relevant was present in the pathological proximal anamnesis. Laboratory analysis revealed a low increase of serous CA 19.9. A total body CT scan was performed and a pancreatic mass of about 13 cm in diameter, located in the tail, was noted. A suspicious pancreatic carcinoma was considered. US-FNC was performed and conventional smears air dried and alcohol fixed were executed and respectively stained with Diff Quik and Papanicolaou. The rapid on site evaluation revealed a spindle cells proliferation, suggestive of mesenchymal type. A second pass for Cell Block preparation was performed. Cell Block sections confirmed the mesenchymal proliferation and immunostained sections revealed positivity for CD34, Bcl2 and CD99; negativity for epithelial markers, pS100, Desmin, CD31 and CD117. The definitive FNC diagnosis was that of a mesenchymal tumor, suggestive for SFT. A US-CB was also performed. Microscopic observation showed a spindle cell tumor, partly organized in a storiform pattern, with variable cellularity. Immunohistochemical study revealed intense positivity for CD34, Bcl2 and CD99; S100, CD117 and Desmin came negative; Actin

positivity was scant; Ki67 index was low (2-3%). Definitive diagnosis was that of SFT.

The patient is 6 months into recovery healthful and is in close follow up. Because of the patient's age, surgery was not proposed as a first treatment choice.

Discussion. Soft tissue pancreatic tumors are rare neoplasms and thus are seldomly suspected on clinical grounds and instrumental analysis.

SFT has been identified in the pancreas less than 20 years ago. Few reports have been made in these years, and less than 3 cases have been diagnosed on the basis of FNC.

In our case the diagnosis was reached by FNC and CB.

Prognosis of SFT is hard to establish on small samples. The described features of malignancy in SFT of different organs are: the presence of necrosis, a high mitotic index, high cellularity, cellular pleomorphism, presence of hemorrhage and size; All these features must be evaluated on the whole surgical specimen because of the elevate neoplastic variability.

The few existing pancreatic reports do not describe an aggressive course.

In our case neither FNC nor CB were useful in establishing the behavior; however, both FNC and CB did not show any of the above-listed features.

The extended age of the patient made it difficult for the possibility of surgical excision. Follow up data will give more information of the course of the neoplasm.

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Reporting of gastric foveolar dysplasia: a survey of gastrointestinal pathologists

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Background. There is considerable variability in the criteria used for diagnosing gastric foveolar dysplasia (FD) amongst pathologists.

Design. The diagnostic criteria for separating FD from reactive change and adenomatous dysplasia (AD) were tested by an online questionnaire that was circulated to 13 gastrointestinal (GI) pathologists

(Canada, UK and USA) The following was ascertained:

- How often do you make a diagnosis of FD per year?
- List the diagnostic criteria you use for FD.
- List the criteria separating low-grade (LGD) from high-grade (HGD) FD
- How do you distinguish LGD FD dysplasia from reactive change?
- How do you distinguish FD from AD dysplasia?
- Do you use immunohistochemistry in the diagnosis of FD? If so, what stains?

Results. Eight out of 13 pathologists never or rarely (1-2 times /year) make the diagnosis of FD, while 5 pathologists make

it 4-12 times/year. The criteria used for the diagnosis of FD include: eosinophilic pale cytoplasm, cytoplasmic features of foveolar type mucin, nuclear size, round monomorphic nuclei, prominent nucleoli, minimal or no nuclear stratification. Criteria used to separate LGD from HGD include: membrane irregularities, loss of polarity, higher N/C ratio, architectural complexity, nuclear size. The distinction between reactive and dysplastic lesions is based on the presence of inflammation, surface maturation, lack of architectural complexity and gradual merging with the surrounding cells, nuclear hyperchromasia and enlargement. Rounded nuclei with minimal atypia and stratification, and lack of intestinal metaplasia are used to distinguish FD from AD. Immunohistochemistry (CDX2, MUC5AC and CD10, AMACR and p53) is used by 6 out of 13 pathologists in difficult cases.

Conclusions. Gastric foveolar dysplasia is rarely diagnosed in this survey of GI pathologists and the criteria used are variable and inconsistently applied. There is a need to standardize criteria, particularly with regard to separating reactive change from LGD.

Blastic plasmacytoid dendritic cell neoplasm (BPDCN): case report

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Introduction. Blastic plasmacytoid dendritic cell neoplasm (BPDCN), formerly known as agranular cluster of differentiation (CD4+/CD56+ hematodermic neoplasm, is a rare and aggressive type of lymphoma, with only ~100 cases reported worldwide. BPDCN is a hematological malignancy with an aggressive clinical course. Due to its biological and clinical features, it could be classified as an intermediate disease between acute myeloid leukemia (WHO 2008) and non Hodgkin lymphomas (WHO-EORTC 2005). Most patients with BPDCN have skin lesions and simultaneous involvement of the peripheral blood, bone marrow, and lymph nodes. **Material and Methods.** We report the case of a 74-year-old man, at diagnosis, in November 2013, showed purplish eruptive nodules, neither painful nor itchy, preferentially located on his trunk and legs, with a circular shape and a diameter of about two-three centimeters. A biopsy specimen taken from thigh skin lesion revealed a dermo-epidermal infiltrate of small sized cells. On the basis of positive immunophenotyping for CD4, CD56 was made a diagnosis of BPDCN. Immunostaining for CD8, CD30, CD20, CD79a, CD3, CD5, CD10, CD23, Bcl-6, TdT, myeloperoxidase, D1 cyclin, CD34, CD117 and CD138 was negative. Mib-1 proliferation index was 70%. The usual differential diagnosis of BPDCN include nasal-type NK-cell lymphoma, cutaneous T-cell lymphoma and leukemia cutis. The patient underwent staging with complete blood counts, serum chemistries, computer tomography scan and bone marrow biopsy. Bone marrow examination showed infiltration (15%) by medium-sized blast-like cells with basophilic cytoplasm and irregular nuclei. The patient underwent therapy with intralésional administration of interferon alpha, subsequently with subcutaneous administration of interferon alpha 3.0 MUI three times per week for two months, oral administration of dexametasone 20 mg on day 1-4 of each month. Results. We detected an improvement of the skin nodules within 3 weeks from the start of the treatment with interferon, and a progressive reduction in lymph node size over the course of 4 weeks from the introduction of corticosteroid therapy. Our patient is alive after

a year and a half from the diagnosis and in complete response. To date skin lesions are significantly reduced and present in the form of small dyschromic alterations. **Conclusions.** BPDCN is a rare aggressive disease that typically affects elderly patients. The most commonly affected non hematopoietic organ is the skin. Although BPDCN is initially sensitive to conventional chemotherapy regimens, this response is relatively short and long-term prognosis is poor. Due to patient's age and comorbidities he didn't undergo multiagent chemotherapy and we have decided to review the applicability of interferon alpha in this case and to date the patient is free from disease.

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Primary squamous cell carcinoma of the middle rectum: a case report

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Introduction. Colorectal cancer is the third most common cause of cancer-related death in the world. Primary squamous cell carcinoma of the colon and rectum are extremely rare, with an incidence of less than 1% of colorectal malignancies. Little is known about the risk factors and natural history, prognosis and treatment of this neoplasm, and the association with human papillomavirus (HPV) infection has not been well established.

Materials and methods. A 44-year-old man presented for anal bleeding. Colonoscopy revealed a mass 10 cm from the dentate line, the biopsies were suspicious for cancer. An anterior resection of the rectum with mesorectal excision and sphincter preservation was performed. The surgical specimen followed the standard procedure for processing, cutting, staining and immunohistochemistry.

Results. Grossly, the rectum showed an ulcerate, polypoid lesion of 5 cm in maximum diameter. Histologic examination revealed moderately differentiated squamous cell carcinoma infiltrating the mesorectum. No metaplastic epithelium was found in the normal mucosa. Polymerase chain reaction (PCR) for HPV detection revealed the presence of HPV-16 infection not only in the neoplastic lesion but also in the adjacent normal tissue, with virus integration detectable only in the tumour, as demonstrated by p16 stain positivity of neoplastic cells. A diagnosis of pT3N0 B/IIA G2 (according to TNM7) squamous cell carcinoma of the rectum was performed. Due to the rarity of the histotype, a whole body CT-scan was carried out to exclude other sites of involvement, with negative results. Oral and anal swabs as well as multiple biopsies of the gastroenteric tract were done to evaluate HPV presence. Anal and oral swabs were HPV negative, whereas

HPV infection was detected in other sites of the colon, with p16 positivity expressed by lymphocytes, endothelial cells and epithelial cells, thus suggesting an “HPV- related colitis”. **Conclusions.** The association of HPV infection with colorectal cancer has been extensively investigated. However, no strong evidence supports the involvement of the virus in its development, and where and how HPV comes to colorectal tissues without giving cancer or pre-neoplastic lesions (i.e. coilocytosis and/or dysplasia) in the anal region remains to be fully investigated. In our case, the absence of HPV in the most common ports of entry for the virus (oral and anogenital

region) suggests that the infection might not be the result of a direct spread. The detection of the virus in endothelial cells and tumour-infiltrating lymphocytes as well as in normal tissues supports the view that the virus was present in blood circulation. It is well known that the colon environment is a complex network composed by stromal, inflammatory and immune cells, microbiota and epithelial cells, in which there is a complex interplay among host-protective and tumor-promoting actors. It is reasonable that the virus has reached middle rectum via blood circulation and elicited its oncogenic role in this site for a transient imbalance of immune response.

Giovedì, 24 Settembre 2015

Aula Blu 2 ore 9.00-10.00

Miscellanea 1

Moderatore: F. Crivelli (Milano)

Role of N-formyl peptide receptors (FPRs) in systemic sclerosis

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Introduction. Systemic sclerosis (SSc) is a heterogeneous group of diseases caused by the combination of vascular dysfunction, inflammatory/autoimmune processes and extensive fibrosis that contribute to cause organ damage. The pathogenesis of SSc is extremely complex and, although recent years have seen many advances in this field, the mechanisms involved remain largely unclear. During the last decade considerable attention has been focused on the formation and functional role of the myofibroblast, considered as the main responsible for the excessive matrix production and deposition. Despite this, in SSc the origin of myofibroblast has not been completely established.

Characterizing the molecular players involved in the genesis of fibrosis and in the fibroblast-to-myofibroblast transition may help to properly manage the patients with SSc and improve treatment options.

N-formyl peptide receptors (FPRs) are a distinct set of cellular receptors acting as Pattern Recognition Receptors (PRRs), which are known to be involved in many cellular processes, such as migration, proliferation, differentiation, growth, and death, and in innate inflammatory responses. Three members of this family have been characterized in humans: FPR1, FPR2, and FPR3. Several studies have signalled a specific role of FPRs in the regulation of a growing number of fundamental cellular processes.

The aim of the present study was to determine whether FPRs could play a crucial role in the fibroblast-to-myofibroblast transition, contributing to excessive matrix production in SSc. **Methods.** Real-time polymerase chain reaction (RT-PCR) and Western Blot were used to evaluate FPR1, FPR2, and FPR3 expression in skin fibroblasts of 10 normal subject and 10 SSc patients. Immunohistochemistry was performed to confirm the data.

Results. SSc fibroblasts showed high levels of FPRs mRNA and a slight increase of protein levels. Immunohistochemistry confirmed FPRs overexpression in SSc.

In addition, as many FPRs functions are activated through the interaction with urokinase receptor (uPAR), and as a cleaved uPAR form (u-PAR-derived uPAR88-92 peptide) induce cell migration and proliferation in both normal and SSc fibroblasts, we evaluated the expression of uPAR in SSc fibroblast. SSc fibroblast showed increased expression of a cleaved uPAR88-92, involved in fibrosis and Endothelial Mesenchymal Transition (EndoMT).

Furthermore, SSc fibroblasts showed high levels of α -smooth muscle actin (α -SMA) expression, myofibroblast phenotype marker.

Conclusion. these results indicate that FPRs may have an important role in the origin of fibrosis and in the fibroblast-to-myofibroblast transition in SSc.

Angiotropic melanoma

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Introduction. Extravascular migratory metastasis (EVMM) in melanoma is a relatively recent field opened from about 15 years by Lugassy and Barnhill. During EVMM, tumor cells migrate along the external surfaces of vascular channels, mostly without intravasation, demonstrating angiotropism and pericytic mimicry. Angiotropic melanoma cells are defined histologically as melanoma cells closely associated with the endothelium of vessels, both blood and lymphatic, in a pericytic location, generally detected at the advancing front of the tumor; massive intravasation is absent, although some authors include early signs of intravascular invasion as part of EVMM definition. The clearly recognizable melanoma cells cuffing the abluminal surfaces of the endothelium of microvascular channels, either in linear array or in aggregates, and the absence of massive intravascular tumor cells groups, are considered two mandatory features in order to define angiotropism on histological basis.

Vascular mimicry is a well-defined feature of melanoma cells that distinguishes from pericytic mimicry being the latter strictly linked to migration along vascular channels.

Angiotropism could be a prognostic factor predictive of tumor metastasis, as shown by Barnhill and Lugassy.

Several studies have been carried out, during last years, in order to define the molecular signature of angiotropic melanoma cells.

Methods. We retrieved a consecutive series of melanoma tissue samples from our archives relative to the last five years by selecting only cases of melanoma showing angiotropism, according on the above-mentioned criteria. Only cases showing clear images of extravasal migratory metastasis and no morphological evidences of massive intravasal invasion, were included into the study. We evaluated the morphology of angiotropic melanoma cells, pointing out in particular to the morphology of the pericytic mimicking cells. We correlated our findings to the follow up, with particular emphasis to distant metastasis. We sub-grouped patients according to the kind of metastasis, subcutaneous versus deep metastasis.

We performed IHC on FFPE tissue with Cancer Stem Cell (CSC) and mesenchimal stem cell markers.

Results and Conclusions. From our analysis we confirmed the original finding of significant correlation between angiotropism and poor prognosis; we also correlated the metastatic behavior with the morphological aspect of the angiotropism, grouping the kind of distant metastasis on the basis of initial signs of vessel brakeage in angiotropic foci. We particularly focused on molecular signature of angiotropic melanoma cells based on BRAF mutation status and stem cell markers expression in order to better stratify the metastatic risk.

Extravascular migratory metastasis is a revolutionary new paradigm about the mechanisms of melanoma metastasis, additional to the extensively studied intravascular dissemination of tumor cells. EVMM has also been described in other solid tumors. Our work let envisage a better definition of angiotropic melanoma cells that could help to unravel new aspects of tumor biology, especially regarding the metastatic feature and resistance to therapy. We intend to extend our investigation on other solid tumor to the aim of detect early signatures of metastasis and therapy response.

Melanocytic hyperplasia in the epidermis overlying trichoblastomas in 100 randomly selected cases

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Melanocytic hyperplasia in trichoblastoma

One hundred cases of trichoblastomas (large nodular, small nodular, cribriform, lymphadenoma and columnar) were randomly selected and studied for the presence of melanocytic hyperplasia in the epidermis overlying the tumors, which was defined as foci of increased melanocytes in the basal layer of the epidermis (more than 1 per 4 basal keratinocytes). Focal melanocytic hyperplasia was detected in a total of 22 cases of trichoblastoma (22%), and this phenomenon was most frequently seen in columnar trichoblastoma (7 cases), followed

by large nodular trichoblastoma (5 cases). The mechanism of epidermal melanocytic hyperplasia overlying trichoblastoma is unclear. As in a third of cases focal melanocytic hyperplasia was detected also in the epidermis overlying uninvolved skin, usually associated with solar elastosis, UV may be a contributing factor. This is further corroborated by the occurrence of the lesions predominantly on the face. This features appears to have no impact on the clinical appearance of the lesion and is recognized only microscopically. In an adequate biopsy specimen containing at least part of trichoblastoma, it should cause no diagnostic problems.

Introduction. Melanocytes are normally present in the basal layer of the skin. The number of melanocytes in the normal skin is constant in all races, with the ratio of one melanocyte for every 4 to 10 basal keratinocytes, depending on the location. Melanocytic hyperplasia refers to increased numbers of melanocytes, which are often appear enlarged, with hyperchromatic nuclei ¹. This phenomenon is often seen in sun-damaged skin (actinic melanocytic hyperplasia or in an etiologically similar condition (PUVA-associated melanocytic hyperplasia) in which it is considered as a reaction to excessive UV radiation. In addition to an increase in number, melanocytes often show starburst appearances. Melanocytic hyperplasia is also seen in recurrent nevi over a scar. In non-melanocytic conditions, it is sometimes observed in prurigo nodularis. Rare observations of melanocytic hyperplasia in the epidermis overlying trichoblastoma have been made ^{2,3,4} but as far as we are ware, no systematic studies were performed. The aim of our study was to investigate the frequency and extent of melanocytic hyperplasia in various types of trichoblastoma.

Material and methods. Twenty of cases of each main type of trichoblastoma (large nodular, small nodular, adamantinoid (lymphadenoma), columnar (desmoplastic trichoeplithelioma), and cribriform (trichoeplithelioma)) were randomly selected from the Pilsen Tumor Registry and personal authors files. Few cases were the epidermis was absent were excluded and substituted by new randomly selected lesions. Thus, 100 trichoblastomas were studied. The clinical information was available for 79 patients, who included 48 women and 31 men, with the age at the time of diagnosis ranging from 4 to 84 years (mean 49 yrs; mediana 48 yrs). In a majority of cases (79%), the lesion were located in the head and neck areas, mostly on the face. All cases with available clinical information were sporadic, without associated syndromes such as multiple familial trichoeplithelioma/Brooke-Spiegler syndrome ⁵.

The histopathological definitions of each trichoblastoma type is provided elsewhere ^{4,6}. Cases with mixed patterns (for example cribriform and retiform, small nodular and large nodular etc.) were not included. Melanocytic hyperplasia was defined as foci of increased melanocytes in the basal layer of the epidermis (more than 1 per 4 basal keratinocytes) and was quantified as follows: focal melanocytic hyperplasia occupying less than 1/3 of the epidermis above the tumor (score 1); melanocytic hyperplasia occupying 1/3 to 1/2 of the epidermis above the lesion (score 2), and melanocytic hyperplasia occupying more than half of the epidermis above the lesion (score 3). Also estimated were absence/presence of melanocytic hyperplasia in the epidermis outside the trichoblastoma, presence of solar elastosis and other relevant features.

Results. As defined above, focal melanocytic hyperplasia was detected in a total of 22 cases of trichoblastoma (22%), and this phenomenon was most frequently seen in columnar trichoblastoma (7 cases), followed by large nodular tricho-

Tab. I. Melanocytic hyperplasia in trichoblastoma.

Variant (total)	Score 1	Score 2	Score 3
Columnar (7)	5	2	0
Large nodular (5)	4	0	1
Cribriform (4)	0	3	1
Lymphadenoma (4)	2	2	0
Small nodular (2)	1	0	1

blastoma (5 cases). In over a half cases (12 cases), the extent of hyperplasia was scored as 1, with only 3 specimens demonstrating melanocytic hyperplasia extending over the half of the epidermis over a trichoblastoma (Table I), (Fig. g. 1, 2). In 6 cases, focal melanocytic hyperplasia was detected also in the epidermis overlying uninvolved skin, and 4 of these cases there was prominent solar elastosis. Basal hyperpigmentation was usually not observed, but in 2 cases well demarcated areas with intraepidermal melanin were present in the spinous layer, focal ascend of melanocytes (Fig. 3). Whereas the epidermis was largely atrophic above the trichoblastomas, in one case of large nodular trichoblastoma, it manifested changes reminiscent of prurigo nodularis and melanocytic hyperplasia was observed in the hyperplastic areas. Two cases of columnar trichoblastoma were associated with a compound melanocytic nevus.

Discussion. To the best of our knowledge, melanocytic hyperplasia in the epidermis overlying trichoblastomas was not specifically studied; however, our research of the literature on trichoblastomas revealed several pictures where melanocytic hyperplasia can be recognized (Fig. g. 22.25, 22.55 in 2; Fig. ure 1 in ⁷ Fig. ure 3 in ⁸).

The mechanism of epidermal melanocytic hyperplasia overlying trichoblastoma is unclear. One can speculate that specific follicular stroma may produce certain factors but this is unlikely given a relative low frequency of this phenomenon. Additionally, in a third of cases in our series focal melanocytic hyperplasia was detected also in the epidermis overlying uninvolved skin, usually associated with solar elastosis, indicating that UV may be a contributing factor. This is further corroborated by the location, inasmuch as most lesions occurred on the face. Of note, in many cases the epidermis overlying the trichoblastomas was thinned, with loss of rete ridges, likely due to the pressure from the underlying expanding tumor, which may not only optically contribute to an increase of melanocytes but also to increase to the insolation area.

That is epidermal melanocytic hyperplasia overlying trichoblastomas is not specific to this entity is evident by its occurrence in association with other epithelial and mesenchymal tumors, including plexiform neurofibroma and Merkel cell carcinoma ^{9 10}. Epidermal melanocytic hyperplasia is distinct from melanocytic colonization, which occasionally seen in various nonmelanocytic tumors ¹¹⁻¹⁵, or maybe an essential component of the lesion like in melanocytic matricoma ¹⁶. Melanocytic colonization was beyond the scope of this study but in a few cases for which staining for melanocytic markers was available, occasional intratumoral melanocytes were present. Associations of trichoblastoma and pigmentary changes/melanocytic lesions has been addressed in the literature. The most common occurrence is large nodule trichoblastoma, which often contains abundant melanin. Columnar trichoblastoma (desmoplastic trichoepithelioma) is associated with a melanocytic nevus in about 10% of cases which in line with our findings in this study ¹⁷. A collision of melanoma and trichoblastoma (sometimes referred as trichoblastomela-

Fig. 1. Increased melanocytes in the basal layer of the epidermis over columnar trichoblastoma.

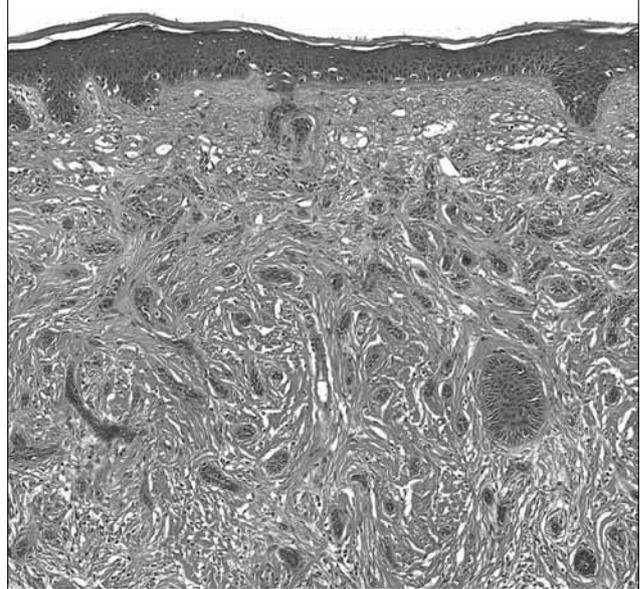


Fig. 2. Epidermal melanocytic hyperplasia overlying small nodular trichoblastoma. Melanocytes are large and some have hyperchromatic nuclei. The epidermis is atrophic. (A, B).

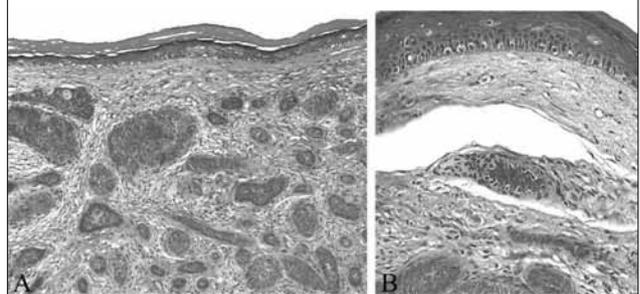
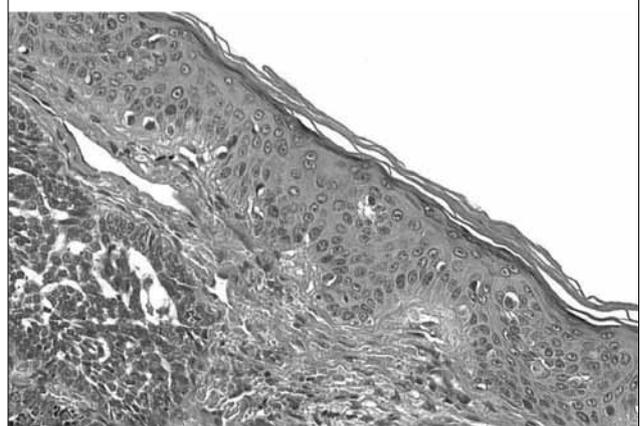


Fig. 3. Intraepidermal clumps of melanin and focal melanocytic ascend of melanocytes above large nodular trichoblastoma. Increased melanocytes in the basal layer were present elsewhere in this case.



noma or METRO (MELanocytic tumor +TRichOblastoma)) has been described ^{18 19 20}.

In conclusion, melanocytic hyperplasia in the epidermis overlying a trichoblastoma can be encountered in lesions located on the face. It appears to have no impact on the clinical appearance of the lesion and is recognized only mi-

croscopically, as an incidental finding. In an adequate biopsy specimen containing at least part of trichoblastoma, it should cause no diagnostic problems.

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The pathologist in the diagnostic and therapeutic path: a new role for a before hidden profession

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Introduction. Regional guidelines and cancer screening programs have designated as priority for each Hospital, the

establishment of diagnostic and therapeutic paths (in Italian: Percorsi Diagnostico-Terapeutici e Assistenziali, PDTA), to optimize the management of patients with chronic or neoplastic diseases. The PDTAs are a tool aimed to lead clinical and organizational processes within a hospital and as a mean to improve the way through which patients face their diseases.

The PDTAs promote a major involvement of patients and a more effective coordination among all the specialists involved. Inside each PDTA, the pathologist is responsible not only for the diagnoses (which must be drafted according to official guidelines), but also for the optimization in resource allocation; a timely and accurate diagnosis means a correct choice of the therapeutic pathway.

The pathologist is also called to a continuous update on biological markers and to acquire and manage biological samples for a "patient-tailored" cancer gene profiling, even according to the rules of the Biobanks.

Material and methods. At our Department of Pathology there are four pathologists in staff and a Director. In Martini Hospital have been activated PDTAs for neoplastic diseases in the following areas: breast, lung, gynecology, gastroenterology and urology; for each of these PDTA have been appointed two pathologists (a referent and a deputy). The referent is called to join to internal meetings of the Interdisciplinary Care Groups (In Italian: Gruppo Interdisciplinare di Cura, GIC), weekly convened to discuss the cases, and to be continuously updated in the topics related to the assigned PDTA.

Results and conclusions. The PDTAs offer a great chance to the pathologist to address challenges of a new approach to his own work.

The first challenge is to achieve effective and shared procedures and a common language that must be used by all pathologists so as to be correctly understandable for all health professionals. The second challenge is a better integration capability of the pathologist with the other health professionals, in order to play his role in the choice of targeted therapies, in the assessment of the cost/benefit ratio and in the new debate which it is opening about the tissue banks.

The last challenge is a new relationship of the pathologist not only with the disease but with the sick, so as to retrieve some features of the "physician", usually neglected by a profession that rarely provides for a direct contact with the patients: no longer the pathologist of the dead but of the living!

The pathologist will also be involved in the problem of timing ("what kind of test before?") and in the problem of the aggregation ("When should we decide that an aggregation of little benefits for a large number of patients accounts more than a great benefit for fewer patients? For which patients?").

We want also to stress the involvement of the pathologists in the choice of the so-called "Target Therapies", through the management of innovative technologies for the cancer diagnostic, such as the molecular biology and the cytogenetic. The pathologist found a conflict between his duty as a citizen in a just society and as a doctor in relation to individual patients, and the temptation, as a researcher and scientist, to run tests only for his pride.

The task of the Scientific Society in this new scenario will then be to collect guidelines produced by a working group and to validate them through a dedicated commission, whose decisions should be taken on the basis of professionalism and expertise in the field; moreover, the Scientific Society should also verify the application of the guidelines in the different Pathology Departments, even keeping an eye on the safeguard of the professional ethics.

Finally, the main role of the Director of the Pathology Departments will be that of reorganize the availability of resources: harmonization of activities among the staff, sharing of resources and equipments and standardization of procedures.

Tumour findings during solid organ transplantation: The Verona Alert Experience

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Introduction. We present a 15-year overview of transplantation activity at University Hospital of Verona. Donors selection was performed in agreement with the National Guidelines established in 2003 by Italian National Transplant Centre. The guidelines identify three risk levels regarding infectious, neoplastic and other transmissible diseases and a “two-step ALERT” multidisciplinary evaluation for the donor risk assessment. First step ALERT 1 consists of pre-operative radiological and laboratory test integrated by clinical multidisciplinary examinations, based both on previous medical history both on emerged findings to investigate. Second step ALERT 2 consists of intra-operative surgical and pathological examinations based on macroscopical and microscopical evaluation of suspected lesions. Postmortem examination was performed routinely up until 2013 and it represents a “third step” of evaluation because it could reveal transmissible diseases undiagnosed at clinical and intra-operative assessment. All these tests are performed by a composite transplant team.

Methods. Neoplastic findings of the 506 patient donors in our transplant work-up were collected from 1999 to 2014. We assessed the overall neoplasms frequency classifying the tumors into benign, borderline and malignant lesions in the transplant setting. The diagnosis was established by histological examination of biopsy, surgical and autopsy specimens in all cases. Tumor types and anatomic location were recorded. A three-tier ALERT subdivision was defined according to the results of the pre-operative and intra-operative examinations: ALERT 1 + (AL1), ALERT 1/ALERT 2 + (AL1/2), ALERT 2 + (AL2). ALERTs values were evaluated for the overall neoplasms and for the three different categories of tumor’s biological behavior. Autoptic diagnosis percent was also estimated.

Results. Neoplastic findings of 506 patient donors were collected from 1999 to 2014. The donors were 275 male and 231 female with a mean age of 52 years and an age range from 32 to 72 years. The main cause of death was cerebral hemorrhage (51.8%) followed by head trauma (30.4%) and post anoxic encephalopathy (8.7%). The other causes of death (9.1%) comprises cerebral ischemia, cerebral primary malignancies, meningitis, pneumococcal encephalitis, acute hydrocephalus and sepsis. Over the whole fifteen years period 359 donors were used for transplantation, equal to 71% of reported potential donors. We detected 37 malignant neoplasms, 39 benign neoplasms and 2 neoplasms of uncertain behavior representing an overall neoplasm frequency of 15.4% (7.3% malignant; 7.7% benign; 0.4% uncertain). We diagnosed 11 different histotypes of malignancy, the most frequent represented by prostate acinar adenocarcinoma (n = 8; 22.8%) fol-

lowed by renal cell carcinoma (n = 7; 21.6%) including four clear cell renal cell carcinoma, two multilocular cystic renal cell carcinoma and one papillary renal cell carcinoma. Other relevant neoplasms diagnosed were four breast carcinomas (n = 4; 10.8%) with tree ductal and one lobular adenocarcinomas and four thyroid papillary/micropapillary carcinomas (n = 4; 10.8%). Leiomyomas were the most common benign neoplasms (n = 8; 20.5%), five arised from the uterus and one from the fallopian tube, displaced by vascular neoplasms (n = 6; 15.4%) five of the liver and one of the skin, thyroid adenomatous nodules (n = 4; 10.25%), renal angiomyolipomas (n = 3; 7.7%) and mammary fibroadenomas (n = 3; 7.7%). We encountered also two neoplasms of uncertain malignant potential, a serous ovary borderline tumor and an oncocytic renal neoplasm with variable cytological atypia and three types of malignant precursors (two prostate intraepithelial neoplasia, one urothelial dysplasia and two displastic nevus). AL1 positive cases were 31/78 (39.8%); among these, 64.5% were malignancies, 32.3% were benign neoplasms and 1 case (3.2%) was a borderline lesion. AL1/2 positive cases were 32/78 (41%); among these 28.1% were malignancies, 68.8% were benign neoplasms and 1 case (3.1%) was a borderline lesion. AL2 positive cases were 9/78 (11.5%); among these, 55.6% were malignancies and 44.4% were benign neoplasms. An autoptic diagnosis was performed in 6/78 cases (7.7%) all were malignancies (2 hepatocellular carcinomas, 2 invasive breast ductal adenocarcinomas, 1 thyroid papillary carcinoma and 1 large cell carcinoma of the liver).

Conclusions. Overall incidence of neoplasms finding during trasplantation was 15.4%: 7.7% benign, 7.3% malignant and 0.4% borderline. The strict application of the “two-step ALERT system” for the risk assessment of transmissible diseases permitted the diagnosis of 72/78 (92.3%) of the neoplastic lesions before transplantation with ALERTs percent distribution 39.8% (AL1), 41% (AL1/2), 11.5% (AL2). Six tumors (7.7%) escaped the “ALERTs protocol” and have been discovered during donor autopsies. Our protocol was designed to minimize the risk of undiagnosed transmissible diseases in the pre-transplant work-up. In this view the goal of the “ALERTs protocol” is to provide a useful tool for reduce the risk of donor/recipient tumors transmission. Prospective institutional and multicenter data are essential for the planning and standardization of more strict cancer control protocols in the pre-transplant screening activity.

The role of bone morphogenetic proteins in delaying the onset of sarcopenia

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Introduction. Age-related bone diseases, such as osteoarthritis (OA) and osteoporosis (OP), are strongly associated with sarcopenia and muscle fiber atrophy¹. Potential mechanisms involved in the reduction of skeletal muscle mass during sarcopenia converge on the failure of satellite cells in replacing and repairing damaged muscle fibers^{2,3}. Myostatin and Bone Morphogenetic Proteins (BMPs) are molecules able to regulate muscle mass homeostasis by activating satellite stem cells⁴. In this study, we investigated the role of BMP2,

BMP4 and myostatin in the patho-physiogenesis of sarcopenia related to osteoporosis and osteoarthritis.

Methods. Muscle atrophy, BMP2, BMP4, and myostatin expression were evaluated in 27 biopsies of osteoarthritic (OA) women and 27 biopsies from osteoporotic (OP) by immunohistochemical reaction. Muscle stem cells niches were investigated by ultrastructural analysis.

Results. We found that OA muscle biopsies showed a significantly higher number both BMP2 positive fibers ($62,79 \pm 6,205$) and BMP4-positive fibers ($37,35 \pm 5,63$) as compared with muscle of OP patients ($9,60 \pm 1,57$ and $13,92 \pm 3,343$). Unlike BMP2 and BMP4 expression, the number of myostatin positive fibers in OP patients ($33,95 \pm 4,10$) was significantly higher compared to OA group ($13,86 \pm 1,68$). The ultrastructural analysis of BMPs positive tissues displayed the presence of a high rate of satellite cells both single or as syncytium giving a proof of muscle regeneration capability.

Discussion. Our results clearly indicated that sarcopenia and osteoporosis shared an impairment of metabolic activity. Conversely, the higher expression of BMPs in OA patients seems to inhibit the onset of age-related sarcopenia.

Conclusion. The characterization of molecular mechanisms underlying the bone-muscle crosstalk could open new therapeutic perspectives in elderly diseases.

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Adrenal collision tumors: an unusual case of adrenocortical carcinoma and adrenal myelolipoma in the same gland

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Introduction. The evidence of myelolipomas or cortical neoplasm in the adrenal glands is not unusual. Conversely adrenal collision tumor (ACT) is an infrequently described tumor entity made by two adjacent, but histologically distinct tumors of the adrenal gland without a substantial histological admixture at the interface. The most frequent is the association of myelolipoma and adrenocortical adenoma. More rare is the association of myelolipoma with pheocromocytoma, hemangioma, metastasis and adrenocortical carcinoma. We report a case of an ACT composed of a myelolipoma and an adrenocortical carcinoma in a 54-years-old man, with morphological and immunophenotypical features. **Methods.** A 54-years-old man presented to the physician with abdominal pain. Ultrasonographic examination showed a heterogeneous hypodense solid mass of 60x40 mm at the level of the upper pole of the right kidney; computerized tomography scan of the abdomen also showed a right retroperitoneal mass of 6 cm with irregular contrast enhancement. The images were suggestive for adrenal tumor. All biological examinations were normal; serum cortisol, urinary metanefrin and normetanefrin were within normal limits, therefore the adrenal neoplasm was

not-functional. The patient was admitted to the Department of General Surgery of the Second University of Naples and a right adrenalectomy was performed. We received a specimen of g 95 and measuring cm 6x5x5; it was a bulky tumor mass showing, in cross section, a quite variegated and coarsely lobulated appearance, with soft bulging nodules ranging from yellow-orange to tan. Grossly, areas of necrosis were not observed. Histologically, the tumor showed mainly a diffuse and solid growth pattern, occasionally mixed with pseudocystic areas lined with atypical cells and filled with proteinaceous material. Cellular morphology was characterized by cells with lipid-depleted and compact acidophilic cytoplasm mixed with cells showing an abundant intensely eosinophilic and finely granular cytoplasm ("oncocyctic" appearance). The nuclei were round to oval with one or more prominent nucleoli (grade 3 according to criteria of Fuhrman) and with some large eosinophilic pseudoinclusion. Nuclear pleomorphism was also observed, consisting of enlarged and hyperchromatic nuclei, often hyperlobated or multiple. The stroma showed, focally, myxoid changes and lipomatous metaplasia. Clear cells and necrosis were not observed; mitotic index was low (<6 mitosis x 50 HPF) and atypical mitotic Fig.s were uncommon. A broad fibrous pseudocapsule surrounded the neoplasm and capsular invasion was not found; vascular invasion was observed, consisting of plugs of tumor within the lumen of large veins. The neoplastic cells showed immunohistochemical positive reaction for vimentin, CD56 and, focally, for MART-1 and synaptophysin and were negative for S-100 protein and chromogranin A. The proliferation index (Ki-67) was about 15%. Peripherally, near the neoplasm (at about 0.6 cm), a myelolipoma was also found. This lesion measured 1,8 cm and was typically characterized by trilinear mature hematopoietic elements in a background of mature fat cells. It was only histologically detected because of its small size and with insufficient macroscopic fat to allow detection by radiological imaging.

Results. On the basis of histological and immunohistochemical features a diagnosis of ACT composed by adrenocortical carcinoma and myelolipoma was made. The malignant potential of the adrenal cortical neoplasm was based on the presence of 4/8 Weiss criteria: presence of high nuclear grade, diffuse architecture, invasion of venous structures and absence of clear cells. The tumor weight and the proliferating index (Ki-67) were also evaluated. The morphological diagnosis of adrenal myelolipoma was confirmed by the pathognomonic immunohistochemical profile of the hematopoietic elements.

Conclusion. ACT is an infrequently described tumor entity. Many ACTs may be undetected because of the small size of one component or/and sampling error. Therefore, the possibility of an ACT should be considered dealing with a heterogeneous adrenal mass in order to appropriately sample the lesion. When detected, the major diagnostic problem is to exclude the malignancy in one or both the neoplastic component.

Molecular pathology screening strategy for the identification of colorectal and endometrial cancer related to Lynch syndrome

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Introduction. Lynch syndrome (LS), also referred to as hereditary non-polyposis colorectal cancer, is an autosomal dominant disease caused by a defect in one of the mismatch

repair (MMR) genes 1. LS is the most common hereditary colorectal cancer (CRC) syndrome, accounting for 1–3% of all CRC cases. In the female population affected by LS, the incidence of endometrial cancer (EC) equals that of CRC², and in many cases EC represents the sentinel form of neoplasia³. It is estimated that 3–4% of ECs arise in women carrying an inherited predisposing mutation.

The multidisciplinary group for the study of hereditary cancers at ULSS2 Veneto has a well-established interest in the diagnosis and molecular characterization of LS-related CRC. This group is now extending its interests to the screening of EC diagnosed by the Anatomic Pathology Unit with the objective of identifying patients at increased risk of LS for subsequent second-level molecular testing.

The aim of the present work is to describe the diagnostic algorithm adopted by the multidisciplinary group for the diagnosis and management of patients with clinical suspicion of LS. This algorithm focuses on the differences in the molecular characterization between colorectal and endometrial neoplasia.

Methods. Molecular characterization of LS-related tumours was performed as described below. Immunohistochemistry (IHC) staining of MMR protein was performed on formalin-fixed, paraffin-embedded tissue using the EnvisionFlex+ Visualization System (Dako). The primary antibodies were Anti-Human MutL Protein Homolog 1 (clone ES05-Dako), Anti-Human Postmeiotic Segregation Increased 2 (clone EP51-Dako), Anti-Human MutS Protein Homolog 2 (clone FE11-Dako), and Anti-Human MutS Protein Homolog 6 (clone EP49-Dako). Positive and negative controls were considered. Microsatellite instability (MSI) analysis was performed on DNA extracted from tissues comprising at least 80% of tumour cells using a QIAamp DNA FFPE Tissue Kit (Qiagen) by analysing a panel of five mononucleotide markers represented by NR21, NR22, NR24, BAT25 and BAT26. The amplified tumour DNA and the matching normal DNA derived from blood were analysed using capillary electrophoresis equipment and GeneMapper software. BRAF V600E mutation analysis was conducted using an Anti-EGFR MoAb Response (BRAF status) kit (Diatech Pharmacogenetics). MLH1 promoter methylation was analysed in DNA extracted from 5 µm EC sections using pyrosequencing technology; the DNA was treated with sodium bisulfite and amplified to assess the region proximal to the transcription initiation site located between nucleotides –248 and –178. Molecular characterization of MMR gene was conducted using the MLPA technique and MRC-Holland specific probe sets. Sequencing analysis of the coding sequence and flanking intronic regions was performed using exon-specific primers and BigDye Terminator v3.1 chemistry (Life Technologies) on an AB3130 Genetic Analyzer (Applied Biosystems Life Technologies). The sequences obtained were compared with the NCBI references.

Results and Discussion. Figure 1 presents the diagnostic algorithm adopted by the multidisciplinary group for the study of hereditary cancers treated at ULSS2 Veneto for the diagnosis and management of patients with a clinical suspicion of LS. Below, we comment on three main points by comparing the performance of the MMR IHC test, MSI analysis test, MLH1 promoter methylation analysis and EPCAM IHC test. The clinical criteria and computational models were used to identify patients with LS. However, the results of studies based on the MMR gene molecular test clarified that also using the most liberal clinical criteria (i.e., the revised Bethesda Guidelines) would mean that a substantial percentage of LS

patients would be missed. A universal screening strategy of all newly diagnosed CRC cases, based only on an IHC test of MMR protein, has been proposed, especially in the last period. We note that the abnormal expression of MMR proteins can be detected when a mutation causes the synthesis of truncated proteins or proteins that are quickly degraded by proteolytic intracellular systems.

Although most mutations of the MMR genes give rise to truncated proteins, the existence of missense mutations that can give rise to catalytically inactive but antigenically detectable proteins has also been documented. This false-positive IHC signal can be unmasked by MSI analysis, which may indicate a high-grade instability.

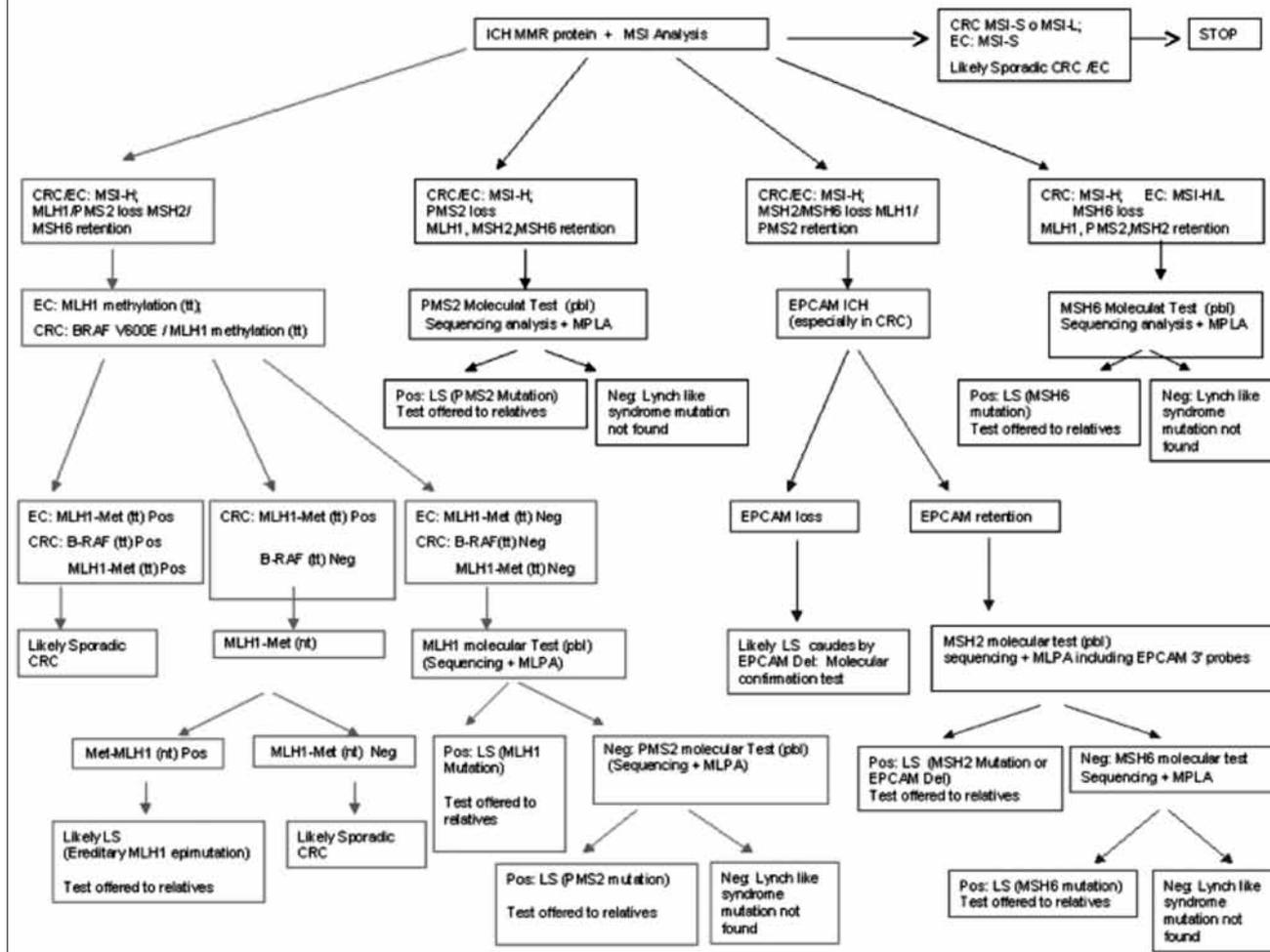
At the same time, a screening strategy based only on MSI analysis appears unsuitable because of low-grade instability, which might lead to the exit of the patient from the LS diagnostic algorithm. This may be associated with isolated loss of MSH6 because of a mutation in the corresponding gene, which is particularly common in LS-related EC. For the reasons mentioned above, we decided to perform both IHC and MSI analysis, which are considered complementary analyses, in all patients with newly diagnosed CRC who are aged <70 years, in patients with CRC who are aged ≥70 years old and have a family history of LS, and in all newly diagnosed patients with EC aged ≥65 years.

In cases of MLH1/PMS2 protein dimer loss, before proceeding to MLH1 gene molecular analysis, it is necessary to exclude the silencing of protein expression caused by somatic MLH1 promoter hypermethylation, which indicates a sporadic origin of the tumour.

As in CRC, MLH1 promoter hypermethylation is often associated with a BRAF mutation, and all CRC cases characterized by MLH1 protein loss and the MSI-H phenotype are tested for the BRAF V600E variant. By contrast, this is a rare event in EC and, for this reason, these tumours are subjected to the MLH1 somatic methylation test using a pyrosequencing assay developed specifically to analyse a region proximal to the transcription initiation site related to the transcriptional silencing of MLH1 gene⁴.

According to Newton and co-worker⁵, BRAF mutation in CRC cancer is closely but not exclusively associated with MLH1 methylation. This evidence prompted us to evaluate the future combined use of both the mutation and methylation test in CRC patients. In cases of CRC in which hypermethylation is detected but the BRAF mutation is absent, it will be interesting to analyse the MLH1 hypermethylation status in normal tissue, which may indicate that hypermethylation is a rare event in LS that reflects constitutional hereditary MLH1 epimutation rather than a germline mutation. MSH2/MSH6 protein loss can occur because of MSH2 germline mutation or because of 3' deletion in a neighbouring gene, EPCAM, which can lead to a transcriptional read-through and mediate epigenetic silencing of the MSH2 allele in a mosaic pattern in EPCAM-expressing cells. This event can be analysed by the MLPA MSH2 assay, which includes probes designed specifically to hybridize to the last exons of the EPCAM gene. A useful approach is represented by EPCAM IHC evaluation, in which a negative result implies the presence of a constitutional EPCAM deletion, which then excludes the need to perform MSH2 mutation analysis. Greater caution must be taken for EPCAM IHC-positive results, which may indicate a second-MSH2-inactivating hit not affecting the EPCAM gene⁶; in such cases, one cannot exclude the presence of a germline deletion in the EPCAM 3' region, which must be analysed by molecular assays.

Fig. 1. Lynch syndrome screening strategy adopted by the multidisciplinary group for the study of hereditary cancers treated at ULSS2 Veneto. (MSI-H, high-grade instability; MSI-L, low-grade instability; MSI-S, stable tumour; tt, tumour tissue; nt, normal tissue; pbl, peripheral blood leukocytes)



Conclusion. The diagnostic algorithm described here makes an important contribution to the diagnosis and management of LS. Identification of individuals with LS is relevant because this allows one to estimate the risk of the patient developing other forms of neoplasia belonging to the LS. This will allow these individuals to benefit from dedicated cancer surveillance programmes whose goal is the detection and treatment of premalignant lesions, which should improve the prognosis for patients. Moreover, efficient screening strategies based on accurate and updated molecular pathology techniques allow the identification of healthy family member carriers of the pathogenetic mutation, which was previously identified only in affected relatives, who could benefit from an early diagnosis or prevention programmes.

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Ion AmpliSeq™ RNA Lung Fusion Panel tested in a series of patients eligible to ALK inhibitor treatment

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Introduction. The knowledge of the molecular characteristics of non-small cell lung cancer (NSCLC) has led to the development of effective targeted therapies for personalized medicine in a subset of patients. The use of the tyrosine kinase inhibitors, gefitinib and erlotinib, was related to mutational status of Epidermal growth factor receptor (EGFR) and more recently the use of crizotinib was approved by the Food and Drug Administration (FDA) for the treatment of patients with locally-advanced or metastatic NSCLC with anaplastic lymphoma kinase (ALK)

gene rearrangements^{1,2}. The predominant partner in ALK fusion is Echinoderm Microtubule-Associated protein Like-4 (EML4) and several different EML4-ALK variants have been identified³. However other fusion partners of ALK, and rearrangements in other genes such as ROS1, RET and NTRK1, have been identified and studies are ongoing in order to better understand their functional implication in the sensitivity to crizotinib^{4,5}. Even if several methods can be used for the detection of abnormalities in these genes, Fluorescence In Situ Hybridization (FISH) assay using dual-labeled break-apart probes⁶ still remains the gold standard for EML4-ALK carrier identification. FISH assay is able to detect an ALK rearrangement but lacks of the ability to identify the fusion partner gene, a clinically important information in lung cancer. This limitation may be overcome by the use of next generation sequencing (NGS), a novel approach for genome wide studies. NGS is able to read DNA sequences simultaneously and in a parallel way, also allowing the detection of low frequency genetic variants. Moreover it is characterized by high speed, relatively low cost and high sensitivity and specificity^{7,8}.

Aim of this study is to test the possibility to use an NGS approach based on the Ion AmpliSeq™ RNA Lung Fusion Panel on Ion PGM Platform as a diagnostic tool for the identification of fusion transcripts implicated in lung cancer.

Material and Methods. Ten patients (6 Female and 4 male) with locally-advanced or metastatic NSCLC (8 adenocarcinomas and 2 squamous cell carcinoma) have been enrolled. All patients were previously tested for ALK rearrangement by break-apart FISH assay. RNA was isolated from formalin fixed paraffin embedded (FFPE) tissue using Ambion® RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE. 10 ng of total RNA for each sample were then processed using the Ion AmpliSeq™ Library Kit and the Ion AmpliSeq™ RNA Lung Fusion Panel. Panel is able to identify 72 known fusion transcripts in 4 acceptor driver genes (ALK, RET, ROS1 and NTRK1). Moreover the presence of 5' and 3' gene expression assays for the driver genes and of five expression control genes allowed us to obtain an imbalance value that can be used to assess the presence of an unknown translocation.

Quantified libraries were sequenced on Ion 316™ chip and data analyzed using the “AmpliSeq RNA Lung Fusion single sample” workflow integrated in Ion Reporter™ Software 4.2.

Results. A fusion event was detected in 3 of 10 samples. In particular: sample 2 presented a double EML4-ALK translocation EML4-ALK.E17A20 and EML4-ALK.E18A20; sample

3 showed the most common variant EML4-ALK.E13A20; sample 8 presented a novel variant HIP1- ALK.H28A20 previously reported only once in patients and in in-vitro models^{9,10}. We observed 100% concordance with FISH.

All sequencing runs were analyzed according to “gene fusion analysis guidelines” of Ion Reporter™ Software. A total of mapped reads per sample between 36.139 and 544.157 have been detected. Considering the imbalance value, that provides a measurement of the presence of a fusion, two translocated samples presented a positive imbalance value and the other one a negative value.

When a specific fusion is called with a read counts sufficiently higher than the threshold (20 reads), despite a negative imbalance value, the presence of translocation is confirmed (Tab. I). When we consider patients behaviour, our preliminary data confirm the presence of ALK rearrangement in non smokers, as previously described.

All translocated patients were treated with crizotinib, standard schedule, and to date computed tomography scans did not reveal any evidence of recurrence or metastasis in these patients. Patients will be followed up to investigate if there is an improvement in progression free survival between the different translocation variants of patient 8 and 2. This is important in particular for patients harboring HIP1-ALK because the response of this variant to ALK inhibitors still remain unknown. Patient 3 was lost to follow-up.

Conclusion. Ion AmpliSeq™ RNA Lung Fusion Panel was able to detect in a single reaction and with a low RNA input the most important fusion variants and fusion partners involved in NSCLC. NGS is able to define the right breakpoint region, novel rearrangements and unknown partners that could be involved in sensitivity to pharmacologic inhibition. The role of the novel HIP1- ALK.H28A20 variant, detected in this small series of patients, needs to be further investigated in order to define its involvement in the therapeutic setting.

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Tab. I.

Sample ID	Fusion Transcript identified with PGM	Read Counts	Total Mapped Fusion Panel Reads	3'/5' Imbalance
1	WT	/	169812	/
2	EML4-ALK.E17A20	601	544157	0,0633
	EML4-ALK.E18A20	21808		
3	EML4-ALK.E13A20	30	57003	0,0117
4	WT	/	177885	/
5	WT	/	376608	/
6	WT	/	505851	/
7	WT	/	36139	/
8	HIP1- ALK.H28A20	86091	526293	-0.0185
9	WT	/	524465	/
10	WT	/	414970	/

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Field cancerization detected in oral brushings from oral mucosa after surgical resection by quantitative DNA methylation using bisulfite Next Generation Sequencing

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Background. Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and it poses a significant public health problem due to its impact on the speech, mastication, taste, swallowing and aesthetics. Despite major progress, the 5-year survival rates is around 60%, a value that remained unchanged during the past three decades. The poor survival rate has been ascribed to a high frequency of locoregional recurrences and the majority of patients present advanced stages of OSCC at the time of diagnosis (two thirds are stage III-IV). The most important prognostic indicator for relapse of OSCC is the presence of metastatic spread to lymph nodes in the neck. In this case, the incidence of distant metastasis can be as high as 50% with worse prognosis and reduced survival rate. These conditions makes it imperative to diagnose early the disease and to identify those patients with worse prognosis to facilitate appropriate therapeutic management to reduce the morbidity and mortality. An important issue in oral carcinogenesis is that carcinomas develop within large preneoplastic field of mucosal epithelium with genetically and epigenetically altered cells, which often extend into the surgical margins when tumors are excised, and then can cause local recurrence and second primary tumors ¹. It has also been shown that histologically normal tissue adjacent to tumours and oral potentially malignant lesions can have an aberrant methylation pattern in candidate genes, suggesting that this kind of epigenetic modifications are early events in oral carcinogenesis. In OSCC various gene promoters were identified previously to show an aberrant methylation pattern related to early diagnosis ²⁻⁶, and prognosis ⁷. Clinically, these epigenetic events have been associated with tumor aggressiveness, invasiveness and with the malignant transformation

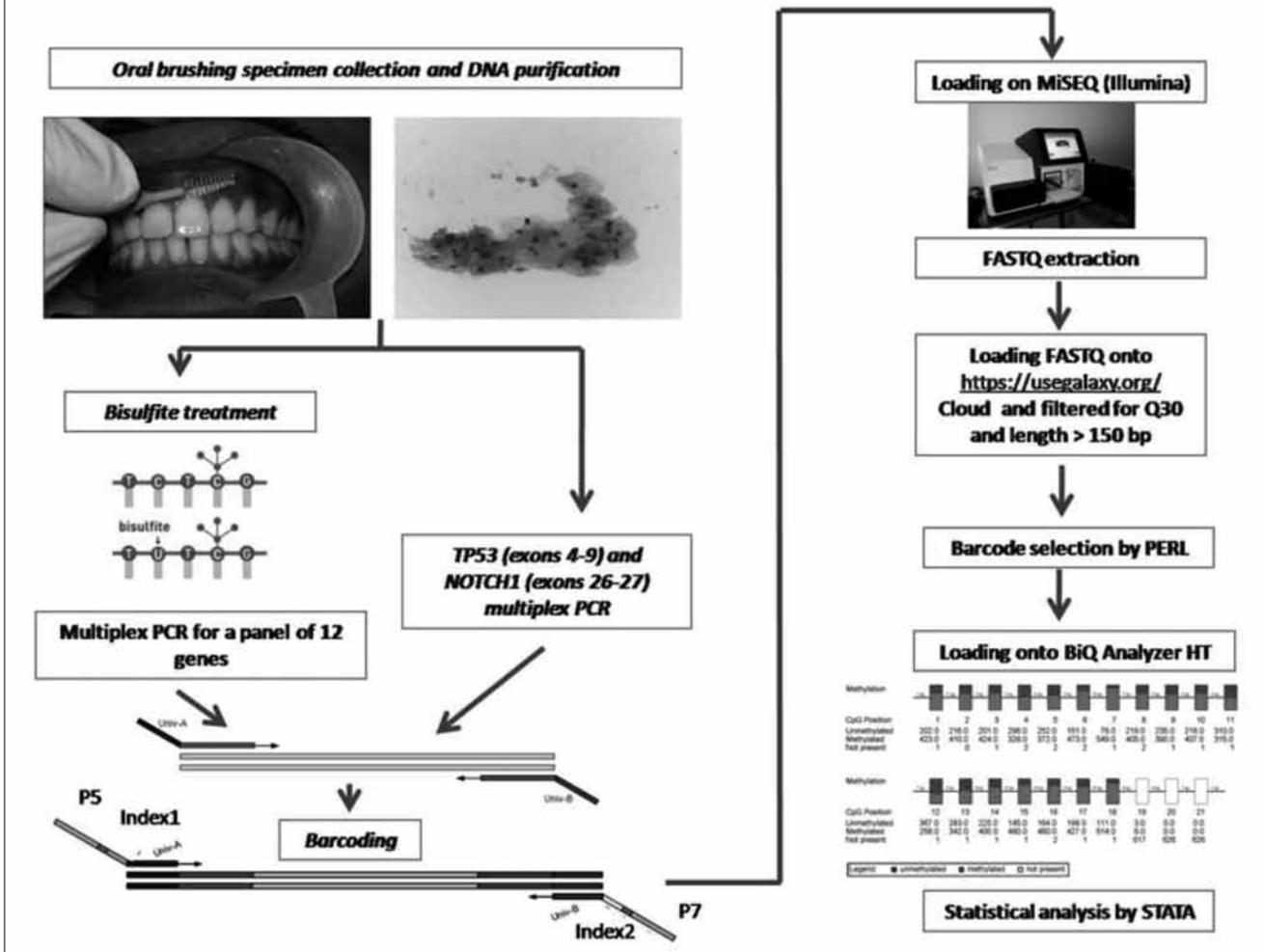
of High Grade Squamous Intraepithelial Lesion (HG SIL) ⁸. The fast development of Next Generation Sequencing (NGS) methods, has brought new opportunities to the wide usage of the bisulfite sequencing method for genome-wide and single gene promoter DNA methylation analysis. This method, respect to other most used such as qMSP that interrogate only a few CpG (usually 2-4), may evaluate in parallel a wider set of CpG increasing the power of analysis. Aim of the present study was to analyze the DNA methylation pattern in a set of candidate genes, evaluating oral brushing specimens collected from oral mucosa in the site of surgical resection after intervention to characterize the presence of field cancerization.

Methods. We collected oral brushing specimens from the following groups: 7 healthy donors (group 1); 5 OSCC (group 2a) and their normal mucosa counterpart (group 2b); 5 normal mucosa in the site of surgical resection with no morphological evidence of lesion (group 3a) and coupled normal mucosa collected in the opposite site of mouth (group 3b); 4 High Grade Squamous intraepithelial Lesion (HG-SIL) in the site of surgical resection (group 4a) and their normal counterpart (group 4b). PAP smear evaluation was done to confirm the presence of lesional cells within the brush. DNA was purified and bisulfite treated. A set of previously described differentially methylated genes in OSCC ⁹⁻¹⁰ (EPHX3, FLII, GP1BB, KIF1A, LINE-1, LRRTM1, MIR124, MIR137, MIR193, NTM, ZAP70, ZNF382) were investigated by bisulfite-Target NGS using MiSEQ platform (Illumina, San Diego, CA). FASTQ files were extracted and loaded onto galaxy cloud (<https://usegalaxy.org/>) and filtered for quality (Q30) and length (> 150 bp). Reads were then loaded on BiQAnalyzer HT (biq-analyzer-ht.bioinf.mpi-inf.mpg.de/). Each CpG ratio value (C/T) was then processed in R and the statistical significance between different groups was investigated by STATA using Wilcoxon-Mann-Whitney test as follows: group 2a vs 2b; 2a vs 1; 3a vs 4a; 3a+4a vs 3b+4b; 3a+4a vs 1. Significance was assumed when the statistical tests returned P-values <0.05. TP53(exons 4-9) and NOTCH1 (exons 26-27) were also investigated by the same NGS Platform. Fig.1 summarize the entire assay.

Results. Comparing OSCC with their coupled normal mucosa (group 2a vs 2b), we identified 8 out of 12 genes with various altered CpGs. Moreover comparison between OSCC (group 2a) vs group 1 (normal healthy donors) revealed a statistical significance in all genes in most of CpGs evaluated. On the contrary, no differences in any of the 12 interrogated genes were found between the group 3a (site of surgical resection after intervention with no morphological evidence of lesion) vs group 4a (the site of surgical resection after intervention with HG-SIL). Due to this, we decided to combine 3a and 4a, as they belong to the same population. We then performed the Mann-Whitney comparison test between 3a+4a group vs 3b+4b (coupled normal mucosa of the same patient collected from the opposite site of mouth): we found 6 genes informative for this purpose. All genes except one (FLII) resulted informative comparing group 3a+4a vs group 1 (normal healthy donors). TP53 and NOTCH1 mutation analysis revealed the presence of non synonymous variants in cases from the group 2a, 3a and 4a.

Conclusions. In our preliminary results, bisulfite target NGS analysis of a set of 12 genes from oral brushing specimens, allowed to discriminate OSCC from normal contralateral mucosa, and OSCC vs normal healthy donors with much more CpGs involved. The early diagnosis of OSCC with high risk of progression using a non-invasive/low-cost method could select patients that can benefit of more aggressive treatment

Fig. 1. Flow chart of the bisulfite target NGS assay for 12 gene promoters:



protocols or intensive follow-up modalities. We identified an aberrant methylation pattern even in all patients in the site of surgical resection after intervention with no morphological evidence of lesions, in addition to patients with HG-SIL. These epigenetic modifications revealed the presence of a cancerization field independently from the presence or absence of HG-SIL. This altered field was confirmed by non synonymous TP53 and NOTCH1 mutations¹¹. A close follow up program with these patients may detect earlier possible local recurrence.

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Giovedì, 24 Settembre 2015

Aula Gialla 3 ore 12.00-13.00

Miscellanea 2

Moderatore: G. Dell'Antonio (Milano)

Immunohistochemical assessment of AURKA expression in ovarian serous carcinoma may be a new prognostic tool for therapy

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Ovarian Serous carcinoma constitute the predominant histological subtype of ovarian malignant epithelial tumors. The high grade form is included in the Type 2 tumors (high grade serous tumor, high grade endometrioid tumor, carcinosarcomas and undifferentiated carcinomas), that are typically characterized by poor prognosis and rapid progression. These tumors are frequently in advanced stadium and platinum treatment is request. The clinical and pathological parameters are often unable to provide a reliable outcome prevision, for these reasons it's essential to look for new prognostic markers. Despite the same histotype and same advanced stadium not all the patient are respondent to platinum treatment. In this retrospective study, an immunohistochemical evaluation for AURKA was performed on 30 cases of ovarian serous carcinoma, subdivided in two groups: platinum-sensitive and platinum resistant patients. A specific grading system for the evaluation of the immunohistochemical results was designed. From the results it appeared that AURKA positivity (with score ranging from 6 to 8) in our series of patients it's able to discriminate significantly platinum-sensitive patients from platinum-resistant patients. The reported findings suggest AURKA as a new sensible tool to evaluate the biological behavior of ovarian high grade serous carcinoma, in order to assess the individual prognosis and the potential efficacy of platinum treatment.

Morphological features of rectal endometriosis and radiological correlations

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Introduction. Endometriosis represent an important issue for women's health and many questions on the pathogenesis and treatment remain open. Symptoms are often vague with consequent delays on diagnosis and treatment. Rectal endometriosis is highly debilitating and may require invasive surgical treatments including segmental bowel resection. Particular attention should be paid on the subtle histopathological lesions of the affected bowel in order to plan the most effective treatment. It also constitute an ideal model for the comparison of

histological findings with the most innovative diagnostic imaging techniques. The morphological features of rectal endometriosis on segmental resection specimens are investigated and compared with the radiological findings of preoperative multidetector computerized tomography combined with the distention of the colon by rectal enema (MDCTe)

Materials and methods. 24 consecutive patients were eligible for the study. Patients underwent MDCTe and then underwent laparoscopic segmental resection for bowel endometriosis. All the surgical specimens were routinely fixed and processed. All the histological sections were reviewed, additional slides were obtained from the most significant paraffin block and stained with Masson Trichromic stain and immunohistochemistry for Smooth Muscle Actin (SMA). A series of morphologic parameters were evaluated, entered on spreadsheet and compared with radiologic findings.

Results. The mean (\pm SD) age of the study population was 36,4 \pm 5 years. In most cases, endometriosis infiltrated the muscle layer of the rectum (submucosa:7/24; muscularis propria:16/24; adventitia:1/24); the lesions were mainly diffuse (diffuse:19/24; focal:5/24) and had mixed histologic architecture (mixed: 12/24; macrocystic: 3/24, microcystic: 7/24, adenomyosis-like: 2/24) and most of them were well-differentiated glandular type (accordingly to Abrao et al.). Patients with macrocystic architecture more frequently showed isolated foci (3 cases). The foci of endometriosis showed a poor or average amount of endometrial stroma (G0:0/24; G1:11/24; G2:11/24; G3:2/24), perilesional fibrosis was unexpectedly mild and occasionally abset (G0:3/24; G1:11/24; G2:7/24; G3:3/24), the endometriotic lesions were constantly accompanied by a variable degree of disarray of the muscle fibers, generally graded from moderate to severe (G0:1/24; G1:9/24; G2:7/24; G3:7/24). In the majority of cases we observed perilesional amputation neuromas (21/24). Patients with pure microcystic architecture more frequently showed a greater degree of disarray of the muscle fibers. Comparing the histopathological features with the radiological data patients with pure macrocystic architecture showed a residual lumen significantly reduced when compared with patients with patients with pure microcystic architecture (mean: 2.33 mm vs. 7.2 mm, $p = 0.044$). Patients with mixed architecture showed intermediate characteristics (average 6.35 mm). The patients with a mild degree of disarray of the muscle fibers (G0-G1) showed a narrower diameter of the residual lumen if compared to patients with a more severe disarray (G2-G3) (mean 3.9 mm vs. 7.41 mm; $p = 0.057$).

Conclusions. Rectal endometriosis showed morphological features somehow more similar to adenomyosis uteri rather than to ovarian endometriosis. These findings seem to support the dis-embryogenetic theory (Mullerian secondary system) rather than the retrograde menstruation theory. The intestinal symptoms complained by the patients, often indication for surgery, seem to be more related to functional rather than to anatomical problems. No case of stenotic scar was observed; instead we often documented a disruption, even severe, of the microanatomy of the muscle layer of the rectum. The documented morphological

alterations seem to support segmental surgery that removing the entire affected area allow to restore a proper bowel function. Only a limited proportion of patients with deep endometriosis may benefit from a less radical surgery showing an illness more similar to ovarian endometriomas with superficial macrocysts and reduced disarray of the muscle fibers.

EGFR mutations in circulating tumor DNA of lung adenocarcinoma patients

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Background. Adenocarcinoma lung patients with EGFR sensitizing mutations receiving targeted agents develop resistance. Proof of concept exist that circulating tumor DNA (ctDNA) could be used for the diagnostic molecular characterization and follow-up of cancer patients. Here we report the analysis of a series of matched tissue and plasma DNAs of 16 lung adenocarcinoma patients.

Methods. DNA from matched microdissected cancer tissue and plasma (6 ml) of 16 patients with advanced lung adenocarcinoma was assayed for EGFR mutations using next-generation sequencing (NGS) on Ion Torrent (Life Technologies) to sequence the coding regions harboring hotspot mutations of the gene and digital PCR (Bio-Rad) for three EGFR mutations (746-750del, L858R and T790M). Descriptive statistics with 95% confidence intervals (CI) was used.

Results. Analysis of cancer tissues revealed that 6 of 16 cancers had EGFR mutations using both NGS and digital PCR analysis: five 746-750del and one L858R. The five deletions were also shown in plasma DNA by digital PCR while only three were detected by NGS. The L858R was not detected in plasma DNA. The 6 patients with EGFR mutations were treated with erlotinib and 5 of these were shown to harbor the EGFR T790M mutation by digital PCR, which was undetected in the original cancer tissue; EGFR T790M mutation was found in 5 of 7 stage IV adenocarcinomas and never in patients at lower stages.

Conclusions. These data confirm that ctDNA holds important information for cancer diagnosis and disease monitoring. However, the question remains whether plasma DNA completely mirrors the genetic footprint of the primary cancer. Contradicting between digital PCR and NGS may originate from the different sensitivity of the 2 techniques requiring validation before clinical application.

Malignant pleural mesothelioma: evaluation on pathology reporting

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Introduction. Several articles have focused on practical guidelines for the pathological diagnosis of malignant pleural mesothelioma (MPM) and on protocols for the pathologic reporting. The aim of this study was to verify the reporting of MM and use of immunohistochemistry (IHC) in the practice of pathology, taking advantage of a population-based mesothelioma registry.

Methods. The source of cases was the mesothelioma registry of Lazio-Italy (6 million people; 2001-2014) and also a pathology-based mesothelioma archive (PathMA: 1980-2000). We reviewed 819 pathology reports as typewritten out in full by pathologists. We evaluated the report content, completeness and the health system context wherein diagnoses were formulated, and whether and how pathologists follow the recommendations of the literature. A study-specific dataset was devised. All pathology information (tumor site; type of specimen; histology; IHC workup) was manually entered in the dataset; data were processed via the statistical software IBM SPSS. It was investigated the approaching to recent guidelines. Reports were measured on the compliance to recent recommendations.

Results. Reports refer to 753 histological diagnoses mainly based on pleural biopsies (n=677, 89.9%) with IHC confirmation (n=599, 88.47%), 66 cytological diagnoses based on pleural effusions (87.9%) with IHC confirmation (77.5%), and 3 autopsies. A total of 60.2% were epithelioid tumors. Sarcomatoid tumors (9.3%) were more frequent than biphasic tumors (7.7%); both variants were never diagnosed by cytology. The microscopic type was not specified in 187 reports (23%). IHC results were available for 705 reports (86%). There is variability in number of antibodies (up to 56 types), in the number of diagnosis per pathologist (median 5), and in the choice of IHC markers. The mean number of antibodies for each diagnosis was 5.89 (range 1-23). The mean number of markers tested by epithelioid (6.01), biphasic (7.02), sarcomatoid (6.39) and unspecified histology (4.83) was significantly different (p<0.000; Kruskal Wallis test). The most used markers were calretinin (82.3%, 93.3% of positivity), TTF-1 (63.4%, 0.9% of positivity), CEA (57.6%, 3% of positivity), pan-cytokeratin (47.4%, 98.2% of positivity) and cytokeratin 5/6 (45.3%, 88.1% of positivity). The combined use of 2 or more carcinoma markers and 2 or more mesothelioma markers reached the 59% for epithelioid MPM. 120 pathologists were involved: 59.2% reported between 1 and 2 diagnoses; 32.5% between 3 and 19 diagnoses; 9 (7.5%) reported more than 20 diagnosis, and a single pathologist (0.8%) performed 160 diagnoses. 114 reports (14%) were diagnoses without IHC.

Conclusions. From our data emerge some critical issues: lack of completeness for relevant data items (laterality, histology classification, information on diagnostic procedures, morphologic coding, ambiguous terminology in the diagnosis field). The free-text format makes the interpretation nonobjective, sometimes even cryptic. A little bit more of 50% of reports regarding epithelioid MPM come close to international/national IHC guidelines (Arch Pathol Lab Med. 2013;137(5):647; Am J Clin Oncol. 2011;34(1):99). It is necessary to standardize the pathologic reporting of MPM, so far not implemented among pathologists in Italy, for the accurate interpretation of diagnoses, therapeutic/prognosis aims and medico-legal implications.

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia with Aguayo-Miller Syndrome: report of a case

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Introduction. Diffuse intrapulmonary neuroendocrine cell hyperplasia (DIPNECH) is a rare, potential precursor le-

sion to typical pulmonary carcinoid tumors. Fewer than 50 cases have been reported in the literature. Their pathogenesis, clinical significance and management is still controversial. Aguayo and Miller in 1992 described a few patients presented with DIPNECH associated with interstitial diffuse pulmonary disease. Here we describe a further case of DIPNECH with interstitial pulmonary disease.

Materials and methods. clinical charts, radiologic and histopathological findings of a patient with Aguayo-Miller syndrome were reviewed. The clinicopathological features of the present patient are discussed and compared to those of similar reported cases.

Results. A 60-year-old woman presented with progressive exertional dyspnea, cough and limited exercise tolerance. She had no history of smoking, or of occupational or other infectious exposure. Additional work-up with thoracic computed tomography (CT) scan revealed mosaic pattern with tiny bilateral indeterminate subcentimetric pulmonary nodules. Bronchoscopy with transbronchial biopsy was performed, but it resulted inconclusive. The patient underwent two wedge resections of the middle lobe of the right lung: the histological findings revealed a diffuse proliferation of small cells, without atypia, located in the airway walls and forming nodular aggregates that tested positive for neuroendocrine markers (chromogranin and synaptophysin). There was concomitant fibrosis, accompanied by constrictive obliterative bronchiolitis. The diagnosis was of DIPNECH associated with Aguayo-Miller syndrome.

Nine years later, the patient underwent a second wedge resection of the lower lobe. This time histopathological examination revealed two well-differentiated neuroendocrine neoplasms of 0.7 cm and 0.5 cm in size in the right lower lobe classified as typical carcinoid tumors. The histological findings showed a proliferation of ovoid to spindle cells arranged in nests and trabeculae with a very low proliferation index (<1 mitosis/10 high-power fields) and no necrosis. There were multiple peribronchiolar and subpleural foci of neuroendocrine cell proliferation exhibiting linear to micronodular subepithelial growth, as well as tumorlets (0.1 cm to 0.4 cm). There was mild septal fibrosis, associated to multifocal obliteration of bronchiolar lumina and mild chronic inflammatory infiltrate. The diagnosis was of two typical carcinoid tumors associated with diffuse intrapulmonary neuroendocrine cell hyperplasia (DIPNECH) and Aguayo-Miller syndrome. The present case was reviewed in thoracic oncology multidisciplinary rounds. The consensus was no adjuvant therapy but continued surveillance with clinical assessment and thoracic CT.

Conclusion. DIPNECH is being increasingly recognized, probably because of an increase in the usage and accuracy of investigative imaging and increased awareness of the entity. Most cases remain stable over many years independent of the mode of presentation, although a few patients progress to severe airflow obstruction. Owing to its rarity, the optimal management of DIPNECH remains poorly defined. Long-term surveillance is generally accepted to monitor for progression to typical carcinoid tumor. However, optimal time intervals for surveillance and the preferred imaging modality is as yet unclear. With respect to the symptoms of obstructive airway disease, inhaled bronchodilators or steroids may prove to be beneficial. The role of surgery in the management of DIPNECH is controversial and, in existing case series, has been limited to patients who develop an associated carcinoid tumour. Given the potential significant morbidity of DIPNECH and its possible neoplastic

transformation, it is important to understand and recognize this rare entity.

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Ki-67 expression and KRAS mutations: prognostic biomarkers for non-small cell lung cancer (NSCLC)

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Background. In non-small cell lung cancer (NSCLC) prognostic/predictive role of KRAS mutations is continuously debated. KRAS mutation subtypes or potential co-vulnerability with other biomarkers may explain controversial results. When mutations or loss of p53 or loss of LKB1 occur, self-sufficiency of growth signals increase and in association with KRAS mutations NSCLC phenotype seems to be more aggressive.

The use of Ki-67 immunostaining in lung cancer is not easy to apply and the feasibility of Ki-67 in specimens of small size like the endoscopic bronchial biopsies is still controversial. Some studies indicated that patients with a higher Ki-67 score (>25% positive cells) at diagnosis had a significantly lower disease-free survival and was associated with unfavourable clinical outcome.

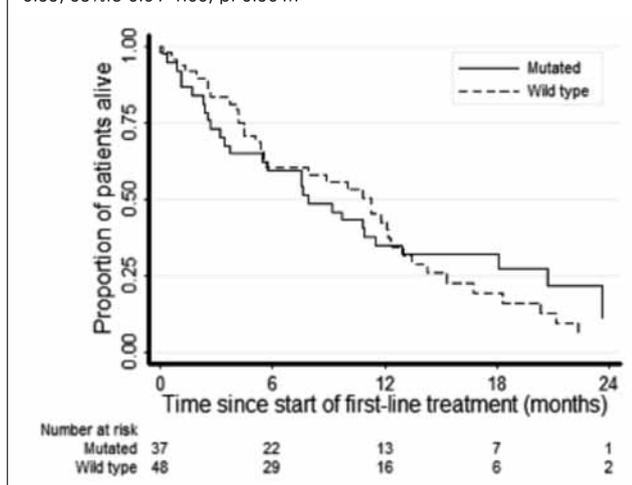
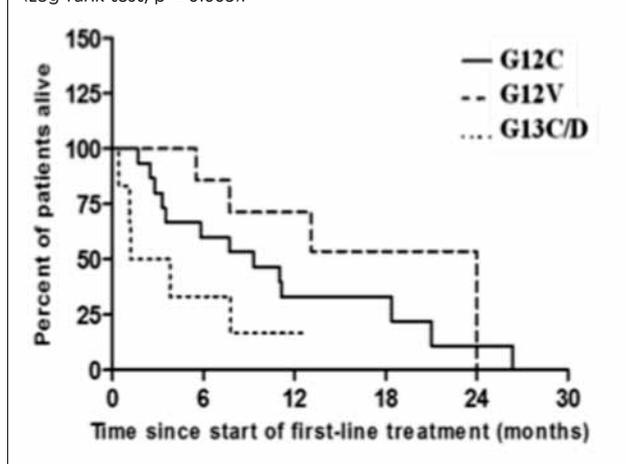
Levels of Ki-67 expression as surrogate biomarker of unchecked growth and prognostic impact of KRAS mutations have been investigated.

Patients and methods. We retrospectively analyzed consecutive patients (pts) with advanced NSCLC treated with a platinum-based chemotherapy at the Department of Oncology of Udine from June 2012 to June 2014. Ki-67 has been assessed by immunohistochemistry and reported as Ki-67 average (Kia) and Ki-67 hotspot (Kih) values. Formalin fixed and paraffin-embedded (FFPE) sections were stained with Ki-67 monoclonal antibody (Mib-1 clone, 1:200; Dako) on Dako Autostainer Link48; antigen retrieval was conducted in citrate buffer at pH 6 in PT Link instrument and Envision Dual Link Kit (Dako) was used as visualization system with diaminobenzidine as chromogen and hematoxylin as counterstain. Ki-67 labelling index was obtained using an automated cellular image analysis system with a validated nuclear algorithm (Aperio, Leica Biosystems). Slides were scanned at 20X magnification using the Aperio Scanscope Console and the images were analyzed using ImageScope Nuclear algorithm software that reports the total number of nuclei counted in each selected areas and the percentage of positive cell nuclei. All tumor areas in each slide were manually marked.

KRAS/EGFR mutational status was evaluated by Agena MassARRAY® Platform with Myriapod® Cancer Status

Tab. I. Ki-67 values (median and hotspot) according to KRAS status and histological subtype (*Min-max: range minimum-maximum).

	KRAS status			
	KRAS mut (N=37)		KRAS wild-type (N=48)	
Ki-67(%)	Median	Min-max*	Median	Min-max*
Ki-67 average (Kia)	41	14-83	46	8-80
Ki-67 hotspot (Kih)	55	19-87	64	16-89
	Histotype			
	Adenocarcinoma (N=57)		Squamous cell carcinoma (N=23)	
Ki-67(%)	Median	Min-max*	Median	Min-max*
Ki-67 average (Kia)	37	8-83	57	29-80
Ki-67 hotspot (Kih)	52	16-89	73	49-88

Fig. 1. Kaplan-Meier curves for overall survival (OS) between KRAS mutant (continuous line) and KRAS wild-type (dashed line) groups (HR 0.99, 95%CI 0.61-1.60, p: 0.961).**Fig. 2.** Kaplan-Meier curves for overall survival (OS) among main KRAS mutant subtypes (G12C, G12V and G13C/D mutations). Median OS for G12C, G12V and G13C/D was 9.3, 24 and 2.5 months, respectively (Log-rank test, p = 0.005).

kit (Diatch pharmacogenetics) according to manufacturer's instructions on DNA extracted from either cytologic or small biopsy sample, depending on the amount and percentage of neoplastic cells available.

ALK rearrangement was performed by Fluorescent In Situ Hybridization (FISH) on FFPE sample or cell-block preparation with ALK Break Apart FISH Probe kit (Abbott).

Higgins index (I2) was used for evaluating Ki-67 heterogeneity. Cox model for progression-free survival (PFS) and overall survival (OS) and logistic model for disease control rate (DCR) were used for univariate analyses. Survival curves were estimated with Kaplan-Meier method.

Results. Eighty-five out of 227 screened pts have been considered for analysis: 48 were KRAS/EGFR wild-type/ALK not rearranged (Raswt) whereas 37 were KRAS mutated/EGFR wt/ALK not rearranged (Rasmut). Median age of all pts was 66 years (range: 38-83). The majority of pts were male (62 pts, 73%), smokers/ex-smokers (77 pts, 91%) and had NSCLC adenocarcinoma histology (57 pts, 67%). KRAS mutations occurred in 44% of pts; G12C (15 pts, 41%), G12V (7 pts, 19%) and G13C/D (6 pts, 16%) were the most frequent detected mutations.

Regarding to KRAS status, median values of Kia and Kih were 46% and 64% in Raswt and 41% and 55% in Rasmut group, respectively. According to histology, Kia and Kih median values were 37% and 52% for adenocarcinoma and 57% and 73% for squamous carcinoma, respectively. (Table I) Pts in Raswt group obtained 74.5% of DCR compared to 55.6% in Rasmut group (OR 2.43, 95%CI 0.93-5.88, p=0.07);

median PFS was 4.9 and 4.5 months (HR 0.88, 95%CI 0.55-1.39, p=0.58) while OS was 11.3 and 8.0 months (HR 1.01, 95%CI 0.62-1.64, p=0.96), in Raswt and Rasmut group, respectively (Fig. 1).

Among common mutations KRAS G13C/D subtypes had a worse prognosis compared to other mutations on codon 12 for PFS (Log-rank test, p=0.03) and for OS (Log-rank test, p=0.005) (Fig. 2).

Similar results have been found between KRAS G13C/D mutations and KRAS wild-type groups for PFS (HR 0.28, 95%CI 0.08-0.96, p=0.04) and OS (HR 0.25, 95%CI 0.06-0.98, p=0.04). No interaction was detected among KRAS status, Kia or Kih (data not shown). Ninety-five percent of pts had an I2 greater than 69.5%; DCR, PFS and OS improved increasing I2 value. (Tab. II).

Tab. II. I2 and patients outcomes. (*OR and HR estimate for each 10% increase of I2 index).

DCR			
	OR	95%IC	p
I2*	1.46	0.98-2.16	0.061
PFS			
	HR	95%IC	p
I2*	0.84	0.72-0.97	0.017
OS			
	HR	95%IC	p
I2*	0.83	0.71-0.96	0.014

Conclusions. The study did not show any statistical interaction between all KRAS mutant pts and Ki-67 expression. Larger controlled studies are needed to confirm the absence of interaction, and to validate the prognostic role of each KRAS mutation subtypes. To the best of our knowledge this is the first study that investigate with this statistical approach the role of heterogeneity of Ki-67 in predicting outcome of NSCLC pts. Intriguingly, results for high Ki-67 heterogeneity and for prognostic relevance of I2 values needed to be verified in further studies.

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Carbonic anhydrase IX is a marker of hypoxia and correlates with higher Gleason scores in prostate cancer

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Background. Carbonic anhydrase IX is a member of α -carbonic anhydrases that is preferentially expressed in solid tumors. It enables bicarbonate transport across the plasma membrane, neutralizing intracellular pH and conferring to cancer cells a survival advantage in hypoxic/acidic micro-environments. Overexpression of CA-IX in cancer tissues is regulated by HIF-1 α mediated transcription, and the enzyme is considered a marker of tumor hypoxia and poor outcome. The role of CA-IX in prostate cancer (PCa) has not been fully clarified and controversy has arisen on whether this enzyme is overexpressed in hypoxic PCa tissues.

Methods. We analyzed the expression of CA-IX in two PCa cell lines, LNCaP and PC-3 by immunocytochemistry and Western blotting, and on 30 non-neoplastic specimens and 110 cancer biopsies from high grade prostatic intraepithelial neoplasia to Gleason score 6 to 10. Then we correlated the level of expression of CA-IX and HIF-1 to the Gleason pattern and score by applying chi-square test and k statistics with a p < 0.05 considered statistically significant.

Results. We found that CA-IX was mostly cytoplasmic/nuclear, with very limited membrane localization in both cell lines. We also observed that the intensity of the staining was higher in PC-3 cells, both under normoxia and hypoxia. Interestingly, in response to hypoxia, the staining became stronger and the protein was expressed intensely in the nucleus. When we analyzed CA-IX expression in human PCa biopsies we found that protein staining positively correlated with HIF-1 α and with Gleason pattern and score. Once more, CA-IX was mainly cytoplasmic in low grade adenocarcinomas, whereas in high grade tumors it was strongly expressed in the nucleus of the neoplastic cells. No staining was detected in non-neoplastic specimens for both antibodies, except for 8 samples with atrophy showing a low level of CA-IX expression. Stromal and endothelial cells intermingled with non-neoplastic glands demonstrated significant CA-IX positivity. **Conclusions.** There is a statistically significant association between CA-IX and HIF-1 α expression in PCa tissues that identifies the enzyme as a reliable marker of tumor hypoxia. In addition, there is a positive correlation between CA-IX expression and Gleason score. The nuclear translocation of the protein in PCa cells may epitomize a biological switch that stirs the tumor towards a worst outcome. Cytoplasmic/nuclear-bound of CA-IX may display previously unrecognized, alternative biological functions that should be considered in order to design novel anti-cancer therapies targeting the enzyme.

Genetic deletion of osteopontin skews spontaneous prostate cancer development towards aggressive human-like neuroendocrine cancers

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Osteopontin (OPN) is a secreted glycoprotein that belongs to the non-structural extracellular matrix (ECM). Its overexpression in human prostate cancer has been associated with disease progression, androgen independence and metastatic capability. Nevertheless, the pathophysiology of OPN in prostate tumorigenesis has never been studied. We crossed Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice with OPN deficient (OPN^{-/-}) mice and followed tumor onset and progression in these double mutants. Ultrasound examination detected the early onset of a rapidly-growing, homogeneous and spherical tumor in about 60% of OPN^{-/-} TRAMP mice. Such neoplasm seldom occurred in parental TRAMP mice otherwise prone to adenocarcinomas and were characterized for being androgen receptor negative, highly proliferative and endowed of neuroendocrine (NE) features. Gene expression profiling showed up-regulation of genes involved in tumor progression, cell-cycle and neuronal differentiation in OPN-deficient versus wild type TRAMP tumors. Down-regulated genes included key genes of TGF β pathway, including SMAD3 and Filamin, which were confirmed at the protein level. Furthermore, NE genes and particularly those characterizing early prostatic lesions of OPN-deficient mice were found to correlate with those of human prostate NE tumours. These data underscore a novel role of OPN in the commitment to a NE fate of prostate carcinomas.

Large cell calcifying sertoli cell tumor of the testis. Report of a case of this rare neoplasm

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Introduction. Large cell calcifying Sertoli cell tumor (LCCSCT) is a rare neoplasm of the testis originating from sperm cord cells. These tumors are usually benign and unlikely proceed to metastasis formation. The lesions may occur in an isolated form or may be associated with multiple neoplasia syndrome and Peutz-Jeghers syndrome (PJS) and Carney complex (CNC) are present in 40% of reported cases. We report a case of LCCSCT of the testis in a 13-year-old boy diagnosed at first on frozen section examination and successfully treated with testicle-sparing tumorectomy.

Methods. A 13-year-old boy was found during a self-examination to have a hard nodule in the right testis. The patient presented no other signs or symptoms and showed no endocrinologic abnormalities. A testicular ultrasound showed a 9 mm round, smooth, nodular lesion in the right testis. The lesion was widely calcified, with prominent posterior acoustic shadowing, and a color Doppler examination shows a not increased vascular flow in proximity to the calcified area. A surgical consultation was suggested. The patient was admitted to the Department of Pediatric Surgery of the University of Naples Federico II and a biopsy of the testicular lesion with an intraoperative examination was performed. After the frozen section examination, a diagnosis of a sex cord stromal tumor of the testis with spread calcification was made. The patient underwent testicle-sparing tumorectomy. The specimen was fixed in 10% buffered formalin and processed for light microscopy. Histologically, the tumor was composed of nests, trabecule, cords and solid tubules composed of large cells with abundant pink eosinophilic and finely granular cytoplasm. The nuclei were usually eccentric and round-to-oval, with finely stripped chromatin and one or two distinct nucleoli. Some tumor cells showed binucleation. The neoplastic aggregates lay in a stroma ranging from myxoid to collagenous and containing a prominent eosinophilic infiltrate in the background. Several calcification were observed, usually in the form of laminated concretion with a typical "mulberry-like" appearance. Mitotic Fig.s were not seen. The neoplastic cells showed immunohistochemical positive reaction for a-inhibin, calretinin, S-100 protein, vimentin and MART-1. The proliferation index (Ki-67) was low (5%).

Results. On the basis of the histological and immunohistochemical features, a diagnosis of LCCSCT of the testis was made, thus confirming the intraoperative diagnosis. The patient had an uneventful postoperative course. The lesion was confined to the right testis and there were no clinical signs of CNC or PJS. Six month later, the routine ultrasound follow-up showed no residual tumor in the right testis and no other lesion in the left testis. Conclusion. LCCSCT is a rare and distinctive neoplasm and presents a diagnostic challenge for pathologists. Relatively few patients have been reported in the literature with LCCSCTs (about 70), ranging from 2 to 73 years old (average 21 years). In children they often present as prepubertal and/or peripubertal gynecomastia. The differential diagnosis is with Sertoli cell tumor NOS, Leydig cell tumor and, mainly, with the Sertoli cell neoplasia characteristic of the PJS. Although these tumors are very rare, they occur with higher frequency among patients with PJS and CNC. Orchiectomy was often performed in the past; however, these tumors are usually benign therefore there is no need for radical surgery.

HPV-related oropharyngeal squamous cell carcinomas: p16INK4A immunohistochemistry or HPV genotyping?

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Background. Infection with high-risk human papillomavirus (HPV) is linked to a subgroup of squamous cell oropharyngeal tumors (OPSCC) ^{1,2}. Recent studies indicated that in HNSCC, patients with HPV-infected tumors have a more favourable prognosis compared with patients whose tumors are virus-negative ³. In analogy with female genital (or cervical) carcinogenesis, HPV testing has been widely recommended in OPSCC, but there is no consensus on which test consider the 'golden standard' ⁴ among the numerous detection methods available either as single test or combinations ⁵. However, although recent publication of Guidelines for Head and Neck Cancer and College of American Pathologists protocol for the Examination of Specimens From Patients With Carcinomas of the Pharynx suggest p16INK4A -IHC as a screening method for HPV detection ^{6,7}, some questions remain regarding the accuracy of the test when used alone, without molecular detection of HPV-DNA. Our aim was to compare an HPV PCR assay and p16INK4A expression status by immunohistochemistry (IHC) as a surrogate marker.

Material and methods. Retrospective study considering patients affected by squamous cell oropharyngeal tumors. All included samples were processed for immunohistochemistry (IHC) for p16INK4A and tested by PCR for detection of HPV DNA and HPV Genotyping.

Results. A total of 84 patients affected by squamous cell oropharyngeal tumors were included and tested. A significant positive correlation was found between HPV PCR and p16INK4A IHC but the agreement was poor (k coefficient of 0.25). In fact, the sensitivity of the p16INK4A IHC positivity to detect the HPV PCR positivity was low (28.21%, 95% CI. 16.54% - 43.78%) (Table I).

Tab. I. Results of the different detection methods for HPV infection in our series.

p16INK4A immunostaining	
Positive	13 (15,5%)
Negative	71 (84,5%)
HPV DNA, no. (%)	
Negative	45 (53,5%)
Positive	39 (46,5%)
HPV-16	34 (85%)
HPV-39	3 (7,5%)
HPV-31	1 (2,5%)
HPV-11	1 (2,5%)
HPV-positive tumor sites of origin, no. (%)	
Base of Tongue	16 (41%)
Pharynx	11 (28,2%)
Tonsils	6 (15,4%)
Retromolar Trigone	4 (10,3%)
Cervical lymph nodes	2 (5,1%)
HPV+/ p16INK4A +	11 (13,1%)
HPV+/ p16INK4A -	28 (33,3%)
HPV-/ p16INK4A -	43 (51,2%)
HPV-/ p16INK4A +	2 (2,4%)

Conclusion. The positivity of p16INK4A by immunohistochemistry had a low sensitivity to detect HPV DNA and our results suggest the need at least to test with HPV PCR the p16INK4A IHC negative samples to increase detection accuracy of a valuable information for the clinical management of these patients. The better characterisation at a molecular level may better define homogeneous groups of patients for prognosis as well as for responsiveness to treatments 8. The growing evidence of the prognostic significance of HPV status in oropharyngeal squamous cell carcinomas^{9,10}, indicating that HPV is associated with a better prognosis and an increased radiosensitivity, suggests that it can be used as a molecular marker in OPSCC. Despite the clinical advances, there are no commercially available, validated, and universally accepted tests for the determination of tumor HPV status, neither guidelines. With our study, we underlay the need of a consensus on the methods to be used for HPV detection in OPSCC^{4,11} and we reinforce the idea, proposed by other authors¹², that the gold standard for the identification of HPV infection on OPSCC should be the molecular method, at least for the negative p16INK4A immunostain.

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Giovedì, 24 Settembre 2015

Aula Gialla 1 ore 9.00-10.00

Miscellanea 3

Moderatore: E. Bonoldi (Lecco)

Primary low-grade angiosarcoma of the breast. Case report

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Background. Angiosarcoma of the breast is a rare lesion, accounting for 0.05% of all breast tumours and between 3% and 9% of all breast sarcomas; however, up to 44% of all angiosarcomas occurs within the breast (it is the second most common mesenchymal malignancy in the breast, after malignant phyllodes tumour) [1]. Angiosarcoma is subdivided into primary and secondary (subsequent to postoperative radiation), the latter being the most frequent. Microscopically, angiosarcoma consists in a heterogeneous group of lesions, including low-, intermediate- and high-grade tumours. In particular, low-grade lesions can be very similar to benign proliferations. We describe a case of primary low-grade breast angiosarcoma with considerations regarding differential diagnosis and management.

Methods. A 50 years old, asymptomatic woman with a negative breast pathology history underwent a senological check-up for familiar cancer history (mother and a sister affected by breast cancer, an other sister affected by osteosarcoma and fa-

ther affected by colonic carcinoma). Magnetic resonance (MR) showed a 15 mm lesion in upper-external quadrant of the right breast and a 20 mm lesion, classified as “suspect”, in lower-external quadrant of the omolateral breast. Mammography (MX) and ultrasound (US) coinformed the 15 mm lesion, but the 20 mm one was not evident. On 15 mm lesion a fine-needle aspiration cytology (FNAC) was performed; on 20 mm lesion a MR-guided core-needle biopsy (CNB) was performed.

Results. On microscopic examination, FNAC specimens from 15 mm lesion was classified as benign-C2 (sec. European Guidelines) [2] (final diagnosis on excisional biopsy was fibroadenoma). CNB material from 20 mm lesion was abundant: 7 cell blocks were prepared. Specimens were mainly fatty and many of them contained a vascular proliferation consisting of open, anastomosing vascular channels coated from a monolayer of hyperchromatic, plump endothelial cells, CD31 and CD34-positive; mitoses were not evident, but Ki67 immunostain showed some positive cell. Vascular channels were mainly empty, only rare red blood cells were seen. The lesion seemed to have an infiltrative growth pattern but, on CNB specimens, the relationship between the vascular proliferation and mammary gland tissue was not assessable, nor its peripheric spread pattern. Our diagnosis was: “Vascular proliferation; differential diagnosis: low-grade angiosarcoma vs angiomatosis”; we suggested a radical excision of the lesion. So, a mammary wide excision was performed (specimen weight was gr 75 and dimensions were cm 9x6x3,3), without

sentinel lymph node sampling. On gross examination, only an hemorrhagic area was seen (because of the CNB), so the specimen was entirely examined. On microscopic examination, a little remaining lesion was found (16 mm, present only in 1 slide). Morphological features were the same as seen in CNB specimens: a vascular proliferation with an infiltrative, anarchic, growth pattern, consisting of anastomosing vascular channels coated from a monolayer of hyperchromatic, plump endothelial cells without evident mitotic Fig.s; vascular channels contained many red blood cells. The lesion infiltrated mammary fat, but no mammary gland tissue was present next to the proliferation. Surgical margins were histologically clear. Our final diagnosis was: "Vascular mesenchymal tumour, most consistent with low-grade angiosarcoma". To date, no other therapy has been performed (this is a very recent case) and the management of the case is still under discussion. Up to date, the patient is well and alive with no evidence of disease.

Conclusions. Angiosarcoma of the breast is a rare neoplasm, accounting for about 0.05% of all primary malignancies of the breast. In particular, primary angiosarcomas are challenging lesions: frequently they are painless and hardly detectable by MX and US; MR is the most useful imaging method to detect them¹. Moreover, on histological examination, low-grade angiosarcomas can mimic other vascular benign proliferations. In particular, low-grade angiosarcomas must be distinguished from hemangiomas and angiomatosis (which are well-circumscribed, typically < 20 mm and grow around breast parenchyma and not infiltrates it; moreover, hemangiomas, except for angiomatosis, do not have a lot of anastomosing vascular channels)^{1,3}. Complete excision with histologically clear margins is the basis of the management of these lesions; mastectomy is often required, but wide local excision may be considered for smaller lesions; axillary dissection is not indicated as lymph node metastases are uncommon. Total histological examination of the proliferation is mandatory, also because high-grade angiosarcomas can have lower-grade areas at their periphery. The role of tumour grade as a predictor factor of recurrence is controversial^{4,7}, as well as the role of adjuvant radiotherapy and chemotherapy in post-surgical treatment of the lesion⁴.

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A comparative analysis between one-step nucleic acid amplification (OSNA) and routine frozen section (FS) histology

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Introduction. Sentinel lymph node biopsy (SLNB) is the standard of care for axillary staging in breast cancer. In

current practice, two methods are employed to evaluate intraoperative SLN: routine frozen section (FS) histology and intraoperative molecular analysis.

Results of a questionnaire-based survey by the European Working Group for Breast Cancer have shown a discrepancy in the current practice of pathological evaluation of SLN by FS.3. In a 2011 review published in the *British Journal of Surgery*, FS sensitivity ranges from 57% to 74% and specificity ranges from 99% to 100%¹. In the literature, protocols for FS are not standardised and thus difficult to be compared. Moreover, the false negative rates of FS histology, mainly resulting from failure to detect micrometastases, have been found to range from 26% to 43% compared with the final pathological results¹.

Until December 2012 our Institution has performed intraoperative multi step section FS analysis of the SLN for early breast cancer. Starting from January 2013, we have introduced a molecular analysis with OSNA method (Sysmex, Kobe, Japan). OSNA assay is a rapid molecular detection procedure that analyses lymph node metastases by detection and amplification of cytokeratin 19 mRNA. OSNA was reported to have a sensitivity of 87.7% and a specificity of 96-97%^{2,3,4}.

A comparative analysis of these two different experiences was conducted to evaluate the performance of both techniques to detect metastases in intraoperative SLNB.

Methods. Frozen Section Histology

During the period from January 1st 2011 to December 31st 2012 a total of 262 consecutive cases of patients with breast cancer were studied to evaluate the intraoperative detection of sentinel lymph node metastases with routine FS histology. A total of 286 SLNB were performed. Excised SLNs were immediately delivered to the laboratory and peri-nodal fat was carefully removed. If the lymph node was less than 5 mm in diameter it was submitted to FS examination without sectioning. If larger than 5 mm, the lymph node was bisected along its longitudinal axis and both halves were taken for FS examination. If two or more SLNs were sent, all lymph nodes were examined accordingly. A total of 10 consecutive sections for each part of the lymph node were taken and rapidly stained with haematoxylin and eosin. To speed up the processing of each SLN, two technicians and two pathologists were employed. Any remaining nodal tissue was fixed in formalin and embedded in paraffin. 4µm-thick sections were taken every 100 micron.

OSNA Assay

During the period from January 1st 2013 to December 31st 2014 a total of 286 consecutive cases of breast cancer patients were submitted to intraoperative evaluation of SLNB by OSNA assay. Three hundred forty SLNs were analyzed according to the manufacturer's instructions (Sysmex, Japan). The number of CK19 mRNA copies per microliter was calculated, and based on this number, the result was assessed: < 250 negative; from 250 to 5000 micrometastases; > 5000 macrometastases.

Results (Tab.1): OSNA assay detected more cases of sentinel lymph node metastases than FS histology. OSNA detected 99/340 metastases (29%) vs FS that detected 50/286 metastases (17,5%). Particularly, 50 micrometastases (14,7%) and 49 macrometastases (17%) were detected with OSNA and 4 micrometastases (1,4%) and 46 macrometastases (16%) with FS histology respectively. Definitive histology detected 75 metastases with 25 false negative case (FN rate of 33%). The overall sensitivity of FS was 75%, the specificity was 100%. The sensitivity was particularly low in micrometastases (61%).

Tab. I.

	Frozen Section		Definitive histology	OSNA	
	N°	%		N°	%
Macrometastases	46	16	46	49	17
Micrometastases	4	1,4	4	50	14,7
Negative	236	82,5	211 (FN 25)	241	70,89
Tot. positive case	75 (26%)			99 (29%)	

OSNA detected 3 cases of micrometastases in intraductal carcinoma that were considered as false positive (specificity 98,8%).

Conclusion. The present study shows that the OSNA assay using whole lymph node can detect more SLN metastases, particularly micrometastases, than FS histology (14,7% vs. 1,4%). These results are reasonable, because only a part of SLN was evaluated by FS and the false-negative rate of intraoperative FS histology, mainly resulting from failure to detect micrometastases, has been found to be about 33% compared with the final pathological results. The turnaround time of the OSNA assay was approximately 40 minutes, which is a similar time to FS histology but, unlike the latter, does not require additional time to complete the procedure. The likely false-positive results of OSNA assay in three cases of intraductal carcinoma of the breast are probably due to OSNA assay not being able to discriminate between benign and malignant epithelial cells showing CK 19 positivity. Therefore, it is possible that lymph nodes with a contamination of epithelial cells, caused by preoperative diagnostic procedures (fine needle aspiration, mammotome etc.) can result in false-positive diagnoses⁵⁻⁸. However, these false positive diagnoses did not lead to any patient overtreatment because CK 19 mRNA copy numbers were low and interpreted as micrometastasis and therefore there was no need to proceed to complete axillary lymph node dissection.

In conclusion, the intraoperative OSNA assay is a more standardized procedure than FS and able to detect more sentinel lymph node metastases, particularly micrometastases. The turnaround time of the OSNA assay is more convenient in daily laboratory practice. The OSNA false-positive cases were small and do not have any clinical consequence.

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Fixation time and HER2/neu assessment in HER2-positive breast cancer

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Background. Accurate determination of HER2/neu status in breast carcinoma is essential. Preanalytic variables, including time of fixation, are critical to obtain reproducible and reliable HER2/neu results. Improper fixation has been related to many false-negative results in breast cancer hormone receptor tests and a lack of reproducibility of HER2 testing.

According to the updated 2013 ASCO/CAP guideline recommendations for HER2 testing, breast specimens that will be subject to ER/PgR and HER2 testing should be fixed in neutral buffered formalin for a minimum of six hours and a maximum of 72 hours. This fixation time begins when the specimen is initially placed in formalin and ends when the cassettes are no longer in formalin. For specimen fixed longer than 72 hours for HER2 or ER and PgR in which negative test results are obtained, the report should state that prolonged fixation could be a possible cause for the negative result, and alternative testing methods should be considered (eg. ISH for HER2). For HER2 testing, labs should also consider confirming by ISH any specimen fixed longer than 72 hours that is not score 3 by immunohistochemistry.

Must report HER2 test result as indeterminate if technical issues prevent one or both test (IHC and ISH) from being reported as positive, negative, or equivocal. Conditions may include inadequate specimen handling. 1

The purpose of this study was to determine the impact of the length of fixation in 10% neutral buffered formalin on the expression of HER2/neu by immunostochastic (IHC) and silver in situ hybridization (SISH) analysis in HER2-positive breast cancer.

Methods. We studied tissue samples from 5 known HER2-positive invasive breast cancer cases after fixation for 1, 24 and 100 hours in 10% neutral buffered formalin. The tissue was processed immediately after fixation, resembling routine practice.

The 15 resulting blocks were then batch stained with routine hematoxylin-eosin (H&E) and then immunohistochemical studies were performed with the commercially available breast cancer kit (HerceptTest Dako). The stained slides were reviewed and scored. Finally SISH was performed also.

Results. For H&E stained slides we found no significant difference in morphological features regardless of the time fixation, but we recorded the presence of numerous crush artifacts at the epithelial-stromal interface for a fixation time of 100 hours.

We found no significant difference in the intensity of the stain or the percentage of cells stained by immunohistochemistry regardless of the time fixation.

About HER2 determination by SISH in all cases we noted the presence of artifacts which limit interpretation of preparation set both for hypo- and hyper-fixation time and so the HER2 test resulted as indeterminate cause to "inadequate" specimen handling.

Conclusions. Fixation times between 1 and 100 hours in 10% buffered formalin do not appear to have an impact on the expression of HER2/neu by immunohistochemical analysis in HER2-positive breast cancer.

On the other hand, an improper fixation time impacts significantly on the outcome of SISH. Both hypo-fixation and hyper-fixation of samples hesitate in an indeterminate result with SISH.

The optimum was represented by a fixation time of 24 hours (from 6 to 72 hours, as recommended by ASCO/CAP guidelines). We appreciated optimally the morphological characteristics of the tumor with H&E stained slides. All cases were confirmed to be HER2-positive breast cancer (score 3+). It was excellent also the quality of the preparation set for SISH.

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HER2 quantification by mass spectrometry is superior to IHC or ISH in predicting clinical benefit from anti-HER2 therapy in HER2-positive breast cancer (BC)

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Background. To be eligible for an anti-HER therapy, tumors have to be HER2-positive as determined by IHC or in situ hybridization (ISH) analyses. Although gene amplification is generally considered the main mechanism of HER2 protein overexpression in BC, the biologic regulation of HER2 expression is complex and gene amplification may not always correlate quantitatively with HER2 protein levels and with response to anti-HER2 therapies.

Methods. HER2-positive (n=123) primary BC samples were microdissected, solubilized and digested in trypsin in Liquid Tissue buffer. Absolute quantitation for HER2 protein was performed using selected reaction monitoring (SRM) mass spectrometry. ISH was centrally performed on all cases. HER2 gene copy number (GCN), HER2/Chr17 ratio, and pattern of amplification were evaluated and correlated with HER2 protein levels. The survival benefit according to protein and gene levels was calculated for patients receiving an adjuvant anti-HER2 therapy (n=68).

Results. HER2 SRM levels showed weak positive correlations with HER2 GCN and HER2/Chr17 ratio. Average HER2 protein levels were significantly higher in tumors amplified with homogeneous stained regions (HSR, n=50) compared to those with double minutes (DM, n=46). Ten amplified cases showed HER2 protein levels similar to HER2-negative tumors. Eight had a DM and 2 a mixed pattern of amplification. None was amplified in HSR. HER2 protein levels >2,200 amol/μg predicted better disease-free survival and overall survival in patients treated with adjuvant trastuzumab. Neither HER2 GCN, HER2/Chr17 ratio nor pattern of amplification correlated with outcome.

Conclusion. By using an objective non-antibody based method we identified a great deal of disparity of HER2 levels in BC patients that are classified as HER2-positive by ISH. Different amplification patterns resulted in significantly different protein levels; with many cases of DM amplification showing no concomitant increase in HER2 protein expression. Our findings indicate that protein abundance rather than gene status predict the clinical benefit from anti-HER2 therapy in HER2-positive BC patients.

Assessing the clinico-pathological behavior of breast carcinomas at young age: a 14-year single institution analysis

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Introduction. Breast cancer in young women has been reported to pursue a more aggressive clinical behavior compared with the disease in older patients.

Although controversy exist about the definition of "young age" and different cut-off have been proposed, it has been shown that younger age is associated with higher risk of relapse and poorer survival. Several factors have been linked to the poor prognosis associated with developing breast cancer at a young age. These include large tumor size at diagnosis, higher tumor grade, vascular invasion, lower estrogen and progesterone receptor expression, increased expression of HER2 and higher Ki67. Moreover the distribution of molecular subtypes among young patients differs from the older patients, with proportionally less Luminal cancer and more HER2-positive and Triple Negative tumors. This has led some investigators to question whether breast cancer diagnosed at a young age has unique biology or whether it is a just surrogate for higher incidence of aggressive subtypes.

However whether young age is associated with unique cancer biology and then whether age itself is an independent prognostic factor remains a matter of debate.

In this study we evaluated the relationship between age and clinico-pathological features and prognosis of the tumors, and determined a cut-off for defining young age-onset breast cancer by exploring outcomes.

Methods. We revised data of 587 patients aged 50 years or less referred to Pathology Department at the Sacro Cuore Hospital, Negrar (Verona) from January 2000 to December 2014. The total cohort was stratified into four age groups; patients aged ≤35 (group I), 36-40 years (group II), 41-45 years (group III) and 46-50 (group IV) years.

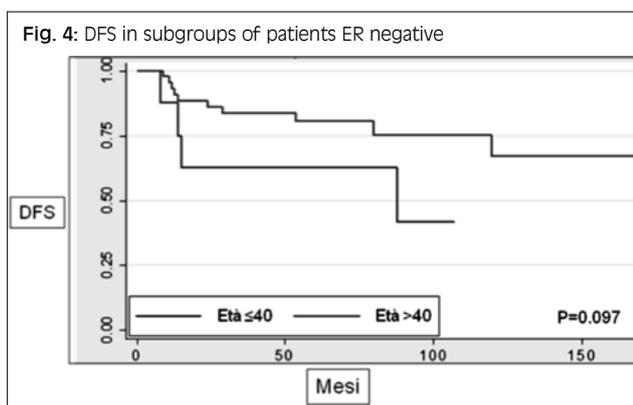
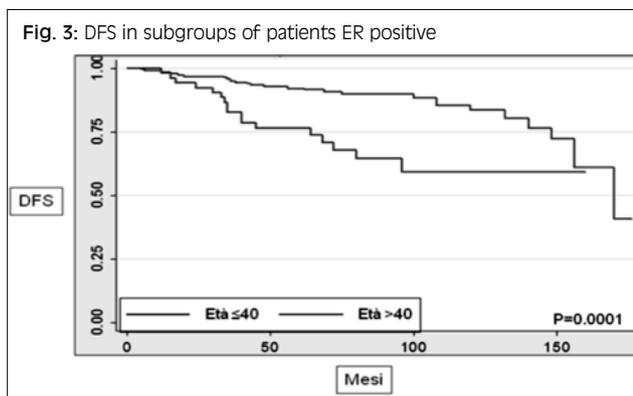
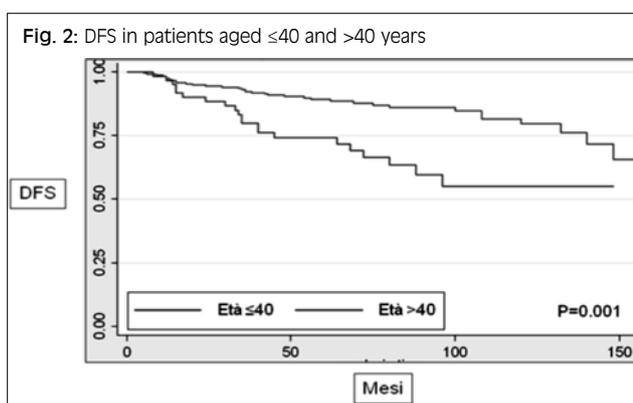
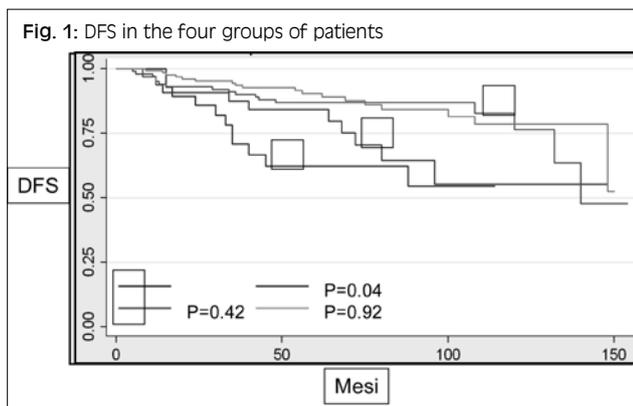
Data from each patient regarding medical history, pathological features, therapy and follow-up were retrieved from the pathology reports and patients' medical records.

All ER/PR/HER2/Ki67 results were expressed quantitatively in the original pathology reports, so that the data could be extracted and interpreted based on current guidelines for this study.

ER and PR cut-off was set at 1%, while Ki67 at 15%.

For statistical analysis, data were imported and merged in STATA/IC for windows version 12.

Chi-square or Fisher exact test, where appropriate, were used to compare the clinicopathological features of breast cancer between different age groups. Cumulative incidence of DFS on matched patient cohorts was described by the Kaplan-



Meier method and compared with the use of the log-rank test. A two-sided P value < 0.05 was considered statistically significant. The Cox proportional hazard regression model was used to evaluate the associations between clinicopathological features and clinical outcome. In multivariate analysis, all variables were initially included into the model and then removed by backward stepwise selection if their P value was > 0.05 .

Results. Of the 587 patients analyzed, 57 (10%) were aged ≤ 35 , 64 (11%) were aged 36-40 years, 190 (32%) were aged 41-45 years and 276 (47%) 46-50 years. The younger patients, when compared with “less young” patients, had higher grade (51% in group I versus 21% in group IV), higher percentage of ER negative tumors (25% in group I versus 10 group IV) and higher Ki67 (65% in group I versus 36% in group IV).

Moreover, in younger patients there were less tumors identified as Luminal A (60% group I versus 79% group IV) and more HER2 (14% group I versus 3% group 4) and Triple Negative (12% group I versus 7% group IV) tumors.

In univariate analysis, group II patients showed a worse DFS than group III patients ($P=0.04$). There was no significant difference in DFS between group I versus group II patients ($P=0.42$) and DFS between group III versus group IV patients ($P=0.92$) (Fig.1).

Subgroups of patients aged ≤ 40 years showed a worse DFS than patients > 40 years ($P=0.001$) (Fig.2). This outcome trend, according to age ≤ 40 versus > 40 years, was more evident in ER positive patients ($P=0.0001$) (Fig. 3), but was not observed in ER negative patients ($P=0.097$) (Fig. 4)

Multivariate analysis, after adjusting for age (≤ 40 versus > 40 years), T and N pathologic stage, tumor grading (G1-2 versus G3), ER PgR status ($< 1\%$ versus $\geq 1\%$), HER2 status and Ki-67 value ($\leq 15\%$ versus $> 15\%$), showed that age ≤ 40 years independently predict a poorer DFS ($P=0.001$; HR 2.5; 95% CI: 1.4%-4.3%).

Conclusions. The results of this study showed that young patients with breast carcinoma presented more frequently tumors with poor prognostic features as high grade, absent expression of ER and high Ki67 than older premenopausal patients. Young patients showed poorer DFS than older premenopausal patients and the risk of relapse increases abruptly in women age younger than 40 years, especially when they have ER positive tumors. Finally, even after correction for stage and tumor features, young age at diagnosis remains an independent risk factor for relapse and then an indication for more aggressive systemic therapy.

Microcalcifications drive breast cancer occurrence and development

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Introduction. Mammary microcalcifications have a crucial role in breast cancer detection, but the processes that induce their formation are unknown. Moreover, recent studies have described the occurrence of the epithelial-mesenchymal transition in breast cancer, but its role is not defined. In this study, we hypothesized that epithelial cells acquire mesenchymal characteristics and differentiated in Breast Osteoblast Like Cells (BOLC) becoming capable to produce breast microcalcifications. In order to verify our hypothesis, we set up an in vitro experiment by performing a co-culture with

breast cancer cells (MCF7), macrophages, and calcium salts. **Methods.** MCF-7 cells were cultured alone, and co-cultured with CO, CO and macrophages, HA, HA and macrophages. Cells were incubated for 48 hours at 37° at an atmospheric pressure of 5% (v/v) carbon dioxide/air before harvesting for both EMT and BOLC characterization. EMT and mineralization markers were studied both by immunostaining for vimentin, RANKL and BMP-2, and by western blot for TGF . The BOLC differentiation and elemental composition of microcalcifications were investigated by transmission electron microscopy and EDX microanalysis.

Results. According to morphological appearance and RANKL and vimentin expression, we found numerous BOLC in Co-Culture of MCF-7, monocytes and CO. Toluidine blue staining allowed us to identify numerous crystal structures similar to breast pleomorphic calcifications. TEM analysis showed that these calcifications were into or next to BOLC. Surprisingly, EDX microanalysis demonstrated that this material was made of HA and/or Mg-HAp. Finally, culture, with large amount of de novo microcalcifications showed higher expression both of TGF and BMP-2. Noteworthy, in all other experimental points, MCF7 do not acquire mesenchymal characteristics and do not become able to product microcalcifications.

Discussion: Our experimental result allowed us to speculate that in vivo, microcalcifications made of CO, mainly associated with BL, attract and activate macrophages that induce the epithelial mesenchymal transition by producing TGF . Moreover, under BMPs induction, mesenchymal like cells could assume an osteoblast phenotype (BOLC) and behave as producers of HA/Mg-HAp microcalcifications. This hypothesis suggests a new role of microcalcifications in breast cancer. Indeed, according to our data, Ha and Mg-HAp calcifications trigger a mechanism of breast cancer development.

Pathological complete response in a patient affected by multiple synchronous, breast and lung primary malignancies: a case report and review of the Literature

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Introduction. Multiple primary malignancies are an emerging problem in clinical practice. Their incidence is increasing likely due to overall longer lifetime and improvements in early diagnosis, treatments and follow-up for many neoplasms. Furthermore a priority is the exclusion of a metastases site rather than a second primary cancer. The distinction of multiple primary malignancies does play a critical role for determining the tumor staging, the correct therapeutic approach and the prognosis of the patients.

Methods. We describe an uncommon case of a patient affected by a breast carcinoma with a pathological complete response after hormonal therapy and radio-chemotherapy for a synchronous pulmonary adenocarcinoma.

To study in deep this important issue, a review of the principal and most recent articles is also reported.

Results. A 63-year-old woman presented with an invasive ductal carcinoma of the breast and a lung adenocarcinoma. After multidisciplinary discussion, the patient underwent pulmonary left lower lobectomy followed by radio-chemotherapy with cisplatin and vinorelbine and started hormone therapy with letrozole. Ten months later, a left mastectomy with axil-

lary lymph nodes dissection was performed. Histologically, a pathological complete response (pCR) was documented.

Conclusion. With a review of the Literature, we discuss the issue of multiple primary malignancies, with its diagnostic and therapeutic implications. Among the principal more recent reported series of multiple primary malignancies, breast carcinoma is one of the cancers most frequently associated with other synchronous or metachronous neoplasms in females. An accurate diagnosis is mandatory for its therapeutic and prognostic valence. Pulmonary metastases are usually detected as multiple pulmonary nodules and/or pleural carcinomatosis. However, in the case of detection of a solitary pulmonary nodule in patients with synchronous or previous diagnosis of an extra-pulmonary malignancy, an accurate diagnosis can be very difficult.

Treatment of synchronous tumors often requires a multidisciplinary approach. The therapeutic decisions depend above all on histology of the tumors, location, stage and general health conditions of the patient.

In cases of multiple synchronous malignancies it has been highlighted the importance of the choice of the best therapeutic approach for both the malignancies, reducing collateral individual effects.

A clinical pulmonary hypertension underlying a rare diffuse developmental lung disease

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Introduction. Full-term infants with severe and prolonged respiratory distress represent a diagnostic challenge. Plain radiographic findings may be nonspecific or similar to classic surfactant deficiency disease for infants with different interstitial lung disease.

Material and methods. An inborn normal female infant was born to a 31-years-old after a normal pregnancy. After the first hour of life the newborn's condition quickly deteriorated, with progressive respiratory distress and cyanosis. Congenital cardiopathy was suspected but echocardiogram revealed a structurally normal heart and severe interstitial lung disease vs pulmonary hypertension. At 20 hours of life the infant presented a severe respiratory failure treated with intensive support with poor results. She died at about 24h of age. After autopsy, diagnosis of primary Congenital Acinar Dysplasia (CAD) was made.

Results. We reported a case of a full term infant with severe respiratory distress who was observed in Neonatal Intensive Care Unit (NICU). After unexplained and unresponsive to treatment respiratory failure, the patient died at 24 hours of life. At autopsy she weighed 2841 g (appropriate weight for gestation). There were no external dysmorphic features. The lungs had normal lobation and their combined weight was within normal limits. The thymus, heart and major blood vessels were all normal, as were the esophagus, larynx, thyroid, trachea and bronchi. The gastrointestinal and genitourinary systems also had a normal appearance. The lungs showed absence of alveolar development, with airways distal to the bronchi composed of irregularly branching ciliated bronchial-like structures lined by pseudo-stratified tall columnar epithelium with goblet cells. The acini were irregular and di-

lated with diffuse growth arrest without alveolar development. Normal alveoli were not present. Both lobules and peripheral airspaces were separated by abundant, thickened loose interstitial connective tissue. These findings were compatible with diagnosis of CAD.

Conclusion. Congenital acinar dysplasia (CAD) is a rare malformation of growth arrest of the lower respiratory tract resulting in critical respiratory insufficiency at birth. It is a rare, apparently sporadic, lethal developmental defect resulting in pulmonary hypoplasia due to failure of lung development beyond the pseudoglandular phase seen at 16 weeks gestation. The etiology is unknown. It is diagnosed by exclusion of all other causes of pulmonary hypertension and a sum of clinical, imaging, and histopathologic findings. There is no cure and clinical treatment is supportive until death of the patient.

In the final analysis, in full-term babies with unexplained progressive respiratory distress from birth and deteriorating radiological changes, congenital acinar dysplasia and other form of interstitial lung diseases should be considered.

Mystery case: a 6-year-old child with visual loss and retrolental lesion. CSHRPE or not?

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Background. Tumors of the retinal pigment epithelium (RPE) are rare challenging lesions that include congenital hamartoma, congenital hypertrophy, combined hamartoma of the retina and RPE, and adenoma or adenocarcinoma. In one study, several authors reported on five cases classified as CSHRPE according to their clinical features; all of these lesions maintained a stable pattern during the follow-up period. Congenital simple hamartoma of the retinal pigment epithelium (CSHRPE) is a rare intraocular finding that is described as a focal, nodular, jet black lesion. These lesions frequently occur at or near the macula and have no known association with changes in the surrounding neurosensory retina, retinal pigment epithelium or choroid, nor have they been related with exudation or hemorrhage.

Case report. We report a unique case of a 6-year-old female child. She was brought to ophthalmologist by her parents, who had noticed leukocoria of right eye since 1 month. At previous ophthalmic visit fundus of right eye was normal and the girl had only esophoria with hyperopia (+1.25 sph). Slit lamp examination of right eye revealed a dense, white, retrolental membrane with vessels emanating from inferior side. BCVA was 20/80 in right eye and 20/20 in left eye. Ultrasonography using 20 MHz probe revealed hyperreflective retrolental membrane and a well circumscribed, about 2 mm lesion originating from the inferior ciliary body. MRI, after contrast administration, revealed enhancement of the inferior region of the ciliary body and of the lens. The rapid growth of the lesion associated with decreased visual acuity led ophthalmologist to perform a biopsy. The proposed clinical diagnosis was medulloepithelioma or persistent hyperplastic primary vitreous (PHPV). Multiple tissue biopsies were performed and showed disorganized retinal tissue with pigmented oval to round cells (s100 protein+, HMB-45+, MART-1+) devoid of significant cytological atypia arranged in cords and aggregates of variable size in the context of cellular collagenous stroma. Glandular-like structures and prominent eosinophilic basement membrane-like material intermixed with vascular and glial elements were observed.

Discussion. On the basis of the clinical and imaging studies, the pathological findings were considered consistent with CSHRPE. CSHRPE is an uncommon condition and few cases have been described in the literature. It was first described by Laqua in 1981, and Gass reported three similar cases that he later published in a review of focal congenital anomalies of the RPE. This lesion has peculiar features on fundoscopic examination, fluorescein angiography (FA) and optical coherence tomography (OCT). Pathologists should be aware of the histopathological and immunohistochemical findings of CSHRPE to avoid misdiagnosis of RPE malignant tumors, including melanoma.

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Venerdì, 25 Settembre 2015

Aula Gialla 1 ore 8.30-9.30

Patologia Molecolare

Moderatore: M. Paulli (Pavia)

Molecular profile of poorly differentiated clusters of neoplastic cells at the invasive margin of colorectal cancer: evaluation of 20 selected cases

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Background. Poorly differentiated clusters (PDC) of neoplas-

tic cells at the invasive margin of colorectal cancer (CRC) are strongly associated with a more aggressive behavior of these tumors. It was recently suggested that KRAS-mutation could be involved in the formation of PDC.

Materials and methods. The aim of the study was to investigate the biomolecular profile of PDC in 20 CRCs that had been previously shown to exhibit KRAS mutation and high PDC counts. Three KRAS wild type tumors were used as controls. Using Laser Micro-dissector Olympus CKX41, we captured the PDC from each tumor. Hotspot mutations were detected in genome-

amplified DNA by using a Mass Spectrometry-based single base extension technique (Mass Array Sequenom Platform - Sequenom, San Diego, CA; Myriapod Colon Status). The occurrence of mutations in KRAS, NRAS, BRAF and PI3KCA genes was evaluated in PDC.

Results. 18/20 cases (90%) showed KRAS mutations in PDC. Mutations were evidenced at exon 2, codon 12 in 10 cases (56%) (5 G12D mut, 3 G12A mut, 1 G12R mut and 1 G12S mut), at exon 2, codon 13 in 3 cases (2 G13D mut and 1 G13C mut), at exon 3, codon 61 in 2 cases (Q61L mut and Q61H mut), at exon 3, codon 59 in 1 (A59T mut), at exon 4, codon 117 in 1 (K117N mut) and at exon 4, codon 146 in 1 (A146T mut). The molecular profile observed in PDC overlapped with that of the tumors from which they were found. The biomolecular profile of PDC in the remaining 2 cases (10%) differed from that of their native tumors, showing KRAS wild type in PDC and KRAS mutations (exon 2, G12A mut and G12C mut) in the tumor. Among cases showing KRAS mutations in PDC, in 3 tumors (2 G12D mut and 1 A59T mut) PDC showed additional PI3KCA mutations, respectively in exon 9, codon 542 (E542K mut), exon 9, codon 545 (E545Q mut), and exon 20, codon 1047 (H1047Y mut). These tumors had high pTstage, lymph-vascular invasion and lymph node metastases, and high PDC counts. On the contrary, tumors with PDC exhibiting wild type status of KRAS, NRAS, BRAF genes showed a lesser PDC-count and a lower tumor grade at WHO classification.

Conclusion. The presence of KRAS mutations in the PDC confirms that KRAS may be involved in tumor growth, tumor progression and PDC formation. The acquisition of additional PI3KCA mutations in some PDC suggests that new clones of cancer cells may be formed at the periphery of the tumor mass and show an aggressive potential.

Mutational analysis of the BRAF and KRAS genes in pediatric Langerhans cell histiocytosis. Introduction

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Introduction. Langerhans cell histiocytosis (LCH) is a pediatric granulomatous disease with an incidence of four to eight cases per million children, characterized by the accumulation of CD1A+/CD207+ mononuclear phagocytes within granulomatous lesions, that can affect nearly all organ systems and may require aggressive chemotherapy. Of note, the clinical features of LCH are not typical of cancer and LCH lesions frequently regress, either spontaneously or after local treatment. The pathophysiology of LCH, the nature of the initiating event(s), and the mechanisms of local tissue destruction by LCH and other inflammatory cells are still largely unknown. Recent studies found that 54% of LCH patients harbored BRAF V600E mutation in the diseased tissue, strongly suggesting a neoplastic etiology in most cases. B-RAF is a protein kinase activated by ras-coupled receptor tyrosine kinases (RTK) that is central to signaling via the Mitogen Activated Kinase (MAPK) and phosphorylates its downstream target MEK and ERK kinases. The RAS-RAF-MAPK pathway coordinates a large variety of cellular responses involved in development, cell cycle regulation, cell proliferation and differentiation, cell survival and apoptosis, and many other physiological processes, by transmitting extracellular signals to various nuclear, cytoplasmic

and membrane-bound targets.

Several studies show reproducible and sustained efficacy of targeted therapy with vemurafenib in patients with BRAF(V600E)-mutated LCH as second-line therapy.

Methods. In our retrospective study, we evaluated the occurrence and prognostic impact of the V600E and KRAS mutation in formaldehyde-fixed, paraffin-embedded samples from 7 children with granuloma of bones, central nervous system involvement, or with early-onset multi-organ disease treated at our institution. Patients were registered in the National Italian Registry for Langerhans Cell Histiocytosis. LCH diagnosis was established on the basis of the patients' clinical history, histological examination and the mandatory presence of CD1a+ histiocytes in clinical biopsy specimens.

Pyrosequencing (system PyroMark Q24) and Therascreen B-RAF and K-RAS Pyro Kit (QIAGEN GmbH, Hilden) were used to demonstrate the presence of B-RAF V600E mutation or K-RAS exon 2 cod.12/13 mutations.

The QIAamp DNA FFPE tissue Kit were used for purification of DNA from FFPE tissue sections.

Biopsies samples were obtained at diagnosis from 7 patients with various clinical forms of the disease: isolated involvement of bone (n=1), multisystem disease(n=2), early-onset multisystem disease (n=1), bone and lung disease (n=1), bone and SNC disease (n=2)

Results. 2 of 7 (28,57%) cases proved to be BRAF mutants and all cases proved to be KRAS wild-type by the methods applied. V600EB-RAF mutations were found in a child with multisystem disease, and in an infant with early-onset multi-organ disease, both with central nervous system involvement.

Conclusions. In conclusion, activating V600E BRAF mutation can be frequently demonstrated in pediatric LCH. The data presented here confirm the association between B-RAF mutation and LCH granuloma and all cases proved to be KRAS wild-type. Unfavorable risk cases potentially also responding to BRAF-inhibitory therapy can be identified by mutational analysis.

The sample size was small and may not be representative of a population based sample of patients.

In our case studies an infant with early-onset multi-organ disease had a chronic relapsing disease and had been subjected to repeated treatments. If there is a recent disease progression at the SNC, the KRAS mutation has made it possible to begin treatment with Vemurafenib

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Molecular Profile, as detected with Mass-Array Spectrometry (Sequenom platform), in primary and metastatic breast carcinoma treated with Exemestane + Everolimus

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Background. PI3K/Akt/mTOR is one of the most important pathways for the regulation of cell survival, proliferation and apoptosis. Mutational events occurring in this pathway could

lead to malignant transformation and endocrine resistance in breast cancer. The mTOR inhibitor Everolimus (EVE) interferes with cellular proliferation by binding FKB12 protein. EVE has been definitely approved thanks to BOLERO-2 phase III study, which showed a significant prolongation of Progression Free Survival (PFS) due to the addition of EVE to Exemestane therapy, compared to Exemestane alone, in Hormonal Receptors-positive (RO+) and HER2-negative (HER2-) metastatic breast cancer patients. Hortobagyi et al. performed Next Generation Sequencing on 227 BOLERO-2 samples of primary breast carcinoma, to study the potential correlation between genetic alterations and EVE efficacy. A greater incidence of mutations in PIK3CA, PTEN, CCND1 and FGFR1/2 genes was detected and it was observed that patients with no or only one genetic alteration in these genes derive the most benefit from EVE therapy. To our knowledge, no previous research has evaluated the mutational status both in primary and metastatic breast cancers.

Materials and methods. Aim of this study was to evaluate the molecular profile in primitive breast cancers (21 ductal carcinomas, 3 lobular carcinomas and 1 colloid carcinoma) and visceral metastases (hepatic and pulmonary), in 25 patients with advanced breast cancer (RO+ HER2-) treated with EVE in combination with Exemestane. Thirty-three DNA samples from 25 patients were examined, 13 from primary breast cancers and 20 from metastatic lesions. In 8 patients, both the primary tumor and the corresponding metachronous metastasis were evaluated. Genomic DNA samples from FFPE tumoral tissue were analyzed by using OncoCarta v2.0 panel on Mass Array Sequenom platform. A preliminary Multiplex-PCR, followed by SAP-dephosphorylating reaction and iPLEX-primer specific extension, was performed to detect more than 150 single nucleotide variations in mutational hotspots from 18 implicated genes (AKT1, BRAF, CTNNB1, FBX4, FBXW7, FGFR2, FGFR3, GNAQ, KIT, KRAS, MAP2K1, MAP2K2, NRAS, PDGFRa, PIK3CA, PTPN11, SOS1, TP53). Differences were evaluated using Chi-Square and Fisher Tests. Survival analysis was conducted using Kaplan-Meier curves.

Results. Overall, 11 DNA samples, out of the 33 examined, were mutated (33%). Mutations were found in 10 ductal carcinomas and in the colloid carcinoma. Five mutations were detected in primary breast lesions and 6 in metastatic ones. All mutations consisted of a single-nucleotide variation resulting in aminoacidic substitution. Among primary lesions, mutations were detected in the following genes: PIK3CA (E545K), FBX4 (G30N), KIT (S709F), MAP2K1 (D67N), FBXW7 (R465C). They occurred with a frequency of 3%, respectively, namely in 1 out of 33 samples each. Only in the AKT1 gene the same mutation (E17K) was found in 2 DNA primary lesion samples. In metastatic lesions, BRAF (R444W), KIT (G565R), TP53 (R273H), FBXW7 (R479Q), CTNNB1 (S45F), PIK3CA (E545K), AKT (E17K) were mutated. Notably, mutations were found exclusively in primary lesions or in metastatic ones, while only in one patient both primary and secondary lesions were mutated; however, these mutations occurred in two different genes: MAP2K1 (D67N) in breast, FBXW7 (R479Q) in metastasis. Of notice, a reduction in PFS was observed in one patient which carried 3 different mutations (FBX4, PIK3CA, KIT) in the primary tumor (3.4 month versus an average of 5 month) whereas a significantly increased PFS (15.9 month) was detected in a case with 2 mutations (PIK3CA, AKT1) in metastatic lesion.

Conclusion. Although the number of patients and samples is quite limited, our findings in mutational status support literature evidence, as genes most frequently mutated were PIK3CA,

AKT1 and FBXW7, even if the percentage of PIK3CA and AKT1 mutations was less than expected. No correlations between primary and metastatic mutational status were detected.

EGFR mutant allelic-specific imbalance assessment in non-small cell lung cancer

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The epidermal growth factor receptor (EGFR) gene, in non-small cell lung cancer (NSCLC), may undergo both mutations and copy number gains. EGFR mutant allele-specific imbalance (MASI) occurs when the ratio of mutant-to-wild-type alleles increases significantly. In this study, by using microfluidic technology, EGFR-MASI was detected in 25/67 mutant cases (37%), being more frequently associated with EGFR exon 19 deletions (p=0.033). In 49 treated patients, we assessed whether MASI is a modifier of anti-EGFR treatment. The difference in progression-free survival and overall survival between EGFR-MASI-positive and EGFR-MASI-negative groups of patients did not show a statistical significance. In conclusion, EGFR-MASI is a significant event in NSCLC, specifically associated with EGFR exon 19 deletions but does not seem to play a role in predicting the response EGFR small molecules inhibitors.

Next Generation Sequencing in predictive medicine practice: a paradigmatic case

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Patients with RAS wild – type (WT) metastatic colorectal cancer (CRC) are potential candidates for targeted therapy with Epidermal Growth Factor (EGFR) tyrosine kinase inhibitors (TKIs). In clinical practice one single tumour sample of a given location was tested for each patient. In our previous study, the analysis of paired primary and metastatic samples obtained from the same patients show a discrepancy in 13.4% of cases. These differences may reflect heterogeneity between primary and metastatic tumours, that, in some cases, may lead to a wrong treatment selection. Here we report a paradigmatic case of a 64-year-old male diagnosed with two synchronous CRC and a concurrent liver metastasis, whose RAS mutational status was assessed by Next Generation Sequencing (NGS) Colon and Lung cancer panel, revealing that liver metastasis was RAS WT and have the same mutational profile of only one synchronous CRC (RAS WT and p53 R175H mutated), while the other synchronous primary tumor showed a KRAS G12V mutation. These results suggest that, in this case, only RAS WT primary tumor had given a distant metastases, an evidence supported also by digital PCR (dPCR) based mutational analysis of circulating tumor DNA (ctDNA) that showing KRAS WT and p53 R175H mutation.

On these bases the patient became eligible for EGFR TKIs treatment.

Next generation sequencing in molecular diagnostic practice

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Aim. To evaluate Next Generation Sequencing (NGS) performance in clinical practice focusing on RAS testing in metastatic colorectal cancer patients for EGFR antagonist treatment.

Methods. An audit of NGS on 844 routine cases was carried out, taking into account age, gender, specimen type (resection vs biopsies), tumour site (primary vs metastasis), tumour stage, neoplastic cells abundance (>30% vs <30%) and, only for biopsies, fixation time (3 hrs/24 h). In a subset of 50 RAS wild-type (WT) patients correlations between NGS findings and response rate (RR) was also evaluated.

Results. Considering as adequate the amplicons represented with a coverage $\geq 500x$, the tests were informative in 711 cases (84.2%). Mutations were detected in 305 cases (51.0%). No significant differences in mutation rates were observed with respect to age ($p > 0.5$), gender ($p > 0.5$), specimen type ($p > 0.5$) and relation to neoplastic cell percentage ($> 30\%$ or $< 30\%$ - $p = > 0.5$). Conversely, adequate rate was higher in the group of biopsies that have 3 h of fixation time ($p = 0.02$) and RAS mutant rate was higher in metastatic tissue (36.5% vs 28%, $p = 0.03$). The RR of NGS RAS WT patients was 35%. The disease control rate was 61%. Data relative to Progression Free Survival and Overall Survival are under collection.

Conclusions. Pathological criteria that make NGS a more robust method for RAS testing and treatment response prediction are neoplastic cell abundance, metastatic tissue sample, stage IV primary tumour and formalin fixation time for biopsies.

Identifying appropriate cutoff value of MGMT gene promoter methylation and its predictive capacity on prognostic outcome of Glioblastoma

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Objective. Glioblastoma (GBM) account for 60-70% of gliomas, but despite advances in therapy these tumour remain associated with poor prognosis. The MGMT gene is epigenetically silenced by promoter hypermethylation in gliomas, and this modification has emerged as a relevant predictor of therapeutic response and of good prognosis. Studies with pyrosequencing (PSQ) showed that this technique has a good prediction of survival in addition to high reproducibility and sensitivity than other techniques. However, cut-off values for the percentage methylation are one of the critical issues to determine methylation status using PSQ analysis. Aim of the study is defining the cut-off value correlated with good favorable prognostic outcome.

Methods. We collected a tumour samples of GBM patients who underwent surgery or biopsy and were/are regularly followed at the Neuro-oncology Unit of National Cancer Institute Regina Elena will be included in this study. We collected demographic, clinical and molecular data, as well as data on response to treatments and outcomes (PFS and OS). For py-

rosequencing method, we will use the modification reagents of MGMT plus kit (Diatech Pharmacogenetics, Jesi Italy) according to the manufacturer's protocol. Modified DNA will be subjected to PCR amplification with a forward primer and a biotinylated reverse primer using the MGMT MGMT PLUS Kit (Diatech pharmacogenetics) and "Rotor-Gene TM 6000" instrument. We will perform pyrosequencing methylation assay to evaluate 10 CpG sites in the following region: chr 10:131,265,507-131,265,556) using sequencing primer of MGMT Kit Diatech Pharmacogenetics, Jesi (Ancona), Italy. The pyrosequencing analysis will be performed with PyroMarker CpG software 1.0.11 (Qiagen). The software give a mean methylation value for each 10 CpG sites and the total mean of all 10 CpG sites.

Results. We enrolled 42 patients of GBM analyzed with PSQ. Of them 14 patients are females (33.3%) and 28 are males (66.7%). We observed in 78.6% patients (n=33) age < 35 years and 19% (n=8) age > 35 years. We defined that the cut off that really predict the best outcome is 35% of methylation determined with PSQ. We reported that patient with cut off > 35% have a rate of PSF at 1 years of 53% conversely only 4.2% with cut off < 35% have a rate at 1 years.

Discussion. Our preliminary data showed that patients with a cut-off < 35% of methylation had a shorter PFS but not significant difference we observed in terms of overall survival. Are necessary other study on large population for confirmed this data.

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A novel CE-IVD kit for routine NGS-based molecular diagnostics of lung and colorectal cancer

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Introduction. Oncological companion diagnostics require testing for mutations in different genes according to tumour type. Traditional methods identify single mutations independently. Next generation sequencing permits simultaneous interrogation of multiple genes on multiple cases. Here we investigate the only CE-IVD kit designed to identify the mutations required by AIOM-SIAPEC guidelines for colorectal and lung cancers that functions on all NGS platforms.

Materials and methods. Forty-eight cases were analysed (23 lung adenocarcinoma and 25 colorectal cancer) for which pyrosequencing diagnostics was performed, testing EGFR for lung cancers and KRAS for colon with NRAS and BRAF where KRAS was negative. All cases were blind tested using the new 4bases BENKitTM on PGM Ion Torrent NGS technology to analyse simultaneously mutations in KRAS, NRAS

and BRAF for colon and EGFR for lung cancers. As a further control, 10 colon and 10 lung cancers were also tested using 454 Roche sequencing, and 10 lung and 5 colon were also sequenced with PGM Ion Torrent NGS using the colon-lung 22-genes Lifetechnologies RUO panel.

Results. There was a 100% correspondence in the identification of mutations between pyrosequencing and the new 4Bases technology. Furthermore for those cases where NGS was performed (454 or Ion Torrent 22-genes kit) there was 100% correlation in the percentage of mutated alleles identified.

Conclusion. The 4Bases CE-IVD kit is a cost and time effective method for molecular diagnostics. It also provides the user the ability to construct the cancer-specific genes diagnostic panel leaving any unused gene-mixes for research use.

Next-generation sequencing techniques in colorectal and non small cell lung cancer mutation profiling for targeted therapy: diagnostic value of a small 6 gene panel

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Introduction. Cancer diagnostics is rapidly relying on molecular profiling in order to choose and tailor the best treatment for each tumor patient based on its genomic or epigenetic alterations 1-3. In particular, next and third-generation sequencing techniques allow to achieve a quick and deep overview of several genes and regions at once⁴. Herein, we present a targeted genomic profiling by NGS of a series of lung and colorectal cancers from patients surgically treated at our Institute using a custom panel of six genes selected on the basis of clinical treatment guidelines. We aimed to verify whether a small panel of genes may produce overlapping results in

comparison to more standardized methodologies like qPCR or Sanger sequencing saving time and reducing costs.

Methods. We designed a custom gene panel for targeted resequencing including 6 genes currently analyzed in routine tumor diagnostics for the detection of somatic variants, namely KRAS; NRAS; BRAF; EGFR; PTEN; PIK3CA. The majority of them play an important role in the response to EGFR-targeted therapy: KRAS, NRAS, BRAF in colorectal cancer (CRC) and EGFR and KRAS in non small cell lung cancer (NSCLC). The amplicon-based panel was designed with the Illumina Design-Studio software. DNA was extracted from FFPE samples and prepared for sequencing with the Truseq Custom Amplicon Kit. FASTQ files were analyzed through the MiSeq Reporter pipeline, that employs BWA5 for read mapping and the Illumina Somatic Variant Caller for variant calling. We further cross-validated the analysis results with the ODESSA pipeline⁶. The annotation procedure was carried out with the Illumina Variant Studio analysis software. Final annotated mutation tables were filtered out with the following parameters through in-house scripting:

1. Overall coverage > 200x
2. AlleleFrequency > 10%
3. Mutation_type: protein_coding and missense

Results. The overall dataset contained 40 samples, deriving 18 from CRC and 22 from NSCLC tissues. Out of 40 cases, we obtained an overall 97% concordance on single point mutations between NGS techniques and standard methodologies. In particular, we found a 90% correlation in KRAS mutations and 100% in NRAS and BRAF (Table I-2). In NSCLC, we observed a 81% concordance in EGFR deletions, missing those that overlap on our primers. This led us to redesign the panel for future use to avoid the potential loss of frequent deletions (e.g, ELREA). Of interest, we observed two novel mutations in NRAS and BRAF, located outside the genomic regions usually tested with diagnostic methods (qPCR) (see Table I-II). Several mutations were also observed in PTEN and PIK3CA genes that are not yet currently in routine clinical guidelines for targeted therapy, but may provide useful information about resistance to targeted therapy.

Tab. I. Comparison of qPCR and NGS results in colon cancer patients, evaluating KRAS-NRAS-BRAF and EGFR mutation status.

ID patients	KRAS		NRAS		BRAF		EGFR	
	qPCR	NGS	qPCR	NGS	qPCR	NGS	qPCR	NGS
2C	WT	WT	WT	WT	V600E	V600E	N/A	WT
3C	WT	WT	Q61L	Q61L	N/A	WT	N/A	WT
4C	G12C	G12C	N/A	WT	N/A	WT	N/A	WT
5C	A146X	A146X	N/A	WT	N/A	WT	N/A	WT
7C	G12C	G12C	WT	WT	WT	WT	N/A	WT
9C	G12S	G12S	WT	WT	WT	WT	WT	WT
11C	WT	WT	WT	WT	N/A	WT	WT	WT
12C	WT	WT	Q61K	Q61K	WT	WT	N/A	WT
14C	G12V	G12V	N/A	WT	N/A	WT	N/A	WT
15C	G12V	WT	N/A	WT	N/A	WT	N/A	WT
16C	G13D	G13D	N/A	WT	N/A	WT	N/A	WT
17C	G12V	G12V	WT	V112A	N/A	WT	N/A	WT
20C	WT	WT	WT	WT	WT	WT	WT	WT
21C	WT	WT	WT	WT	WT	WT	WT	WT
22C	WT	WT	N/A	WT	N/A	WT	N/A	WT
23C	G12D	G12D	WT	WT	N/A	WT	N/A	WT
24C	G12D	G12D	WT	WT	N/A	WT	WT	WT
25C	Q61H	Q61H	WT	WT	WT	WT	N/A	WT

◻ : confirmed mutations; ◼ : discordant calls; ◻ : novel mutations.

Tab. II. Comparison of qPCR and NGS results in lung cancer patients, evaluating KRAS-NRAS-BRAF and EGFR mutation status.

ID patients	KRAS		NRAS		BRAF		EGFR	
	qPCR	NGS	qPCR	NGS	qPCR	NGS	qPCR	NGS
1L	WT	WT	N/A	WT	N/A	WT	WT	WT
2L	G12A	G12A	N/A	WT	N/A	WT	WT	WT
3L	G12R	G12R	N/A	WT	N/A	WT	WT	WT
4L	G12V	G12V	N/A	WT	N/A	WT	WT	WT
5L	WT	WT	N/A	WT	N/A	WT	DEL	WT
6L	G12C	G12C	N/A	WT	N/A	WT	WT	WT
7L	G12V	G12V	N/A	WT	N/A	WT	WT	WT
9L	WT	WT	N/A	WT	N/A	WT	DEL	WT
10L	G12A	G12A	N/A	WT	N/A	WT	WT	WT
12L	WT	WT	N/A	WT	N/A	WT	WT	WT
14L	WT	WT	N/A	WT	N/A	WT	WT	WT
15L	WT	WT	N/A	WT	N/A	WT	WT	WT
16L	G12C	G12C	N/A	WT	N/A	WT	WT	WT
17L	WT	WT	N/A	WT	N/A	WT	DEL	DEL
18L	WT	WT	N/A	WT	N/A	A497T	WT	WT
19L	WT	WT	N/A	WT	N/A	WT	WT	WT
20L	WT	WT	N/A	WT	N/A	WT	DEL	DEL
21L	G12D	WT	N/A	WT	N/A	WT	WT	WT
22L	WT	WT	N/A	WT	N/A	WT	DEL	DEL
23L	WT	WT	N/A	WT	N/A	WT	WT	WT
24L	G12S	G12S	N/A	WT	N/A	WT	WT	WT
25L	WT	WT	N/A	WT	N/A	WT	WT	WT

■: confirmed mutations; ■: discordant calls; ■: novel mutations.

Conclusions. In a series of 40 cancer patients, we compared the routine techniques for CRC and NSCLC hotspot mutation profiling with NGS using a custom panel of 6 genes obtaining a 97% overlapping results, also detecting 2 missed mutational events. The system is ongoing further testing with additional tumor samples to better validate our panel and make it available and ready for clinical diagnostics. Some issues still remain to tackle: 1. clean DNA availability starting from FFPE tumor tissues, 2. standard procedures for bioinformatics analysis 3. the minimum coverage necessary to obtain accurate results for clinical application. We believe that the final validation and approval of our small custom NGS panel which concomitantly detects gene alterations in CRC and NSCLC, will lead to speed up the workflow of the molecular pathology laboratory, improving cost-effectiveness for testing gene

mutations before administering targeted therapies for patients with advanced mCRC and NSCLC.

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Patologia Testa-Collo

Moderatore: A. Parafioriti (Milano)

Screening for oral cancer: if not now, when? oral cytology, DNA-HPV HR and oral microhistology for the screening of malignant and potentially malignant oral lesions

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Introduction. The survival rate for squamous cell carcinoma of the oral cavity (OSCC), the 6th cause of cancer-related

mortality worldwide, is still low. Indeed, this frequent lesion is often diagnosed late as there is a lack of simple and reproducible diagnostic tests able to identify early stage precancerous potentially malignant lesions (PMLs). These are clinically subdivided into classes I and II: the former are manifestly clinically suspicious and the latter have an apparently innocent appearance. To date, the diagnosis of oral cavity OSCC and PMLs has been based exclusively on the scalpel (surgical) biopsy. However, as this is an invasive technique, it can be used only on a small restricted area and is, therefore, of difficult application with multiple lesions and, last but not least, generally only used for class I lesions. Oral diagnostic cytology alone, whilst providing useful information (sensitivity is higher than the Pap test, while specificity is similar), does not suffice for the diagnosis of OSCC and PMLs in patients with identifiable oral lesions such as leucoplakias, erythroplakias, red dots, ulcers, etc. ¹.

Methods. An original, less invasive sampling method, which does not generally require anaesthetic and uses a dermatological disposable curette, has more recently been used to remove small epithelial fragments from oral mucosa and provides results comparable to the scalpel biopsy in patients with oral macroscopically evident class II lesions ². However, only experts in specialised centres were able to use this technique. As the territorial (private practise) dentist is the first to observe an apparently innocent lesion i.e. class II PMLs, it was decided to set up a clinical trial in collaboration with them, after a brief training period. Samples were obtained according to our instructions with the curette technique by 50 dentists and treated for histological examination (paraffin inclusion, haematoxylin-eosin staining) as routine small biopsies.

Results. Ten of the 152 samples were inadequate (6.6%), 131/142 negative (hyperkeratosis, parakeratosis or simple hyperplasia), 6/142 (4.3%) low-grade dysplasia (OIN 1), 2/142 (1.4%) high-grade dysplasia (OIN 2-3) and 1/142 (0.7%) OSCC.

Our aforementioned study ², carried out by experts in a specialised centre, reported a 3.6% rate of inadequate samples (6/164). Although inadequate samples in the field trial with the territorial dentists are higher (6.6%, 10/152), it must be considered that “inexpert persons” did the sampling and that the results are still very good as this is a second level test but not the final one, which is the scalpel (surgical) biopsy.

Conclusions. Sampling with the “curette technique” and the use of “microhistology” may well be an effective second-level method to distinguish those reactive, or inflammatory oral lesions requiring only follow-up, from positive lesions (dysplasia and OSCC) to be sent to the specialised centres for routine scalpel biopsy or treatment. Moreover, the material obtained with this technique can also be assessed by flow cytometry to evaluate ploidy. The finding of aneuploidy has not only allowed for the identification of lesions that were at risk of evolution as described in another report ³, but also the selection of individuals who required a stricter follow-up regime. Moreover, curette sampling, which was used to cover ample surface areas and/or multiple lesions, led to a reduction in the number of patients that had to return for further investigation, as well as in the number of surgical (scalpel) biopsies. Consequently, there is a positive cost/benefit ratio for the hospital and less discomfort for patients. Therefore, the adoption of this technique will allow the dentist, to manage even those apparently innocent oral lesions (class II) of difficult definition and/or not yet considered for biopsy, in the most appropriate manner.

Work in progress. We have now started screening using, as a first level method, liquid-based cytology associated with

investigation for four groups of high-risk DNA-HPV (16, 18, 31, 45, 51, 52; 33, 58; 35, 39, 68; 56, 59, 66), as it is known that in the “field cancerization” oral microscopic lesions can precede macroscopic ones (i.e. identifiable by inspection) by long periods, even years. As aforementioned, this is particularly important for patients “at risk”, both for lifestyle (smoking, alcohol, etc.) and in presence of oral infections with high risk HPVs, also in totally asymptomatic subjects, i.e. those who have no class I or II oral lesions and have an apparently normal mucosa on inspection by the dentist. The sampling is carried out using the Cytobrush on the sites which are most commonly involved in oral carcinoma, such as the floor of the mouth, the tongue, gums and cheek lining. As liquid-based cytology alone in experts hands has provided a good sensitivity, specificity and positive predictive values (94.7%, 98.9% and 95.9% respectively in 927 patients, all with histological confirmation ¹⁻⁵, this method can effectively be used as a first level screening tool in totally asymptomatic patients. The patients with macroscopic class II lesions could well benefit from a second level microhistological examination ² and only class I lesions would be subjected to scalpel biopsy.

As the mortality rate for oral carcinoma in industrialized countries is now higher than that of uterine cervical carcinoma, which has been drastically reduced thanks to the screening with Pap test and DNA-HPV, the introduction of screening programmes for oral carcinoma, as advocated for years now by numerous epidemiologists like Silvia Franceschi et al ⁶, is imperative.

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Mass forming lesions in pediatric age. Role of ultrasound guided fine needle aspiration cytology (US-FNAC)

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Background. Fine needle aspiration cytology (FNAC) is a useful, highly sensitive and minimally invasive technique in the diagnosis of superficial and deep seated lesions in adult. In pediatric age, head and neck lesions, particularly lymph nodes and the thyroid gland, are mainly investigated by FNAC. We have evaluated the role of FNAC in pediatric lesions in a tertiary hospital with an onco-haematological pediatric center.

Material and methods. We retrospectively evaluated 101 FNAC performed in children and adolescents ≤18 years of age presenting with a palpable mass or a deep seated focal lesion in the period extending from January 2000 to December 2013. After obtaining consent from parent, at least two ultrasound guided FNAC (US-FNAC) procedures were done by an experienced cytopathologist using a 25 Gauge needle without aspiration. Smears were fixed in absolute alcohol and stained by Papanicolau method.

In all the cases, the on-site evaluation of the material obtained was performed; in the cases with not diagnostic or scanty material, a repeated FNAC procedure was immediately performed to ensure adequate smears.

Results. Among 101 pediatric patients submitted to a US-FNAC procedure, 55 were younger than 14 years old (54.4%) and 46 (45.5%) were aged between 14 and 18 years old. Male to female ratio was 0,7:1. The most common superficial site submitted to FNAC was thyroid (34/101; 33.6%), followed by lymph node (29/101; 28.7%), breast (14/101; 13.8%) and salivary glands (5/101; 4.9%). Deep sited lesions were 5/101 (4.9%), 3 in the liver and 2 in the pancreas.

The category most represented in our series was that related to thyroid disease; into this group, there was a predominance of women over males (26 females / 8 males). Benign lesions were 85.3% (struma 13/29 cases; thyroiditis 7/29 cases; thymic ectopic tissue 3/29; nodular hyperplasia 4/29; thyroglossal cyst 2/29). Malignancy was detected in 5/34 cases, 4 papillary carcinoma and 1 histiocytosis x thyroid localization.

Acute inflammatory disease was detected in 26 over 29 lymph node FNAC (89.6%).

Fibroadenoma was the most frequent diagnosis in breast FNAC (7/14 cases), followed by 6 non-specific inflammatory conditions and 1 ductal papilloma.

The pathology of the salivary glands in 2 of 5 cases consisted in a pleomorphic adenoma. 2 cases of aspecific inflammation and 1 localization of non-Hodgkin lymphoma constituted the remaining three cases.

In our series no inadequate cases were observed due to on-site smears examination.

Conclusions. The simultaneous presence of the radiologist and the pathologist with on-side evaluation of the FNAC material is useful to reduce the quote of inadequate smears. In pediatric age, benign lesions of superficial tissue and organs are the majority of cases. FNAC is an appropriate technique to screen mass forming lesions in children and young adult to avoid unhelpful surgery.

IDH1/2 gene mutation analysis in laryngeal chondrosarcomas

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Introduction. Chondrosarcomas (CS) are malignant tumors of chondrocytes and represent the second most common type of primary bone tumors. They are most commonly found in the medullary regions of long bones, in which case they are classified as central chondrosarcomas. Usually presenting de novo as primary tumors, chondrosarcomas that develop from benign precursor lesions are called secondary chondrosarcomas. Laryngeal chondrosarcoma (LCS) is a rare tumor of the head and neck accounting for 0.2% of all head and neck malignancies and 1% of all laryngeal tumors. Their etiology is unknown. LCS arises in the cricoid cartilage in 70–75 % of the cases, particularly the posterior lamina and cricoarytenoid joint. In decreasing order of frequency, other sites are the thyroid cartilage (20%), the arytenoids, the epiglottis and the corniculate cartilage. Regional and distant metastases are rare and involve the lung and the cervical lymph nodes; they are usually identified in high-grade lesions that are less common (Damiani et al., 2014). LCS tends to be low-grade, well-differentiated, and less aggressive. Even higher grade histologies are relatively indolent in the larynx, with a low

probability for metastasis (Dubal et al., 2014). The mean age at diagnosis is between the sixth and seventh decade of life and it affects more males than females, at a ratio of 3-4:1. The molecular pathogenesis of LCS and its possible signaling pathways are largely unknown. Isocitrate dehydrogenase 1 (IDH1), and IDH2 mutations were originally reported as frequent events in gliomas and acute myeloid leukaemia. Subsequently the same mutations were revealed in at least 56% of central chondrosarcomas (Amary et al., 2014). IDH1 and 2 are metabolic enzymes involved in the citric acid cycle, participate in cytosolic NADPH production necessary for the regeneration of reduced glutathione, a major antioxidant in mammalian cells. The most common mutations affect the exon 4 in IDH1 and IDH2 genes and involve R132 of IDH1 and codon R172 of IDH2. These mutations hit an arginine residue leading to reorganization of the active site which results in loss of enzymatic function for oxidative decarboxylation of isocitrate; they induce an oncogenic gain-of-function for the NADPH dependent reduction of α -ketoglutarate in D-2-hydroxyglutarate (D-2HG). The accumulating D-2HG competitively inhibits α -KG-dependent enzymes, causing cellular alterations in epigenetics, collagen maturation, and hypoxia signaling (Patel et al., 2011). We analyzed IDH1 and IDH2 genes in a small series of laryngeal chondrosarcomas in the attempt to gain some molecular insight in this poorly defined tumor category.

Materials and methods. We reviewed the case files of our institution to identify all LCS cases. After review of the histological slides to identify areas with the highest tumor cell density, genomic DNA was extracted from formalin-fixed, paraffin-embedded archival samples using the QuickGene DNA tissue kit S (Kurabo, Immagini&Computer-Milano-Italy) on the QuickGene-810 extraction platform (Fujifilm, Immagini&Computer-Milano-Italy) according to the manufacturer's instructions. In order to verify the integrity of genomic DNA, Polymerase Chain Reactions (PCRs) were carried out for β -Globin standard laboratory gene, using different primers that give different PCR fragments size: 95bp, 165bp and 268bp. IDH1 and IDH2 genes were analyzed by direct sequencing. For IDH1 and IDH2 sequencing, we used primers that amplified the exon 4 of genes. Genomic DNA was amplified with the following PCR primers: IDH1 fragments were 214-bp (Forward: TGAGAAGAGGGTTGAGGAGTT; Reverse: 5'-AACATGCAAAATCACATTATTGCC-3' by Patel et al., 2011); IDH2 fragments were 150 bp (Forward: 5'-AGCCCATCATCTGCAAAAAC-3'; Reverse: 5'-CTAG-GCGAGGAGCTCCAGT-3' by Catteau et al., 2014). PCR condition were: 10-minute denaturation at 95°C followed by 40 cycles of 30 seconds at 95°C; 30 seconds at 60°C; 30 seconds at 72°C, and final extension of 7 minutes at 72°C. PCR products were separated by agarose gel electrophoresis for quality and quantity assessment and subsequently purified with ExoStar enzyme (GE Healthcare Europe GmbH, Freiburg-Germany). PCR products were labeled with BigDye Terminator v1.1 (Life Technologies, Applied Biosystem, Monza, Italy). Finally, the labeled samples were purified with DyeEx 2.0 Spin Kit (QIAGEN, Milan-Italy), and sequenced with AB 3130 Genetic Analyzer (Life Technologies, Applied Biosystem, Monza, Italy).

Results. Six patients had been treated for LCS in our institution from 1996 to 2013. All patients were male; mean age was 60 years (range 50 to 65). Five cases (83%) originated from the chrycoid cartilage, the last one from the epiglottis. According to WHO criteria, 3 LCS were classified as well-differentiated (G1); 1 as moderately differentiated (G2), and

2 as poorly differentiated (G3). No mutation was found in the IDH1/2 genes in the investigated cases.

Conclusions. This is the first study addressing the molecular profile of IDH1/2 genes in the subset of chondrosarcomas originating from the larynx. Despite the small size of our series, the lack of IDH1/2 mutations is interesting since it clearly distinguished LCS from CS occurring at more common sites. IDH genes are known to be involved in central and peripheral cartilaginous tumors, in fact IDH mutations have been reported in 50% of conventional central CS and in 15% of the periosteal CS (Cleven et al., 2015), but also in dedifferentiated CS. The different pathways that have been proposed to be involved in the histogenesis of LCS, as compared to central and periosteal CS may underlie the extremely different prevalence of IDH1/2 in different subgroups of the tumor. IDH1/2 mutations are considered to be early mutational events in the development of subsets of CS, although their oncogenic mechanism are poorly elucidated: a recent in vitro study suggested that while IDH1/2 mutations cause enchondromas, malignant progression towards central CS is independent of these mutations (Suijker et al., 2015). Interestingly, peripheral CS that develop from benign osteochondromas have not been associated with IDH1/2 mutations (Damato et al., 2012). The pathogenesis of chondral tumours is poorly understood, but our findings imply that LCS, that are believed to originate from the disordered ossification of the hyaline cartilage of laryngeal tissue (Brandwein et al., 1992; Thome et al., 2001), follow a tumorigenic pathway that is distinct from that of their morphological counterparts in the other districts. This hypothesis is indirectly supported by the fact that central chondrosarcomas and enchondromas are found in the setting of Ollier and Maffucci syndrome, whereas both laryngeal and peripheral CS have not been reported to occur in these syndromes (Damato et al., 2012). Other molecular pathways involved in neoplastic transformation of chondrocytes need to be explored in order to find key oncogenetic events in laryngeal chondrosarcomas.

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Cytological features of cystadenocarcinoma of the parotid gland

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Goal. Cystadenocarcinoma of the salivary glands is a very rare, slow growing, low-grade malignant neoplasm. However, when it occurs, it poses a difficult but important diagnostic challenge on cytological samples and a correct diagnosis is important to perform a correct therapeutic treatment. Fine needle aspiration cytology (FNAC) is a reliable diagnostic tool in the management of salivary glands tumours.

Aim of the present study is to describe the FNAC features of two cases of parotid cystadenocarcinoma.

Methods and materials. A 45-year-old woman and 48 year-old man, presented a slowly growing left parotid lesion. Both patients underwent ultrasound-guided FNA, performed with a 22-gaugeneedle. An on site evaluation was performed to evaluate the adequacy of the cytological samples and then the FNA material was processed for routine cytological analysis (including smears and cell blocks) and stained with Papanicolaou stain.

Results. In both cases cytological smears showed a background rich in red blood cells and proteinaceous material. Cellularity was scanty, composed of scattered cells aggregated in papillary clusters. Cells were columnar and cuboidal, small to medium size, had abundant and occasionally vacuolated cytoplasm and a low nuclear to cytoplasmic ratio. In one case the cells showed a strongly eosinophilic cytoplasm that suggest oncocyctic features.

Differential diagnosis included Warthin tumour, mucoepidermoid carcinoma, and papillary cystadenoma.

Based on the presence of papillary structures and presence of mild cellular atypia, the diagnosis of a papillary tumor of low grade malignancy was rendered in both cases and the patients underwent to parotidectomy.

On macroscopy, the lesions measured 1,2 and 1,4 cm respectively in major diameter; both presented a multicystic appearance and cysts were filled with blood and fluid. Microscopically, the tumors were well circumscribed but not encapsulated and showed variably sized multicystic and ductal spaces containing blood and cystic fluid. Cystic spaces were lined by papillary structures, composed of cells columnar and cuboidal, with bland atypia. Oncocyctic differentiation in one case was confirmed on histology and on immunohistochemistry with anti-mitochondrial antibody.

Stromal invasion was present, leading to the final diagnosis of papillary cystadenocarcinoma of the parotid gland.

Conclusion. FNAC is helpful for the differential diagnosis between a benign cystic tumor, as Warthin tumor, papillary cystadenoma and a cystic tumor of low grade malignancy like the cystadenocarcinoma. This distinction is very important because cystadenocarcinoma is typically slowly growing but complete surgical excision is the treatment of choice. FNAC is frequently performed in the pre-operative diagnosis of salivary gland tumours. Therefore knowledge of the FNAC features of rare salivary gland tumours is of utmost importance to reach a correct treatment.

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Fig. 1 and 2: FNAC smears with papillary clusters and eosinophilic cytoplasm that suggest oncocytic features (Fig. 2).

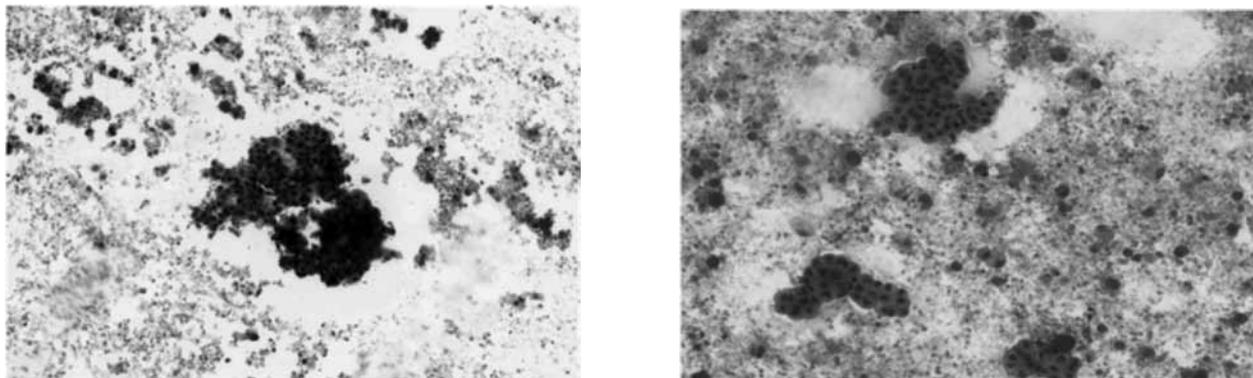


Fig. 3 and 4: Multicystic and ductal spaces containing blood and cystic fluid and lined by papillary structures, composed of cells columnar and cuboidal.

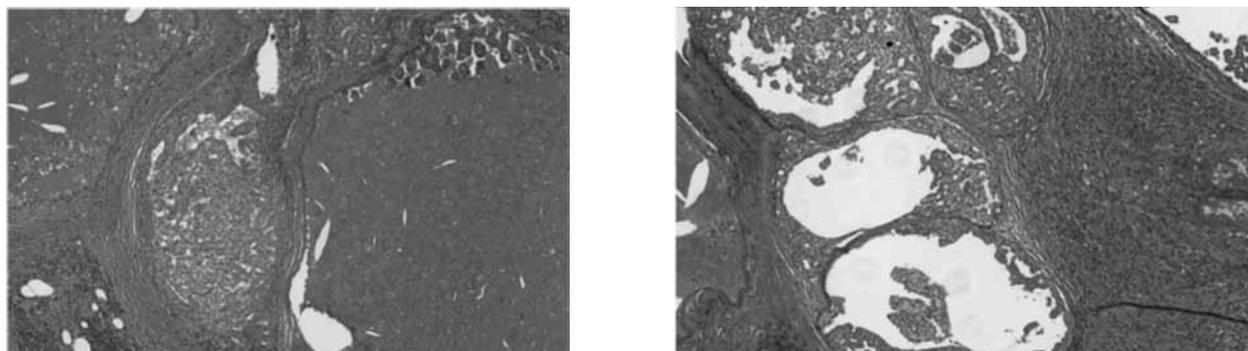


Fig. 5 and 6: A clear stromal invasion is necessary for a diagnosis of malignancy.

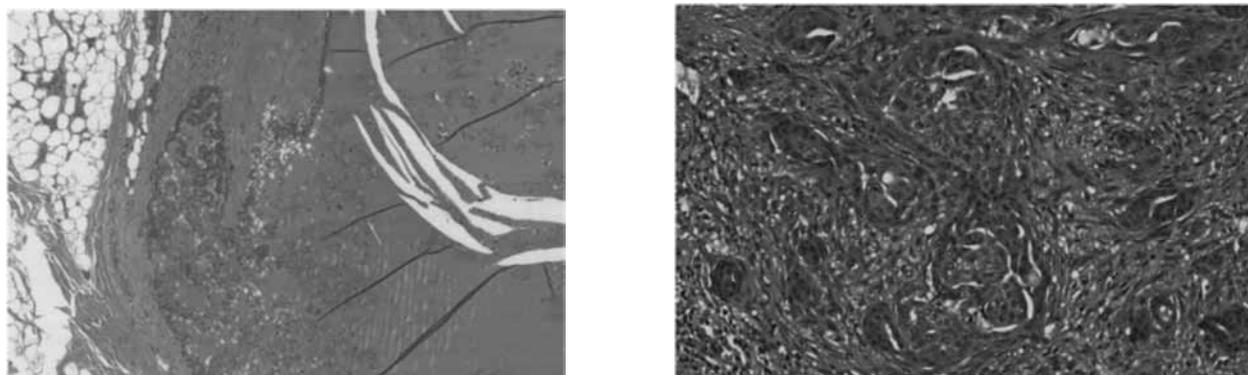
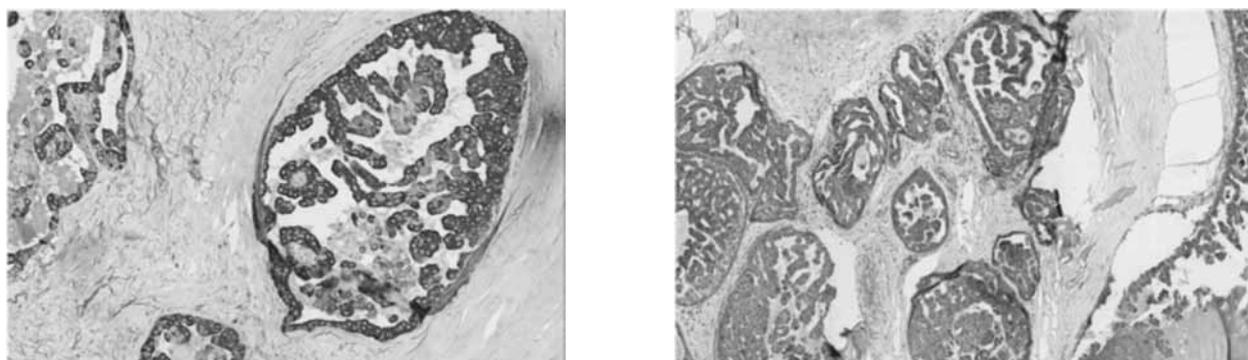


Fig. 7: CK14 diffuse and strong staining. **Fig. 8:** Oncocytic differentiation confirmed on IHC with anti-mitochondrial antibody.



Ck19 expression and HR-HPV infection in OSCC: a new insight in viral carcinogenesis

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Introduction. CK19, the lowest molecular weight keratin, is expressed in basal layer of squamous epithelia of mucosal surfaces. Previous works have shown that HR-HPV induces cell immortalization via E6 and E7 and this, in turn, deregulates cytokeratin expression in cancerous cells lines derived from uterine cervix. Here, we shown for the first time that CK19 is strongly over-expressed in oropharyngeal cancers infected by HR-HPV, and therefore identifies HPV-associated OSCC. **Methods.** We analyzed 38 cases of OSCCs (10 cases HR-HPV positive and 28 cases HR-HPV negative as evaluated by both ISH and PCR based methods). Immunohistochemistry with LSAB-HRP technique has been performed to detect CK19 expression.

Results. CK19 OSCC score (identified multiplying percentage of cancer cell expression to staining intensity) was very different between HPV+ (mean: 267.9 ± 82.5) and HPV- cancers (mean: 66.2 ± 96.9) (p < .001) with a strong evidence of correlation (p < .001; spearman's R: +0.72). ROC curve analysis was performed on CK19 expression index related to HPV positivity.

Conclusions. Strong association between CK19 up-regulation and HR-HPV+ OSCCs has been demonstrated. We identified ROC curve with a cut-off > 195 for HR-HPV positive results (Sensitivity: 92.3%; Specificity: 89.3%). Finally, CK19 test may be useful and accurate in identifying HR-HPV integration status in OSCC.

Cyclooxygenase-2 and E-Cadherin expression in oral squamous cell carcinomas: a tissue microarray study with clinico-pathological considerations

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Introduction. Cyclooxygenase 2 (COX-2) has an important role both in angiogenic mechanisms, but also in promoting cancer invasion. It is able to decrease the E-cadherin expression and to determine to phenotypic changes in epithelial cells (EMT) enhancing their carcinogenic potential.

Methods. We have evaluated the interplay between E-cadherin cytoplasmic delocalization, COX-2 up-regulation and COX-2 induced neo-angiogenesis, in 120 cases of OSCC. The distribution and the number of neo-formed endothelial buds surrounding infiltrating cells that express COX-2, as well as the neo-formed vessels in perilesional chronic inflammatory infiltrate have been studied. A double immunostaining method has been performed to verify co-localization of endothelial cell marker (CD34) and COX-2. IHC has also been used to assess E-cadherin expression.

Results. OSCC cells, which lose membranous E-cadherin staining, acquiring a cytoplasmic delocalization, hyper-expressed COX-2. A new CD34+ vessel formation (sprouting

angiogenesis) has been observed. Only the basaloid type of OSCC showed low COX-2 expression together with very low level of neo-angiogenesis and consequent tumor necrosis.

Conclusions. The COX-2 inhibitors anti-metastatic effect suggests that these molecules might have clinical utility in the clinic-therapeutic management of advanced OSCC cancers.

Inverted papilloma of the nose associated with an adenoid cystic carcinoma

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Introduction. Inverted papillomas are considered rare benign sinonasal lesions; nevertheless, invasive growth and malignant transformation are described. Only few cases of tumours of the salivary glands are reported in association with Ewing papilloma. We reported a case of a 41-year-old man with an inverted papilloma and an adenoid cystic carcinoma of the nasal mucosa.

Materials and methods. A 41-year-old man presented with exophthalmos and loss of vision of the left. The endoscopy revealed a polypoid mass in the left middle meatus. The patient underwent biopsy in the left maxillary sinus, middle turbinate and ethmoid.

Results. Histology revealed two neoplastic populations in the different fragments. The first one was represented by a polypoid neoplasia with a proliferation of pseudostratified ciliated epithelium with the few mucous cells engrowing into a fibromyxoid and vascular stroma. This aspect is consistent with an inverted nose papilloma. The second proliferation was diagnosed as a typical adenoid-cystic carcinoma arising from the minor salivary glands of nasal mucosa.

Conclusions. Inverted papillomas are occasionally associated with a carcinoma. The most frequent histotype is transitional carcinoma. Patients with simultaneous IP and carcinoma are classified into three groups. In the first one small areas of carcinoma are found in the contest of an IP. In the second group foci of IP are admixed with carcinoma. In the last one carcinoma follows IP in the same site.

In this paper we reported a unique pathologic presentation of a coexistence of IP and adenoid cystic carcinoma. This association has never been reported in literature and it is suspected to be a case of collision tumour.

Intra-operative frozen section (FS): an essential diagnostic procedure in the surgery of the major salivary glands

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Introduction. Salivary glands tumours are uncommon lesions, frequently benign, located in the parotid gland (80%). These glands not only have lesions from their own glandular cells, but can also host lesions arising from other tissues or sites, such as the lymph nodes, skin, etc. Difficulty in diagnosing the exact nature of salivary lesions is due to their histological complexity and numerous subtypes.

The surgical decision making is based on the patient's history, examination findings, imaging (CT-scan and NRI-scan) and fine needle aspiration (FNA). FNA is a pre-operative method

that has a success rate of 65% in detecting malignancy. Consequently, the aim of Frozen Section (FS) during surgery is to differentiate benign lesions from malignant tumours, to reduce the over or under treatments, to increase the chances of saving the facial nerve and to evaluate surgical margins. The aim of our study is to demonstrate the accuracy of FS procedure in the surgery of major salivary glands and to stress the need for dedicated units of medical specialist of pathology of the oral cavity.

Methods. We enrolled 499 patients (275 males and 224 females) that underwent surgery from May 2005 and October 2014. An intra-operative frozen section procedure was done for 288 of them. All of frozen sections were compared with the permanent results. The cases were classified for site, nature of the lesion and histotype according to WHO classification. The comparison between intra-operative and permanent diagnosis was done for all of them.

Results. Among the 288 FS procedures, 259 were neoplastic and 29 non neoplastic, of which 199 were benign and 60 malignant. Of 29 non neoplastic FS results, 4 were false negative. Of the 259 neoplastic FS results 2 were false positive and 2 were diagnosed as different malignant types.

Conclusions. Our results showed that the accuracy of frozen section procedure is 98% for tumours of the salivary glands. The highest concordance between frozen section and permanent diagnosis was for inflammatory process (99%), pleomorphic adenoma (98%), Warthin's tumour (97%) and malignant neoplasms (96%). In conclusion, based on these findings, frozen section of the salivary glands can be proposed as a routine procedure and it should be used in decision-making, in particular where a group of expert pathologists with years of experience is available.

Extensive necrosis and infarction in benign lesions of salivary glands: cytological and morphological study

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Introduction. Ischemic or hemorrhagic infarction has been described as an uncommon but possible complication of fine-needle cytology sampling in numerous organs, among which the salivary glands are comprised. In these situations, infarction appears to be directly related to the vascular disturbances caused by needle sampling. Spontaneous infarction of salivary glands benign or malignant lesions remains a very rare event.

Materials and methods. Two cases of extensive necrosis and infarction of salivary gland tumors correlating differences between Fine Needle Aspiration cytology (FNAC) samples and histological specimens are presented.

Results. Case 1: A 60-yr-old man presented with a 4 cm right parotid mass. Smears obtained by FNAC revealed only abundant necrotic debris and granular calcification. Due to the inconclusive cytopathologic diagnosis, parotidectomy was performed. Histologic examination of the whole lesion, demonstrated a well-capsulated nodule entirely constituted by necrotic cells and diffuse microcalcifications. Immunohistochemistry was unhelpful to define the lesion. The histopathological diagnosis was of spontaneous infarcted adenoma.

Case 2: A 79-year-old woman presented with a 2.5cm mass in the right parotid gland. The FNAC revealed a highly cellular smear composed of clusters of oncocytic cells without atypia, compatible with oncocytic adenoma. Histologic ex-

amination of the specimen demonstrated extensive necrosis, hemorrhage, fibrosis, chronic and acute inflammation with pseudoxanthomatous reaction associated with metaplastic squamous cells.

In case 1 the necrotic features were present at the time of presentation, as were seen on FNAC, thus suggesting that benign lesions can undergo spontaneous infarction. In the second case, the changes seen in the surgical resection specimen were not present in the aspiration material, indicating that they were most likely due to the traumatic injury of needling.

Conclusions. Therefore, it is our purpose to underline that in the salivary glands, extensive infarction may be caused by FNA sampling or may be a spontaneous event and may concern benign tumors. Since necrosis can obscure the nature of pathology and mimic carcinoma, pathologists should be aware of these possibilities to avoid misinterpretation that will cause adverse clinical impacts.

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Fig. 1. Case 1. Low power magnification, well-capsulated nodule entirely constituted by necrotic cells and diffuse microcalcifications.

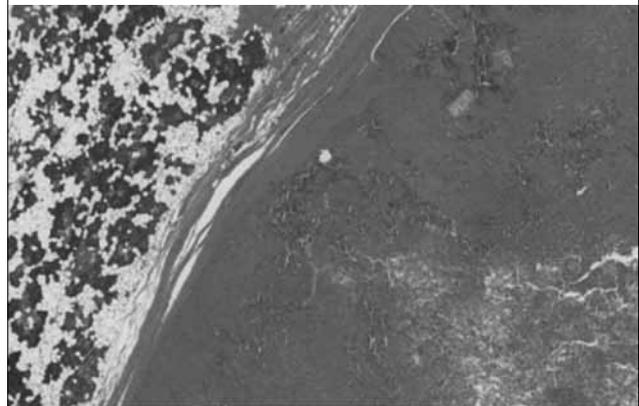
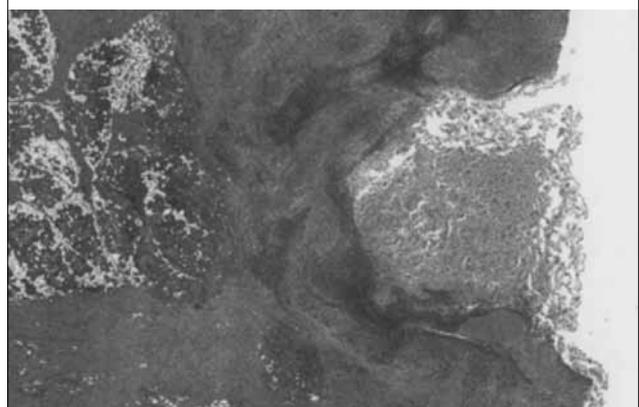


Fig. 2. Case 2: Low power magnification, extensive necrosis, hemorrhage, fibrosis, chronic and acute inflammation with pseudoxanthomatous reaction associated with metaplastic squamous cells.



Extraventricular anaplastic ependymoma in an adult: a case report

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Introduction. Ependymomas are tumors of the central nervous system that account for about 3-5% of all adult intracranial gliomas, arising from ependymal cells of the ventricular system, choroid plexus, filum terminale, or central canal of the spinal cord. They may occur outside the ventricular structures, without any relationship with the ventricular system, representing the rare group of ectopic ependymoma. Less than thirty cases of ectopic ependymomas were reported, and almost fifteen were purely cortical, and only five cases were anaplastic lesions. We present a case of an 25-year-old man with a large ectopic anaplastic ependymoma.

Methods. A 25-year-old man presented with progressively increasing headache and vomiting of six months duration. He also had right-side motor weakness and seizures. Contrast computerized tomography (CT) brain scan showed a left frontal located mixed density lesion with heterogeneous enhancement. A left-side craniotomy and gross total excision of the tumor was done. The tumor had no connection to the ventricular ependymal lining. Margins were well defined.

Results. Hystological examination revealed that the lesion was very cellular and well vascularized. Vascular proliferation and focal necrosis were seen. The nuclei were polymorphic; there were some mitotic Fig.s and perivascular pseudorosettes formations. Immunohistochemical study revealed positive staining of the tumor cells for GFAP, S100 protein,

vimentin, L1CAM and EMA “dot-like”, negative staining for Synaptophysin and Olig2. The MIB-1 labeling index was 5%. The pathological diagnosis was anaplastic ependymoma (WHO grade III).

Conclusions. Although approximately half of the supratentorial ependymomas arise from ependymal cells of the ventricular system or choroid plexus and are purely intraventricular, the remaining has extension through adjacent cerebral tissue, representing extraventricular forms of ependymoma. Only few cases occur in distant places of the ventricular system, representing rare cases of ectopic lesions. The pure cortical ependymomas may arise from embryonic rests of ependymal tissue trapped in the developing cerebral hemispheres. The supratentorial ependymoma tends to be larger in size at the time of diagnosis. The principal differential diagnosis of pure cortical ependymoma should include astrocytoma (both low grade and glioblastoma multiforme), supratentorial primitive neuroectodermal tumor (PNET), ganglioglioma, gangliocytoma, oligodendroglioma and astroblastoma. In pathologic examination, the tumor cells are characteristically organized in perivascular pseudorosettes and, less commonly, ependymal rosettes. Although ependymomas are moderately cellular tumors with rare mitotic Fig. s (World Health Organization (WHO) grade II lesions), our patient had a more aggressive tumor, classified as WHO grade III.

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Dermatopatologia

Braf V600E mutational status in melanoma: comparison of molecular analysis and immunohistochemical expression

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Introduction. The BRAF V600E mutation represents one of the most common alterations in cutaneous melanoma. The mutated protein has become the target of treatment with small-molecule inhibitors and this hot spot is predictive of response in patients with metastatic melanoma. Several molecular methods are currently available to determine BRAF mutational status. Recently, a monoclonal antibody (VE1), directed against the V600E protein, was generated. Our aim was to determine the sensitivity and specificity of the VE1 antibody (Roche-Ventana).

Material and Methods. We analyzed a retrospective series of 60 melanoma patients (54 metastatic and 6 primitive), treated with medical therapies, using a fully automatized immunohistochemical method in order to implement the use of this antibody in routine diagnostic procedures. These samples have been previously investigated for BRAF status with molecular techniques: Sanger sequencing, pyrosequencing or allele specific PCR (COBAS). Regarding the immunohistochemical analyses, the stained slides were reviewed by two observers in a blinded fashion with respect to molecular BRAF status.

Results. The tumors with the BRAF V600E mutation, but not with other BRAF mutations (ex. BRAF V600K), showed cytoplasmic positivity (score 2-3) with the VE1 antibody. Comparing immunohistochemical and molecular methods, 55/60 cases showed concordant Results. Thirty-one of them were BRAF V600E mutated and also showed positive IHC staining, and they had been treated with BRAF inhibitors according to molecular data. Statistical analyses demonstrated that the sensitivity of immunohistochemistry (IHC) was 88.6% and specificity was 96.0%. The overall concordance was 91.7%. IHC positive predictive value for BRAF V600E detection was 96.9%, while the negative predictive value was 85.7%.

Conclusion. Findings from the current study support that IHC is an accurate, rapid ancillary screening tool to assess BRAF V600E mutation status in melanoma patients who could be guided rapidly towards an appropriate clinical therapy.

Focal mucinosis: beyond the mucin deposits

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Background. The clinician sent us a cutaneous sample with the presumptive diagnosis of achromic atypical nevus of the right leg. The formalin fixed sample showed at macroscopical examination a slightly elevated whitish lesion measuring 0,4 mm in greatest diameter.

Material and Methods. On H&E stained section we observed a dome shaped lesion with normal epidermis and

focal accumulation of myxoid material in the reticular and mid dermis, giving it the appearance of "empty dermis" (Fig. 1). In the context of this material we found a rarefaction of collagen fibers and an increased number of small vessels (Fig. 3).

At strongest magnification a population of scattered fibroblast with spindle and star-shaped becomes apparent. The amorphous substance had smooth and not sharp contours and almost reached the hypodermis, sparing the adnexa. In order to confirm the composition in mucin of the myxoid material, we stained the sample with ALCIAN 2.5 and to exclude other diagnosis we perform also AMART-1, SMA, Cytokeratin, S100, cKIT, Vimentin and Desmin immunohistochemical staining.

Results. The histochemical staining for ALCIAN BLUE revealed wide mucin deposits in dermis, probably composed of abundant hyaluronic acid (Fig. 2).

The negative staining for AMART-1 allowed us to exclude the diagnosis of mucinous nevus, whose overlying epidermis usually show acanthosis.

Negativity for cytokeratin, SMA, Desmin, s100 and positive stain for Vimentin (Fig. 4) confirmed the fibroblastic nature of spindle and stellated cells floating in mucin pools, also excluding a form of ancient schwannoma, a nerve sheath myxoma or other soft tissue tumors.

The increase of vascularization was not enough to support the diagnosis of angiomyxoma and, generally, the absence of cKIT positive mast cells sustained the decision of excluding the cutaneous myxomas from differential diagnosis.

However SMA, with its strong staining of vessels, indirectly oriented us to consider the lesion as benign because for the nodular, expansive and not infiltrating way of growth (Fig. 5). The homogeneous dislocation of vascular structure clearly revealed this not aggressive behavior.

Conclusion. The ultimate diagnosis was focal mucinosis, because for its benign nature and the unequivocal fibroblastic nature of the most represented cellular population in mucinous matrix.

We concerned about some degenerative aspect of these cells, like ballooning, but the same staining properties led us to consider them different stages of fibroblasts.

The importance of the diagnosis is given by the correlation of multiple lesions with hypothyroidism, and the clinician should investigate the presence of similar flesh colored papules.

Fig. 1. 10x Hematoxylin and Eosin.

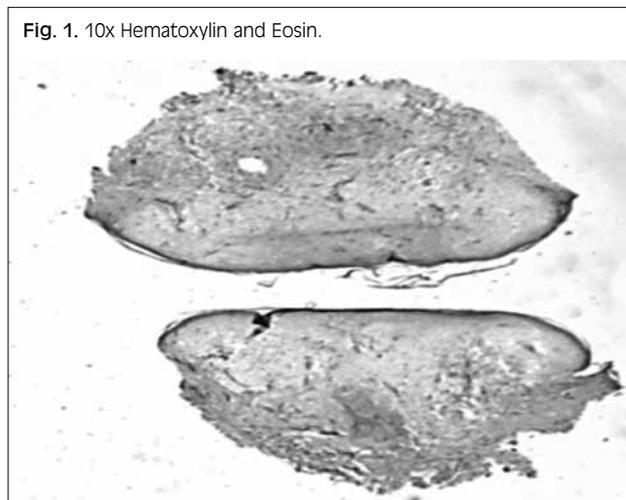


Fig. 2. 10x ALCIAN BLUE.

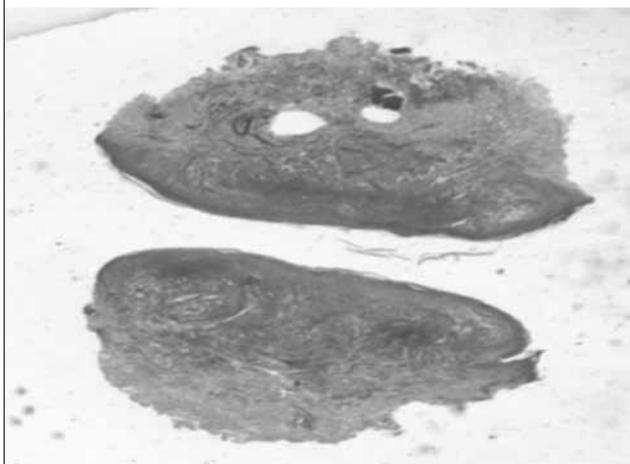


Fig. 3. Hematoxylin and Eosin 40x.



Fig. 4. 40x Vimentin.

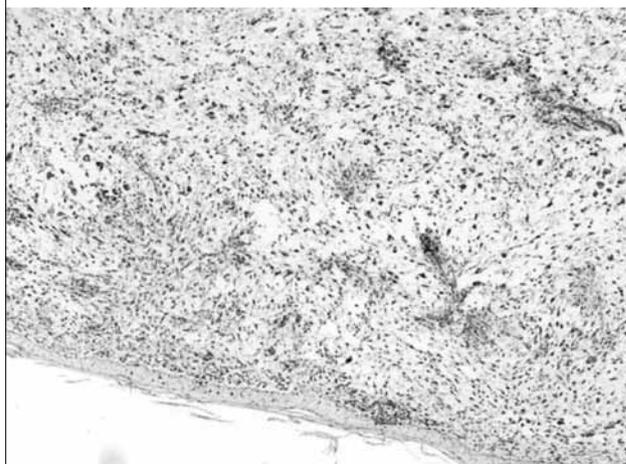
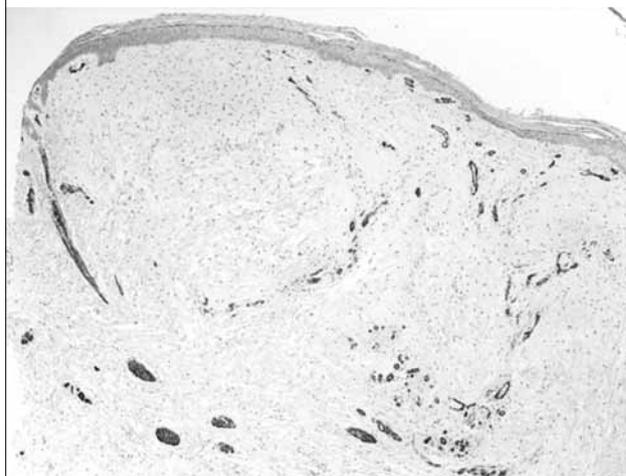


Fig. 4. 40x SMA.



Nasal glial heterotopia in a female child

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Introduction. Nasal glial heterotopia (NGH) and nasal glioma are both correct terms used to describe rare, nonhereditary, benign, congenital malformations, composed of mature brain tissue isolated from the cranial cavity. The majority of these benign congenital tumors are found in the nasal region and occur on the bridge of the nose. Three types of clinical presentation have been recognized: extranasal (60%), intranasal (30%), or both (10%). The remaining few are seen elsewhere in the facial region. These malformations do not usually have any anatomical continuity with central nervous system tissue, a characteristic that distinguishes them from cases of encephalocele.

Nasal glial heterotopia is frequently diagnosed in newborn infants, however, it may rarely be found in adults. Clinically, external nasal gliomas present as masses that do not transilluminate, are not affected by crying or straining, and do not distend with jugular venous compression (Furstenberg sign). At present, approximately 250 cases have been reported. We report recent case of NGH in a female child, presenting as mass located on the left nasopalpebral region.

Methods. A two and a half year old male infant presented at birth with a congenital, 1,3x1,3x 0,7 cm, mass located on the left side of the nose.

Physical examination revealed round, solid, non pulsating, painless tumor covered by erythematous skin. This mass showed no growth or change in size during crying or jugular vein compression (Furstenberg sign). Specific information regarding the relationship of the lesions to the CNS was documented by radiographic studies and intraoperative findings. Doppler flow studies reveal a low arterial flow velocity during the end-diastolic phase. MRI reconstruction images showed that the lesion did not exhibit intracranial extension.

Surgical excision was performed to prevent secondary distortion of the visual development sequelae.

Results. Pathologic evaluation of the excised mass showed tissue consisted of astrocytes without mitosis and neuroglial fibers intermixed with a fibrovascular connective tissue stroma. Inflammatory cells were identified occasionally. Immunohistochemical reactivity with glial fibrillary acidic protein and S-100 protein confirm the histologic diagnosis, consistent with neuroglial heterotopia, while collagen type IV and laminin highlight the reactive fibrosis.

However, no neuronal or other elements like choroid, ependyma or retinal tissue was noted in our case.

Conclusions. Clinical examination and CT and MRI images did not contribute in the diagnosis. Differential diagnosis includes neurogenic tumors, ectodermal tumors, mesodermal tumors, teratomas and lymphatic malformations.

These benign lesions can be distinguished only by histopathological and immunohistochemical examination. The histology of nasal glial heterotopia may be difficult to identify with hematoxylin and eosin stain alone. Special stains and immunohistochemistry, such as glial fibrillary acid protein (GFAP) immunostain, which enhances the glial tissue, and positive synaptophysin and neurofilament which confirms the presence of neuronal cells, are thus of great utility. In summary, the greatest difficulty in yielding a diagnosis of nasal glial heterotopia is not considering this condition. It should be noted that there are no significant histologic differences between lesions with and without demonstrable CNS connection. Therefore, an accurate diagnosis of heterotopia versus encephalocele requires knowledge of the patient's radiographic and/or operative findings.

The potential for an intracranial connection must always be kept in mind when dealing with a congenital midline mass. MRI imaging should be requested and preoperative biopsies avoided.

Ultimately, complete surgical excision provides a definitive diagnosis and curative treatment.

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Ematopatologia

Langerhans cell histiocytosis with spinal involvement in young man: case report

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Background. Langerhans cell histiocytosis (LCH) is a rare condition characterized by a clonal proliferation and accumulation of a specific histiocyte: the dendritic Langerhans cell. LCH manifested either as localized (unifocal or multifocal) or systemic. Clinical variants of LCH include eosinophilic granuloma (EG, a benign variant that primarily affects children and young adults, single or multifocal bone lesion either without visceral involvement), Hand-Schüller-Christian disease (typically in children with classic triad of skull lesions, exophthalmos and diabetes insipidus) and Letter-Siwe disease (life-threatening, disseminated lesions involving visceral organs which presents during infancy or early childhood).

Methods. A 18 years old man showed up at the emergency room physicians because he complained acute lombar pain and paraparesis. Computer tomography scan (CT) total body showed an expansive process measuring 4x3 cm in size which occupied L5 extradural rear space in the median and bilateral paramedian, larger on left side where it was interested epidural space with footprint on dural sac and root left nerve L5-S1. At L5 level, the injury resulted also partial erosion of spinous process and vertebral arch. There was no involvement

of the abdominal and thoracic viscera. The patient underwent an surgical excision of neoplastic proliferation and deep soft tissues near the tumor.

Results. Several surgical specimens came for histological examination: fragments of L5-S1 lamina, epidural adherence, epidural and intradural tissues fragments and tumour-like mass of L5 spinous process.

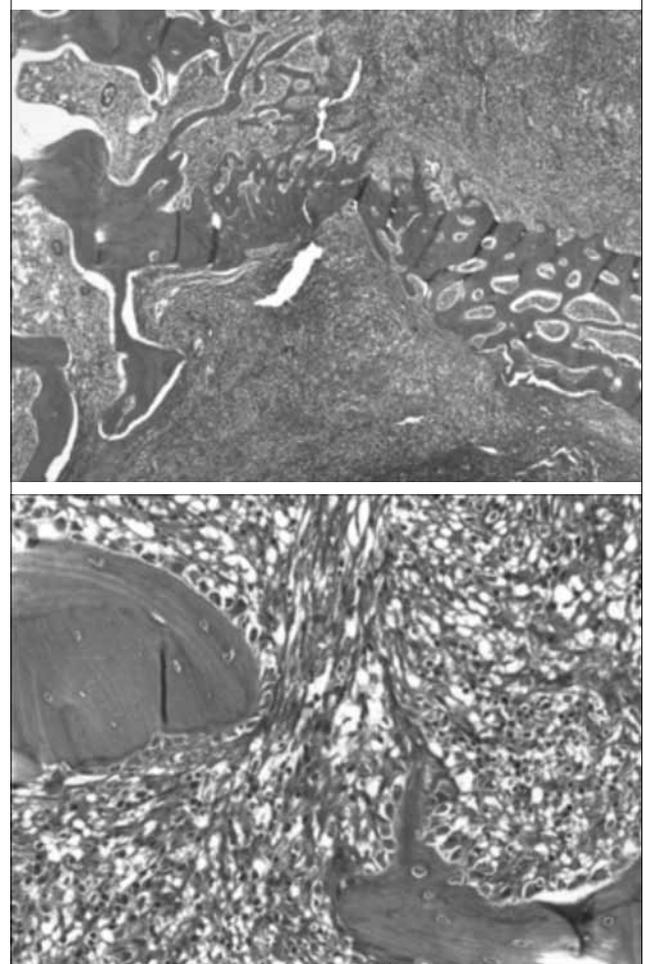
On gross examination, the mass (size 4x3x2 cm) was ill-defined, brownish-red in colour, and jarring to cut for bone presence inside.

Histological evaluation revealed vertebral bone and surrounding deep soft tissues infiltration by loose aggregates of abnormal histiocytic apparent cells in a mixed inflammatory background (large numbers of eosinophils occasionally aggregated in microabscesses, lymphocytes, plasma cells, macrophages and multinucleated giant cells). Histiocytic-like cells were intermediate size with indistinct cytoplasmic borders, eosinophilic to clear cytoplasm with oval nuclei which frequently was indented, irregular in outline and possessed nuclear grooves. Mitotic index was < 5 mitoses per 10HPF. These cells expressed vimentin, CD68, S100, lisozime and intensely CD1a and, therefore, they were interpreted as Langerhans cells.

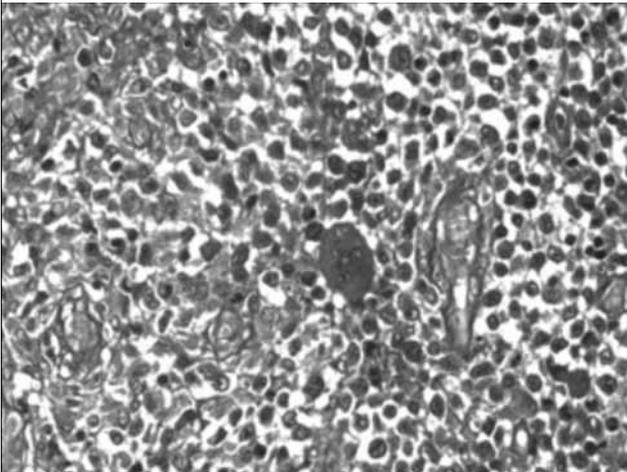
A diagnosis of spinal bone localization of Langerhans cell histiocytosis was made.

Conclusions: LCH represents a spectrum of a rare disorders characterized by idiopathic infiltration and accumulation of

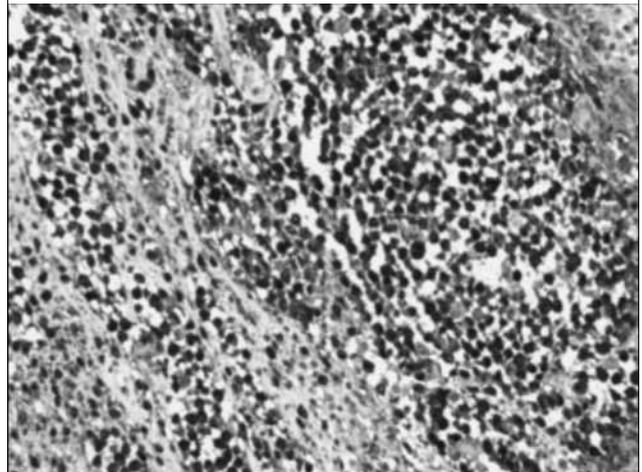
1-2) Tumor-like infiltration of the vertebral spongy bone, E/E



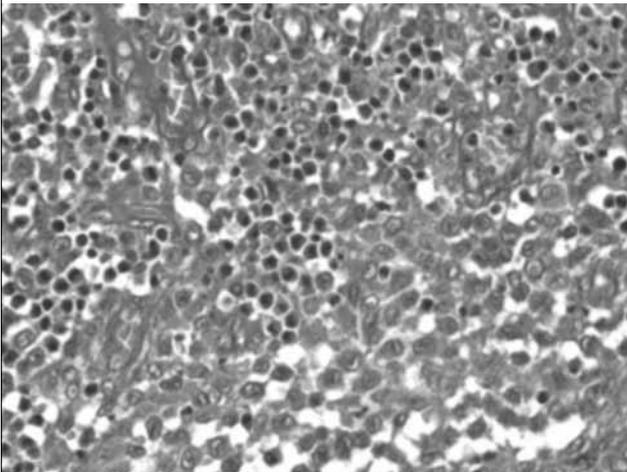
3) Langerhans cells on a rich inflammatory background with eosinophils and giant cells, E/E



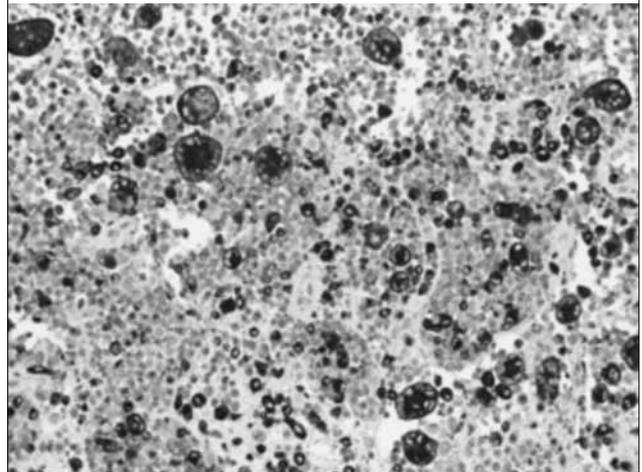
6) S-100



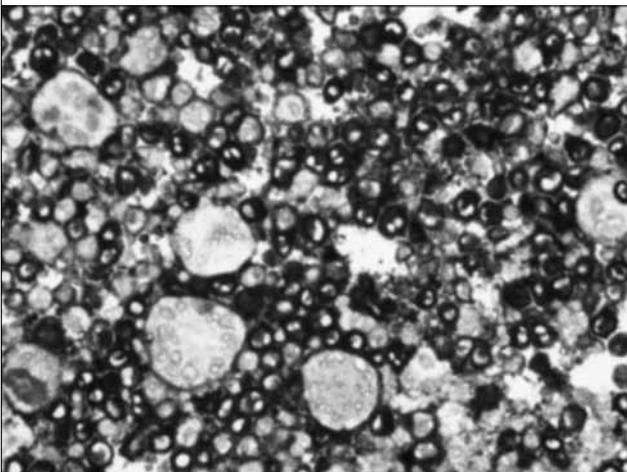
4) Eosinophils microabscesses, E/E



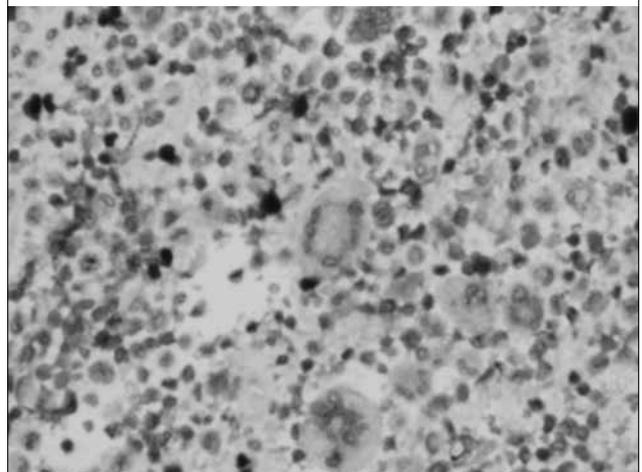
7) CD68



5) CD1a



8) Ki-67



abnormal histiocytes (i.e. the Langerhans cells) within various tissues (bone marrow, skin, central nervous system, lung, liver, spleen, lymph nodes), and it caused focal or systemic effects. LCH predominates in children and its annual incidence is estimated at 4.6 per million in children under 14 years of

age. LCH was formerly known as “histiocytosis X”, a term that grouped three major syndromes, which are now considered as clinical variants of the same disease: eosinophilic granuloma (benign unifocal LCH), Hand-Schüller-Christian disease (multifocal LCH with classic triad of skull lesions,

exophthalmos and diabetes insipidus) and Letter-Siwe disease (fulminant LCH with multiple organ involvement). The key feature is the identification of the LCH cells CD1a positive in a mixed inflammatory background with a large numbers of eosinophils. The ultrastructural hallmark is the cytoplasmic Birbeck granules.

The aetiology of LCH remains unknown, and it is still uncertain whether LCH is a neoplastic disorder, suggested by the monoclonality in lesions, or a reactive disorder resulting from a dysregulation of the immune system.

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BOM histopathological diagnosis: identification of blast cells using CD34/clone QBEND 10 antibody

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Background. Anti-CD34 clone was used for scientific study and in transplantations, to quantify and purify lymphohaematopoietic stem and progenitor cells and it is also a useful marker for the identification of vascular and lymphatic tumors and sub-classification of leukemias, because it brand selectively marrow progenitors (blasts), stem cells and endothelial cells. CD34 is a single-chain transmembrane protein of approximate weight of 116 kDa, expressed on immature hematopoietic stem/progenitor cells, capillary endothelial cells, embryonic fibroblasts and rare glial cells of the nervous tissue. CD34 appears to be expressed at highest levels on the earliest progenitor cells and to decreases progressively with maturation. CD34 is a "stage specific", rather than a lineage-specific, leucocyte differentiation antigen. Approximately 60% of acute B lymphoid leukemia, 40% of acute myeloid leukemia and from 1 to 5% of acute T-lymphoid leukemias express CD34, while it is negative in chronic lymphoid leukemias, lymphomas and multiple myelomas. Monoclonal antibodies to CD34 can be confined to three main classes defined by the specific sensitivity of epitopes of the same and from degradation by specific enzymes. The QBEnd is a class II monoclonal antibody that recognizes an epitope of the CD34-resistant neuraminidase, but which is responsive to the glycoprotease and chymopain¹⁴.

The purposes of our study were: i) to evaluate CD34 expression in BOM diagnosis to identify a CD34+ blast cells standard range, in order to discriminate between myelodysplastic syndromes/myeloproliferative diseases or frankly neoplastic bone marrow; ii) to establish an association between the percentage of existing CD34+ blast cells and disease severity and its development.

Methods. One hundred ninety-three bone marrow biopsies of patients in follow-up for blood diseases, oncological and non-oncological, received in the year 2013 in our center, entered to the study. Each bone marrow biopsy was submitted to decalcification for twenty-four hours and it were processed

and included in paraffin block successively. Fifteen sections were obtained from each paraffin block and used for Hematoxylin-Eosin stain, PAS reaction, Gömöri stain and for immunohistochemical assay: FATT-8, CD61, CD138, CD5, CD23, Myeloperoxidase, CLA, CD20, CD3, CD34 and CD68. CD34/clone QBEND 10 immunostaining was used for blast cells identification in histopathologic diagnosis of bone marrow biopsies. Data of 193 BOM converted into histograms and showed in figure 1.

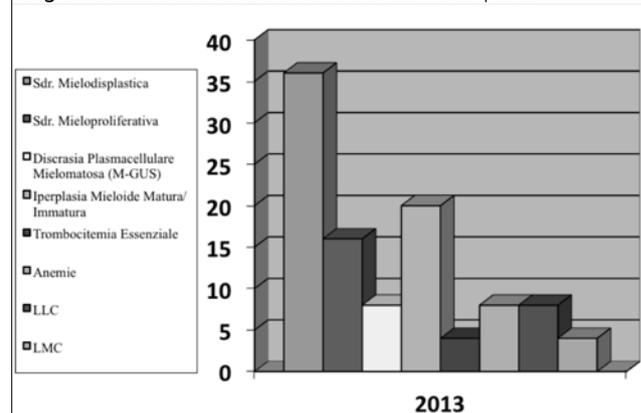
Results. Twenty-five out of 193 cases showed CD34+ blast cells in a range from 1% to 10%, and were distributed as follows: 9 (36%) out of the 25 cases had a myelodysplastic syndrome; 4 (16%) had a myeloproliferative syndrome; 2 (8%) had a myeloma plasma cell dyscrasia (M-GUS); 5 (20%) had a myeloid hyperplasia mature/immature; 1 (4%) had an essential thrombocythemia; 2 (8%) had an anemia (1 patient had an acquired variant and 1 patient had a pernicious variant); 2 (8%) had a chronic lymphocytic leukemia, B-type (B-CLL) and "null" and the last 1 case (4%) had a chronic myeloid leukemia (CML) (Figure 1). In our study we established three standard range ratio: $\leq 1\%$; 2-5%; 6-10% and our data showed that the myelodysplastic syndromes and myeloproliferative and mature/immature variants myeloid hyperplasia, in which blasts ratio is in the third range, showed a clonal activation and any recurrence or reactivation of disease and a poorer prognosis because of the presence of immature, undifferentiated and totipotent cells.

Therefore, to quantify blast cells in the samples and to bring it back in a ratio range could be useful to identify the disease aggressiveness and to affirm that the disease variant in which range of blast cells was less or equal than to the 1% was irrelevant in the diagnosis and prognosis of the cases (Table 1). Conclusions: Our data show that using anti-CD34/clone QBEND 10 immunoreaction for the identification of blast cells in BOM histopathological diagnosis it is possible to detect, to count and to include in a standard range blast cells to improve BOM differential diagnosis.

In this study we find that there is an association between the percentage of existing CD34+ blast cells and disease prognosis.

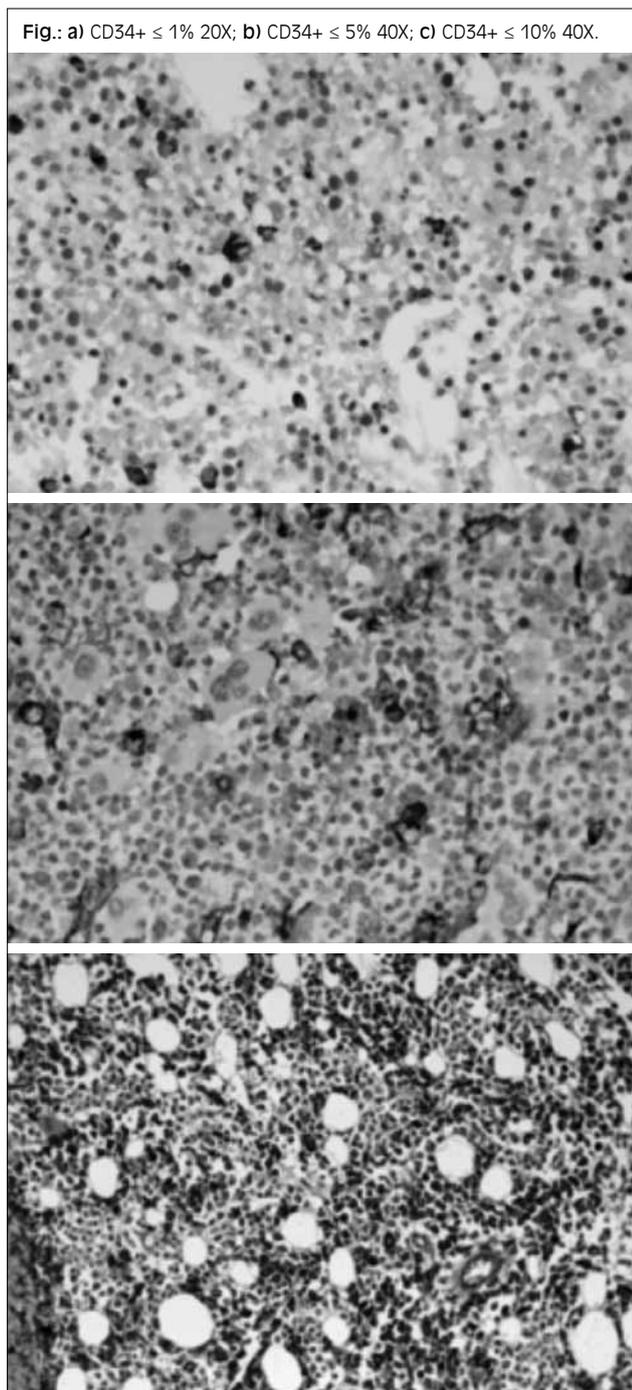
In conclusion, the ratio of CD34+ blast cells is a useful marker in aggressiveness and disease development, this could explain the resistance in targeted treatments because the high percentage of immature elements and lack of differentiation and cell immaturity were negative prognostic factors.

Figure 1. Distribution of Data (193 bone marrow biopsies).



Tab. I. Standard range ratio: ≤ 1%; 2-5%; 6-10% and distribution of disease variants.

DISEASE	RANGE CD34+ (%)		
	≤ 1%	2-5%	6-10%
myelodysplastic syndrome	3	3	3
myeloproliferative Syndrome	1	2	1
M-GUS	0	2	0
hyperplasia myeloid	3	2	0
essential thrombocythemia	1	0	0
ANEMIA	2	0	0
LLC	0	2	0
LMC	1	0	0



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Optimal minimal panels of immunohistochemistry for diagnosis Of B-cell lymphoma for application in countries with limited resources and for triaging cases before referral to specialist centres

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Introduction. Lymphomas are a collection of different malignancies ‘arising’ from lymphoid cells. It includes about 49 entities and over 19 provisional entities and subsets. About 85% of lymphomas are of B-cell origin. Precision in lymphoma diagnosis requires expertise and infrastructure. The entities are defined based on morphology, immunohistochemistry (on some occasions, in situ hybridization), cytogenetics /fluorescent in situ hybridization (FISH), molecular genetics and clinical information. The primary aim of the study was to establish and validate optimal minimal panels of immunohistochemistry that can be used in a staged algorithmic manner to arrive at a precise diagnosis in most cases of suspected B-cell lymphomas in countries with limited resources. Through this work, we also wanted to suggest short panels of immunostains that can be used in referring units who send suspected lymphomas to specialist diagnostic centers in resourceful countries. Overall, the approach we suggest could help in cutting costs and improving quality in both resource poor and resourceful countries.

Methods. The study was initially performed on 296 cases where a B-cell lymphoma or a HIV-associated lymphoma was suspected on initial morphological evaluation during the January 2012 to June 2012 at the Department of Histopathology, of Hammersmith, an extended and validated with an additional 516 cases of suspected B-cell lymphomas seen during the period January 2005 and June 2012 at Department of Medical Biotechnologies of the University of Siena, Italy. Cases where the initial morphological description suggested Hodgkin lymphoma or a T cell lymphoma were excluded. Extensive immunohistochemical work-up had been undertaken on these cases. Where required, in situ hybridization for EBER and light chains, FISH analysis for MYC, BCL2, BCL6 and IG genes, and antigen receptor gene rearrangement studies had been undertaken. The diagnostic process was employed by two phases, phase 1 and phase 2. In phase 1 we identified six types of the B-cell lymphomas having very characteristic morphology and thus requiring a short panel of immunostains to confirm the diagnosis (suspected chronic lymphocytic leukaemia / small lymphocytic lymphoma; suspected follicular lymphoma; suspected mantle cell lymphoma; suspected diffuse large B-cell lymphoma-DLBCL; suspected Burkitt lymphoma-BL). B cell lymphomas that were not diagnosticable by phase

1, slot to phase 2 characterized by five different algorithms or decision trees (nodular pattern; diffuse/interfollicular pattern with small to medium sized cells; diffuse blastic infiltrates of medium sized cells; cases with features overlapping between BL and DLBCL; Biopsies from HIV-positive patients) based on morphology and clinical information.

Results. 812 cases where a B-cell lymphoma or a HIV-associated lymphoma was suspected on morphological grounds were evaluated. This led to arriving at a specific diagnosis of 799 B-cell lymphomas. A correct diagnosis was achievable in 69% cases with the application of 3-5 antibodies; others required additional work-up.

Conclusion. The panels/algorithms suggested assist: 1) well trained pathologists to practice lymphoma diagnostics in countries with limited resources; 2) lymphoma referrals to specialist centres.

Metabolic reprogramming in B-cell lymphomas

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Background. Cell proliferation requires nutrients, energy and biosynthetic activity to duplicate all macromolecular components during each passage through the cell cycle. It is therefore not surprising that metabolic activities in neoplastic cells are fundamentally different from those in non-proliferating cells. Although cancer represents a heterogeneous group of diseases with a wide variety of alterations, one common feature is the ability of tumor cells to use glycolysis instead of oxidative phosphorylation for their energy production (phenomenon termed the Warburg effect). Functionally related, tumor cells show an increased protein, DNA, and fatty acid synthesis to sustain the rapid proliferation and to counteract the hostile environment. The tumor environment creates a unique collection of stresses to which cell must adapt in order to survive. This environment is formed by the uncontrolled proliferation of cells, which ignore the cues that should create normal tissue architecture. As a result, the cells forming the tumor are exposed to low oxygen and nutrients, as well as high levels of toxic cellular waste products, which is thought to propel cells toward a more transformed pro-metastatic phenotype, resistant to cell death. The change in metabolism cannot be purely attributed to alterations in allosteric and product/substrate regulation of the metabolic enzymes. A concerted energy response also occurs, involving factors such as mammalian targets of rapamycin (mTOR), MYC and the hypoxia-inducible factors (HIFs), which are vital for the long term metabolic transformation of tumours. Despite these new insights into the metabolomics of cancer, the extent to which metabolic reprogramming plays a role in lymphomagenesis is still poorly explored. Starting from our previous results in Burkitt Lymphomas (BL), we aimed to deeply characterize the metabolic fingerprint of aggressive B-cell lymphomas.

Material and methods. We studied a total of 400 lymphoma cases including 60 BL, 289 diffuse large B-cell lymphoma-DLBCL; 20 high proliferating (Ki-67>90%) DLBCL, 30 plasmablastic lymphoma-PBL and 60 primary mediastinal B-cell lymphoma (PMBCL). On these cases immunohistochemical evaluation for MYC protein expression, Ki-67, HIF-1 α , adipophilin,

fatty acid synthase (FASN) was carried out. On all the cases FISH analysis for MYC translocation was performed.

Results. We detected a strong Ki-67/MYC/HIF-1 α /adipophilin/FASN correlation. Apart from the histological subtypes, activation of metabolic reprogramming was found in high proliferating cases showing an over-expression of MYC at protein level. In fact only DLBCL cases with a high proliferation rate and with MYC protein over-expression presented adipophilin and FASN expression. Interestingly, among these cases, 13 samples also exhibited BCL-2 protein expression (i.e. double-hit DLBCL). On the contrary, PBL cases with no MYC over-expression showed no lipid metabolism activation. PMBL showed no expression of adipophilin and FASN. We are collecting all the clinical data necessary to evaluate whether the activation of lipid metabolism may impact on patients' outcome. Finally, we are performing in vitro studies on different lymphoma cell lines to assess the effect of drugs commonly used as inhibitors of glycolysis and fatty acid synthesis. **Conclusions.** Our results by identifying the biological mechanisms sustaining the metabolic profiles changes could evaluate whether these deregulated signatures might serve as a biomarker for cancer detection and could potentially provide novel therapeutic approaches for treatment, by using less toxic and expensive drugs.

Patologia endocrina

Microapillary variant of papillary thyroid carcinoma: an observational study of clinicopathological characteristics in a single institution

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Introduction. Among thyroid tumours, papillary carcinoma (PTC) represents the most common histotype. Typically, PTC has a good prognosis with a 10-yr survival greater than 90%. However, rare variants of PTC exist, which feature tall or columnar cells. These rare subtypes are referred to as aggressive PTC variants, in that they portend an unfavourable clinical course. Recently, a micro-papillary pattern in PTC has been indicated as poor prognostic characteristic, but this suggestion is based only on a few series of cases.

Methods. A total of 295 consecutive PTC cases were collected between the years 1992 and 2014. Of these, 242 (82%) were females, while 53 (18%) were males (F:M ratio= 4.7:1), with a mean age of 50 yrs (range 14-92). The corresponding histologic sections (at least 6 for each case) were stained with haematoxylin and eosin. Next, two pathologists reviewed independently the sections to reach a consensus on the identification and quantification of the micro-papillary pattern, as defined by its presence in at least 20% of the tumour. Moreover, on silane-coated serial sections, the immunohistochemical expression of thyroglobulin (TG), antimucin 1 (MUC1), epithelial membrane antigen (EMA), podoplanin (D2-40), thyroid-transcription-factor-1 (TTF-1) and MIB-1 was tested; the nuclear counterstain was performed by Mayer's haematoxylin.

Results. Of the 295 PTC, 124 (42.5%) were codified as follicular variant, 104 (35%) as classic PTC, 34 (11.5%) as sclerosing, 15 (5%) as tall cells, 10 (3.4%) as Warthin-like, and 8 (2.6%) as

micropapillary variant. In this last group, the F:M ratio was 6:2 (= 3:1), and mean age was 55 yrs; spread to lymph nodes was detected in 2/8 cases (25.0%). In 4/8 cases (50%) the malignant nodule was localized in the left lobe, and only in 1/8 (12.5%) it was bilateral. Coexisting lesions were only of two types: hyperplastic nodules (6/8= 75%) or chronic lymphocytic thyroiditis (CLT; 2/8= 25%). Of interest, the 2 cancers with associated CLT had the highest rate of hobnail features (60 and 70%), the rate in the other 6 cases ranging from 25 to 40%. Of additional interest, only the same 2 cases spread to cervical lymph nodes. These 2 cases and another 2 cases (all 4 being women) showed vascular invasion. Within a mean follow-up duration of 40 months, all 8 cases experienced neither local recurrence nor distant metastases. Immunohistochemical findings were indistinguishable from those of the classical PTC, although a constant linear MUC1 reactivity stroma-facing surface with an inside-out pattern was detected in all 8 cases.

Conclusions. Further investigations, are needed to better define the clinical significance of the association with other thyroid pathological associations, such as chronic thyroiditis or hyperplastic goiter by us additionally observed. A prolonged follow-up period is needed to verify the hypothesized aggressive clinical behavior of micropapillary PTC variant, although the lymph node metastatic involvement as well as extrathyroidal extension appear more frequent in this variant.

Patologia feto-placentare

Diagnosis of non-compacted myocardium and cystic adenomatoid malformation in a fetus with gestational age of 22 weeks: a rare association

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Non-compacted myocardium (NCM) is a rare form of genetical and congenital cardiomyopathy characterized by wall trabeculation and intertrabecular recesses communicating with the ventricular cavity secondary to intrauterine arrest of myocardial fiber compaction. Usually its manifestation is isolated, but rarely it can be in association with other malformation (facial dysmorphism) or complex syndrome (Becker dystrophy, Duchenne syndrome or Barth syndrome). It is characterized by a wide variety of clinical presentations: it can be totally asymptomatic or can show severe clinical features (thromboembolic manifestations, ventricular arrhythmias and congestive heart failure). The gold standard for the diagnosis is it's the echocardiography, thug the High Resolution RMN is a very useful instrument for diagnosis. We report a case of NCM, with family history of this disease, in association with Congenital Cystic Adenomatoid Malformation (CCAM), a fairly common hamartomatous malformation of the lung. These diseases were diagnosed by prenatal ultrasound with confirmation during perinatal autopsy. At the best of our knowledge it's the first case in literature of association between NCM and CCAM.

A case of twin to twin transfusion syndrome (TTTS) complicated by an angiodystopic vessel

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Introduction. Twin to twin transfusion syndrome (TTTS) is a specific complication of the monochorionic diamniotic pregnancy caused by an unbalanced blood exchange between the two fetuses through placental anastomoses. Potential sites of arteriovenous anastomoses can be suspected when an unpaired artery from one twin penetrates the chorionic plate in close proximity to an unpaired vein from the co-twin. TTTS is characterized by severe amniotic fluid unbalance associated with growth discrepancy among the two twins who are called recipient and donor. Current standard therapy is represented by lasercoagulation of the vascular anastomoses under fetoscopic guidance. This therapy has changed dramatically the outcome of TTTS, but a surgical failure rate of 10-15% has been reported by various surgery unit. This is due to the fact that the anastomoses responsible for the TTTS may sometimes be located out of the fetoscopic field, either in the membranes or in a peripheral part of the placenta. We describe a case of TTTS in which the fetal surgical approach failed, due to a very unusual course and connection of the main anastomosis. **Materials and methods.** The patient is a 24 years old pregnant woman at 22 gestational weeks with a monochorionic diamniotic pregnancy complicated by TTTS and treated, consequently, by laser ablation of anastomoses. Unfortunately, the procedure failed, and the day after the treatment, both twins died. Detailed assessment of the vascular architecture of the placenta was carried out by dye injection in the two territories, as described in the literature for this type of post-partum evaluation of the laser ablation successes and, especially, failures.

Results. Gross and histological examination of the placenta was performed together with autopsy of both twins. The donor twin was smaller (g 642 vs g 875 of the recipient) and pale, likely due to the severe anemia. The recipient was edematous and plethoric. Gross examination of the placenta demonstrated an anomalous velamentous insertion of the recipient's umbilical cord with the presence of a very unusual vessel that traversed the fetal membrane alone before reaching the chorionic plate. A particular perfusion technique to document the laser ablation intervention and to document arteriovenous anastomoses was performed during placental gross examination. The chorionic plate of the donor side was pale in contrast with the congestion shown by the recipient side. In the chorionic plate, on the fetal surface, there were signs of laser coagulation such as plaques of fibrin deposition and infarction of villous tissue underlining the anastomotic vessels and the injection of colors didn't demonstrate any remaining patent anastomoses on the chorionic plate in these regions. But an unexpected residual anastomoses was documented which connected a donor's vessel directly with the umbilical vein of the recipient in proximity of the cord insertion. This big vessel was impossible to see through the fetoscope, coursing along the membranes and was responsible, following the successful ablation of the chorionic plate anastomoses, for the demise of one twin followed, due to acute ex-sanguino of the surviving fetus into the dead one, by demise of the co-twin. In this case, the assessment of the vascular architecture by means of dye injection in the umbilical artery and vein of the two twins was

of extreme importance in demonstrating how in such a case the laser procedure could have never been successful

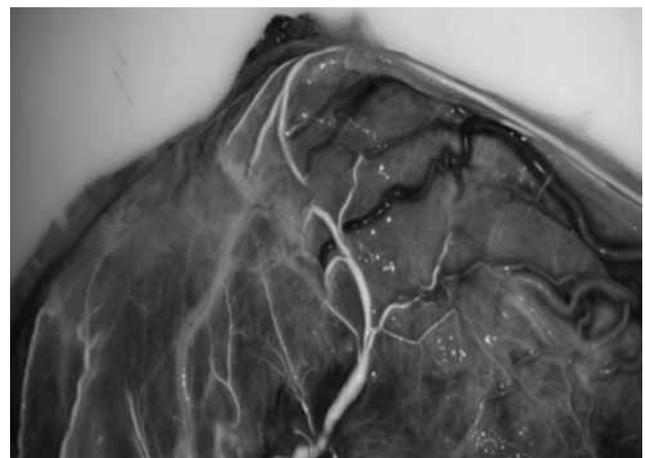
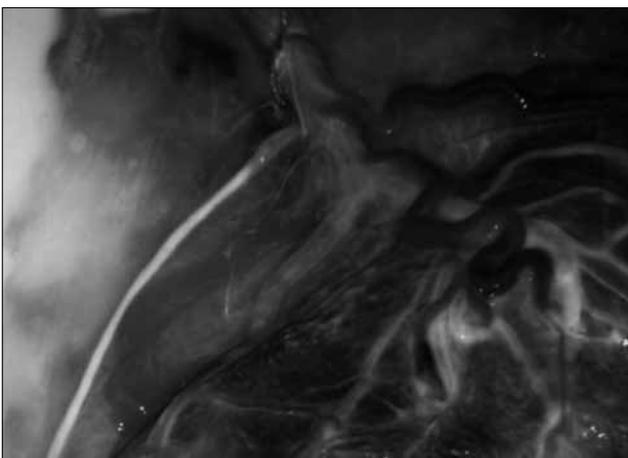
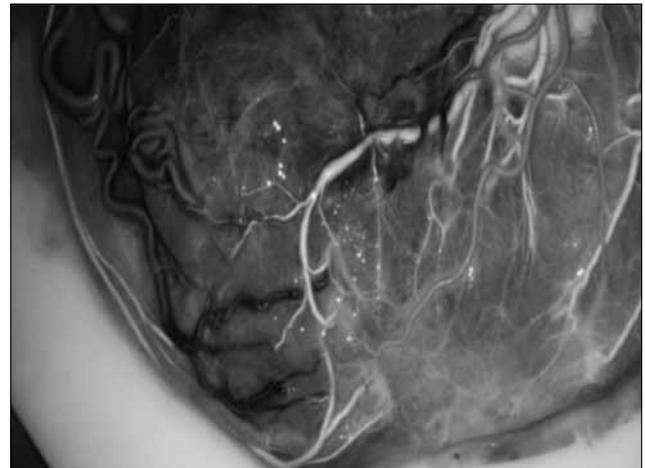
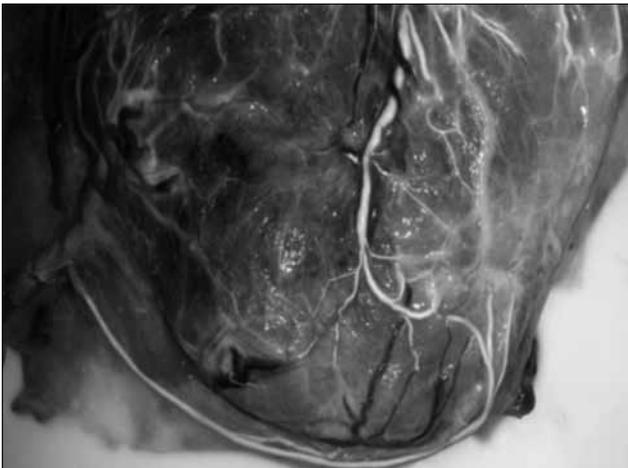
Conclusions. The study of the vascular architecture and anastomosis pattern between the two placental territories is of crucial importance in the analysis of surgical failures in the fetal surgical approach to TTTS. Schatz proposed that the chronic twin-twin transfusion syndrome result when there is an unbalanced flow blood from one twin (the donor) to its co-twin (the recipient) through arterio-venous anastomoses deep within shared placental lobules. The donor will be pale, anemic, and small, with small organ weights as well as oligohydramnios. The recipient will be plethoric, possibly polycytemic, much larger than the co-twin and associated with polyhydramnios. The frequency of the transfusion syndrome is difficult to determine but it's estimate to occur in 5-30% of monochorionic twins. For unknown reasons the syndrome is much more common in female twins. Typically it is first recognize by the finding of polyhydramnios. The syndrome usually develops around midgestation. In general, the early clinical manifestations are present, the poorer the prognosis, although overall the prognosis is poor, particularly if untreated. Management strategies for TTTS treatment have included use of indomethacin to reduce fetal urine output and polyhydramnios, digoxin to treat congestive heart failure, decompressive amniocentesis to prolong pregnancy and affect fetal blood flow, division of intervening membranes and selective feticide. The laser ablation is the latest approach and although the limit of technical feasibility, this procedure is successful in many cases. Pathologic examination of the placenta, both gross and microscopic, plays an important

part in this kind of treatment strategy. A variety of perfusion techniques to document arteriovenous anastomoses have been described (injection of air, milk, ink). In our institution during the last year we examine seven cases of TTTS treated with laser ablation and we try to state a protocol of gross examination. We perform perfusion in umbilical cord vein and arteries of specific colored substances (tempera paints) that can show immediately the links between the two sides of the chorionic plate. We try different kind of ink in order to find the perfect match of ease of execution, reliability and reproducibility.

With this case we want to point out the importance of an appropriate protocol of placental examination in TTTS in supporting and even sometimes guiding the clinical work.

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Patologia ginecologica

The cervical cancer screening in Asl-Ba

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UOSVD Servizio Centralizzato Aziendale di Citopatologia-Screening ASL Ba (Ospedale Di Venere, Bari – Italy)

Introduction. The ASL-Ba (Azienda Sanitaria Locale della Provincia di Bari), coherently with the Italian “Piano Sanitario Nazionale”, offers a public health cytology based cancer screening to women aged from 25 to 64 for the prevention of cervical cancer. Actually there is a peculiar organization in the ASL-Ba: “Piano organizzativo screening oncologici” based on an organized and spontaneous screening.

Materials and methods. The target population is of 350.928 women (Istat 01-01-2013) and in the last three years the adhesion was about 45%. In our screening program, via “Centro Regionale Screening” (Department of Prevention), there is a systematic personal invitation of women by letter to have a Pap test each third year. The program is a multidisciplinary and multistep process, that includes not only a cytological test from the uterine cervix, and involves numerous professionals: secretaries, midwives, cytotechnicians, cytopathologist, biologist cytoscreeners, pathologist, gynecologist, epidemiologist, family doctors, etc. The coordinator of the screening program is the ASL Department of Prevention, that has responsibilities in organization. This is supported by the ASL Maternal-Childish Department Director, with responsibilities in clinical governance and in coordination of the Technical-Scientific Group (tab 1). The Laboratories Department Director has responsibilities concerning cytological and pathological aspects of the screening that have been centralized in the UOSVD Servizio Centralizzato Aziendale di Citopatologia-Screening since July 2013.

Costs of every screening activity are attributed to the corresponding department budget.

The screening program is managed with a specific screening software, EUROSOFIT-DEDALUS, with a network connecting the central secretary (Centro Regionale Screening), all the smear taking centers, the surgery centers and the cytology and pathology laboratory (tab 2 e 3). All the screening activities are standardized in a shared diagnostic and therapeutic protocol similar to Emilia Romagna’s one [(PDT Regione Emilia Romagna 2012; Flow chart GISCI 26-6-2011]

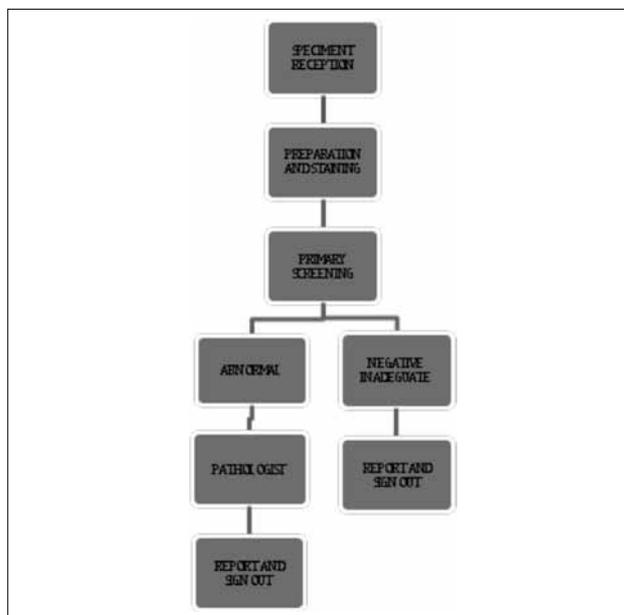
Tab. I. Nucleo Tecnico –Scientifico per lo Screening per la prevenzione del cervicocarcinoma.

Coordinatore del Nucleo Tecnico, Trattamento e Follow-up
Referente e rappresentante per i DDS
Referente Ginecologa Territoriale, Coordinatrice 1° livello
Referente Anatomia Patologica, Coordinatore lettura Screening
Referente Coordinatore 2° livello
Referente per la Medicina Generale
Referente Ginecologo Specialistica Ambulatoriale
Referente Organizzativo del Dipartimento di Prevenzione
Referente Informatico del Dipartimento di Prevenzione
Referente Ginecologo Territoriale per il 1° livello
Referente Ginecologo Territoriale per 1° e 2° livello

Tab. II. Rete 1° livello.

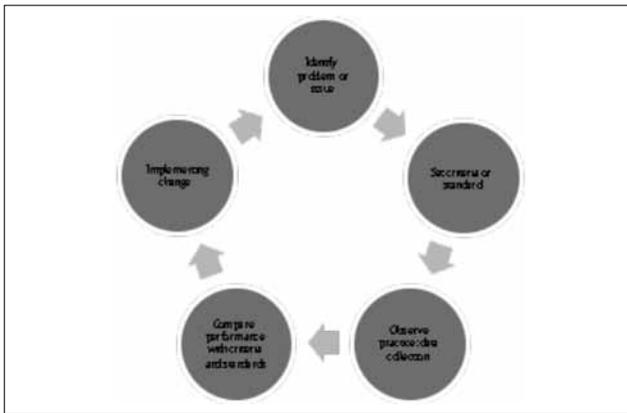
39 Consultori: presentazione delle donne dei 41 Comuni della ASL-Ba
5 Poliambulatori: per l’adesione spontanea
Servizio Centralizzato Aziendale di Citopatologia-Screening: lettura Pap-test
tab 3 – Rete 2° livello
Ambulatorio di colposcopia P.O. Di Venere - Bari
Ambulatorio di colposcopia P.O. San Paolo - Bari
Ambulatorio di colposcopia P.O. Putignano
Ambulatorio di colposcopia P.O. Terlizzi
Ambulatorio di colposcopia – Poliambulatorio Santeramo
Ambulatorio di colposcopia e Anatomia Patologica – Policlinico Bari
Servizio Centralizzato Aziendale di Citopatologia-Screening
3° livello: UO di Ostetricia e Ginecologia della ASL e del Policlinico di Bari

The laboratory team consists of three pathologists, four biologists, three technicians and one secretary that examine about 50.000 cervical smears by year, conventional and LBC in the workflow shown below.

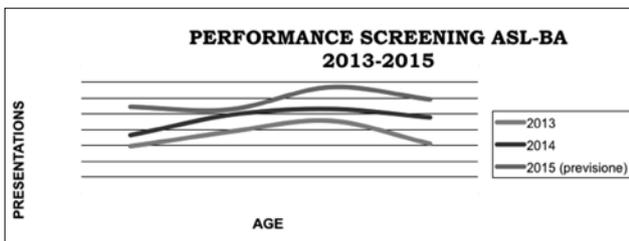


For reporting cervical cytological diagnoses we use the 2001 Bethesda Classification (TBS,2001) of gynecological cytology, and for reporting histology we use TNM classification (Seventh Ed., 2010).

To assume accuracy and diagnostic reliability, our laboratory has in place a quality assurance program. We compound a quarterly report of laboratory performance in terms of workload, staffing, distribution of smears in the different reporting categories, biopsy-cytology correlation and a comparison of these findings with national standards. Periodically we select a topic to be audited and adopt an adapted Deming’s cycle for continuous improvement (W. Edwards Deming, 1950) (fig 1). We don’t have a typical external quality control program, but there are periodical audits with an external professional international expert in gynecological pathology from University of Bari, Prof. L. Resta, especially concerning biopsy-cytology correlations.



Results. The laboratory workload is about 40.000 LBC and 10.000 conventional cervical smears by year (fig 2).



According to Bethesda System (2001) and TNM and WHO Classification, we have standardized the reporting computerized system (“Armonia” – DEDALUS) with the codification shown below:

Cytology

Atipia squamosa di incerto significato (ASC-US)

Atipia severa delle cellule squamose (ASC-H)

SIL di basso grado (L-SIL)

SIL di alto grado (H-SIL)

Atipia ghiandolare di incerto significato (AGC)

Adenocarcinoma in situ, NAS (AIS), ecc

Histology

Condiloma, NAS (coilocitosi)

Neoplasia cervicale intraepiteliale grado I (CIN 1)

Neoplasia cervicale intraepiteliale grado II (CIN 2)

Neoplasia intraepiteliale di grado III della cervice, vulva e vagina (CIN 3)

Carcinoma spino cellulare in situ con dubbia infiltrazione stromale

Carcinoma spino cellulare microinvasivo, ecc

Thanks to the system of auditing we have standardized all the laboratory procedures and workflow and have established the standard of reporting categories:

Casi/lettore per anno= 7.500-8.640

Positivi = 2-7%

Inadeguati = 0,9-1,5% Thin Prep; 5-7% convenzionali

ASC-US = 3-5%; L-SIL = 1,6-3%; ASC-H = 0,3-0,5%; H-SIL = 0,5-0,5%; Ca sq = 0,1%; AGC = 0,1-0,4%, ecc

Now we are working to achieve these standards, because we have not many ASC-US and not many glandular lesions. So we have programmed an audit concerning cellular pathology of glandular lesions. The biopsy-cytology correlation on 279 cases, between 2014 and the first three months of 2015, is found to 7,5% of nonconformity.

The staff is not sufficient for the workload and the employment contracts are, mostly, precarious.

Conclusions. The UOSVD Servizio Centralizzato Aziendale di Citopatologia-Screening of the ASL-Ba is the only one Centralized Screening Unit in Puglia, we are studying the passage to hrHPV as primary test for screening. But we have two big problems to solve: the personnel and the laboratory automation.

Gratefulness to Professor Leonardo Resta.

Vaginal angiomatosis: a rare case

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Vaginal angiomatosis is regarded as a very rare entity of benign vascular tumors of female genital tract. The incidence of these tumors is extremely low and this percentage is further reduced if we take into account only vaginal angiomatosis. The rarity of this disease and the lack of distinctive features poses a problem of differential diagnosis. The aim of our study is to present a rare but emblematic case, in order to rise to suspicion of this disease in similar clinical cases.

High-risk human papilloma virus load and p16INK4a/ki-67 expression in cervical ASC-US, L-SIL and ASC-H cytology as predicting factors of high grade cervical lesions

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Background. Negative predictive value (NPV) of HPV testing for CIN 3 is high among women of all ages (reproducing its high sensitivity). However, the positive predictive value (PPV) of HPV testing for CIN 3 is low among young women, in which transient infections and their pathological manifestations (cytological L-SIL and histological CIN 1) are very common.

HPV load was considered as a means for improving the positive predictive value of HPV testing based on the hypothesis that higher load values are more strongly associated with severe disease. Previous study have established that higher HPV load levels are more strongly associated with prevalent histo-pathological CIN, as compared with clinically occult infections (only detectable with DNA testing), however, it is uncertain whether high HPV load is specifically associated with severe grades of CIN, especially when measured using Hybrid Capture 2 (Digene, Gaithersburg, MD), a clinical test that uses a probe mixture to detect 13 oncogenic HPV types^{1,2,3}.

Another supporter marker in cervical pathology is p16INK4a/ki-67 protein expression. Concomitant expression of p16INK4a, a cyclin-dependent kinase-4 inhibitor which has an anti-proliferative effect, and Ki-67, a proliferation-associated protein, in atypical cervical sample may be used as a marker of deregulation of the cell cycle. Diagnostic application of p16INK4a/ki-67 has been investigated in cervical pathology being expressed in HPV-associated lesions, in low-grade cervical intraepithelial neoplasia (CIN) and in a high percentage of high-grade CIN.

In this study, we investigated: i) the relationship between HPV load, measured using Hybrid Capture 2 (Figure 1), and

p16INK4a/ki-67 expression in cervical ASC-US, L-SIL and ASC-H cytology as predicting factor of high grade cervical lesions and ii) we compare cytological data with definitive histological diagnosis.

Methods. Fifty-one LBC PAP cervical smears, with a diagnosis of ASC-US (n= 26), L-SIL (n= 7), ASC-H (n= 16) and H-SIL (n= 2, used as positive control) and with corresponding cervical biopsy, were selected (between January 2014 and May 2015, in Unit of Anatomic Histology and Cytopathology of the Bonomo Hospital) among 12000 LBC PAP smears. All cytological samples were assessed for HR-HPV DNA Test, with Hybrid Capture 2 (Digene, Gaithersburg, MD). All cytological and corresponding histological samples were assessed for p16INK4a/ki-67 expression, using CINtec Plus immunostaining Kit.

Results. Our data showed that 17 out of 26 ASC-US cytological samples had a low/medium HPV load (range 1-200 RLU/CO) and 3/17 were confirmed as negative after histological diagnosis, whereas 10 were CIN 1 and 4 were CIN 2. In these cases, p16INK4a/ki-67 were expressed focally in 5 out of 10 CIN 1 and strongly in 3 out 4 CIN2 samples in cytology and histology both. Regarding the last 9 out of 26 ASC-US cases with a high viral load (> 200 RLU/CO), 1/9 was Negative, one was CIN 1, 6 were CIN 2 and 1 was CIN 3. In these cases p16INK4a/ki-67 were expressed focally in the only CIN 1 and strongly in all six CIN 2 and in the only CIN 3 samples in cytology and histology both (Table 1, 2 and 3).

As regards the last 5 cases had a high HPV viral load and of

these 1/5 was negative at histological diagnosis, 2 were confirmed as CIN 1, only 1 was a CIN 2 and 1 CIN 3. In these cases p16INK4a/ki-67 were expressed focally in 3/3 CIN 1 and strongly in the only one CIN 2 and in the only one CIN 3 cases, in cytology and histology both (Table 1, 2 and 3).

As regards ASC-H category, 8 out 16 had low/medium HPV load and 4/8 were diagnosis as CIN 1 at histological diagnosis, whereas 2 were diagnosis as CIN 2 and 2 as CIN 3. In these cases p16INK4a/ki-67 were highly expressed in CIN 2-3 cases in cytology and histology both.

Whereas, the remaining 8/16 ASC-H cases with a high HPV viral load were diagnosed 1 as Negative, 1 as CIN 1, 4 as CIN 2 and 2 as CIN 3. In these cases, p16INK4a/ki-67 was highly expressed in CIN 2-3 cases in cytology and histology both (Table 1, 2 and 3).

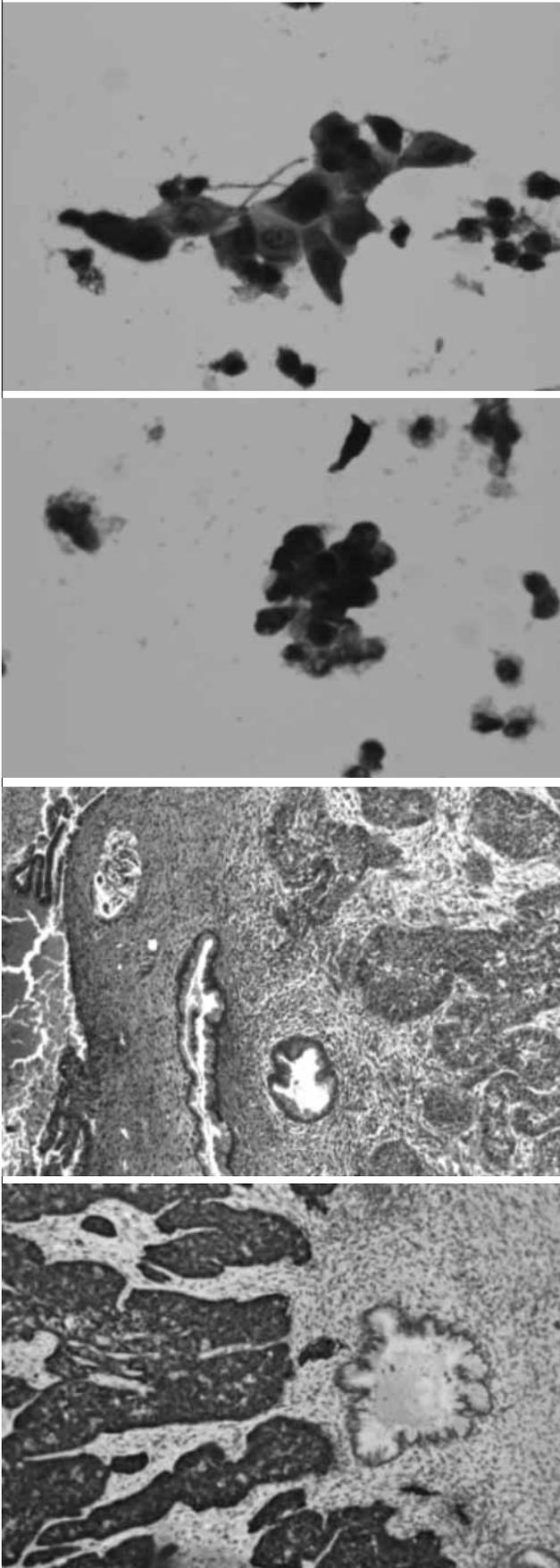
The only two H-SIL cases, used as positive control, had a high HPV viral load, they revealed a diagnosis of CIN 3 and were highly positive for p16INK4a/ki-67 expression in cytology and histology both (Figure 2).

Conclusions. Our data showed that HPV viral load and p16INK4a/ki-67 in ASC-US, L-SIL and ASC-H cases significantly correlates with the severity of cervical cancer precursors (17/24, see Table 3). These data suggest that high viral load and p16INK4a/ki-67 protein expression may have a prognostic value in identifying cytological precancerous lesions, as they correlate with the probability of a CIN2+. Conclusively, HPV load and p16INK4a/ki-67 could be useful in predicting the severity of HPV-related cervical disease.

Figure 1: Digene HPV plate.

ASL BAT Servizio di Anatomia Patologica Andria		Plate ID: 20150526		28/05/2015 13:54:03		Page 1 of 1						
Test Name	Area	Operator	Kit Lot #	Valid Run	Validated By	Primary		Secondary				
						Positive Cutoff	Negative Cutoff	Positive Cutoff	Negative Cutoff			
HPV-HR-HuRTZ-Q	A1_H12	Super	548012190	Yes	Digene	258.00	258.00	258.00	0.03			
A	NC	40	3387-677-15 3387-685-15 3883-683-15	1236-701-15	1247-708-15	1271-717-15	1308-725-15	1318-733-15	1363-A-741	1409-740-15	1417-756-15	12803 518.83 High Risk
B	NC	50	3277-678-15 3410-686-15 4387-694-15	1237-702-15	1248-710-15	1272-718-15	1310-726-15	1319-734-15	1363-B-741	1410-748-15	1418-757-15	2424 93.38 High Risk
C	NC	58	3292-679-15 3429-687-15 4034-695-15	1238-703-15	1249-711-15	1276-719-15	1311-727-15	1320-735-15	1366-743-15	1411-750-15	1419-758-15	1044 4.86 High Risk
D	WRC	279	3295-680-15 3429-688-15 4036-696-15	1239-704-15	1250-712-15	1283-720-15	1312-728-15	1323-736-15	1400-743-15	1412-751-15	1420-759-15	389 1.83 High Risk 2.89 High Risk
E	WRC	264	3302-681-15 3431-689-15 4036-697-15	1240-705-15	1257-713-15	1304-721-15	1313-729-15	1332-737-15	1401-744-15	1413-752-15	1508-760-15	32578 297.83 High Risk 32578 108.26 High Risk
F	WRC	248	3317-682-15 3438-690-15 4205-698-15	1241-706-15	1262-714-15	1305-722-15	1315-730-15	1343-738-15	1402-745-15	1414-753-15	1504-761-15	42000 1718.28 High Risk 32578 108.26 High Risk
G	QC14R - V	58	3332-683-15 3480-691-15 4236-699-15	1242-707-15	1288-715-15	1306-723-15	1316-731-15	1348-739-15	1403-746-15	1415-754-15	1600-762-15	271814 409.38 High Risk
H	QC3-HR - V	1264	3372-684-15 3486-692-15 4235-700-15	1243-708-15	1289-716-15	1308-724-15	1317-732-15	1350-740-15	1408-747-15	1416-755-15	1483-763-15	20174 78.79 High Risk 32578 108.26 High Risk

Figure 2: a), H-SIL EE, 63X, b) p16INK4a/ki-67 positive H-SIL, 63X; a) CIN 3 EE, 10X, d) p16INK4a/ki-67 positive



Tab. I. Correlation between cytological category and histological diagnosis.

CYTOLOGICAL CATEGORY	HISTOLOGICAL DIAGNOSIS				TOTAL
	NEG.	CIN 1	CIN 2	CIN 3	
ASC-US	4	11	10	1	26
L-SIL	1	4	1	1	7
ASC-H	1	5	6	4	16
H-SIL	-	-	-	2	2
TOTAL	6	20	17	8	51

Tab. II. Correlation between cytological category and HPV viral load.

CYTOLOGICAL CATEGORY	HPV VIRAL LOAD		TOTAL
	LOW/MEDIUM (1-200 RLU)	HIGH LOAD (>200 RLU)	
ASC-US	17	9	26
L-SIL	2	5	7
ASC-H	8	8	16
H-SIL	-	2	2
TOTAL	27	24	51

Tab. III. Correlation between High HPV viral Load and p16INK4a/ki-67 expression.

HIGH LOAD (>200 RLU)	p16INK4a/ki-67 Positive expression
ASC-US (n°9)	7/9
L-SIL (n°5)	2/5
ASC-H (n°8)	6/8
H-SIL (n°2)	2/2
TOTAL	17/24

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Chronic endometritis in women with pelvic endometriosis

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Introduction. Chronic endometritis (CE) is a condition not always recognized, but it may cause various kinds of distress: irregular uterine bleeding, infertility, dysmenorrhea, etc.. Presence of signs of CE in women with pelvic endometriosis was investigated in a few cases.

Material and methods. We reviewed 78 cases of women who underwent to hysterectomy or endometrial sampling after a documented history of pelvic endometriosis. The women ranged from 21 to 66 year. A cohort of 78 women submitted to hysterectomy and salpingo-oophorectomy, without foci of pelvic endometriosis was considered as control. These women were affected of uterin leiomyoma or genital prolapse: the age ranged from 35 to 57 year.

Diagnosis and grading of CE were performed according our previous criteria (Resta et al 2012) and in these cases a subsequent slide was submitted to immunohistochemical incubation with anti-syndecan-1 antibodies to reveal the presence of plasmacells and to confirm the diagnosis of endometrial inflammation.

Results. CE was observed in 30 cases (mild in 16 cases, moderate in 13 and severe in 1). The last 48 women does not show signs of CE. In the control group, the presence of inflammatory cells (with a component of plasmacells) was observed in 11 cases (mild in 10 and moderate in 1 case).

Conclusions. The different incidence of CE in women with or without pelvic endometriosis may suggest a possible role of cytokines in the pathogenesis and growth of the pelvic foci of endometriosis.

Pediatric dysgerminoma of the ovary associated with high serum levels of neuron-specific enolase

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Introduction. Dysgerminoma constitutes less than 1% of all ovarian tumors and approximately 5% of malignant ones. Most patients are young. Dysgerminoma is somewhat more common on the right side and is bilateral in 15% of cases. Metastases of dysgerminoma occur more commonly in the contralateral ovary, retroperitoneal nodes, and peritoneal cavity, the latter being associated with a decreased survival rate.

Immunohistochemically, the tumor cells are consistently reactive for PLAP and CD117, variably for keratin (erratically and focally), and sometimes for GFAP and desmin, but not for CD30. Some patients show elevated serum levels of placental alkaline phosphatase (PLAP), neuron-specific enolase (NSE) and prolactin (PRL). Few studies have been done to detecting these markers in the tumor tissues and to evaluate its prognostic significance.

Case report: A 10-year-old girl presented with an abdominal mass from about ten days.

Subsequent abdominal magnetic resonance imaging (MRI) and TC total body revealed a 13,5x6,5x5 cm left pelvic mass suggestive of ovarian tumor. Dysgerminoma was suspected. Tumor markers placental alkaline phosphatase (PLAP) and neuron-specific enolase (NSE) were elevated. The patient was operated on and treatment consisted of simple left salpingo-oophorectomy. No metastasis to adjacent structures was identified during surgery.

Results. The tumor appeared macroscopically large and encapsulated, with a smooth, bosselated surface. The cut surface was solid and gray with foci of hemorrhage and necrosis. Microscopically, the tumor cells group themselves in well-defined nests separated by fibrous strands infiltrated by lymphocytes. The individual tumor cells (PAS positive) were uniform and had large "squared – off" nuclei, one or more prominent elongated nucleoli, and abundant clear to finely granular cytoplasm that contains glycogen and fine droplets of fat. The cell membrane was prominent.

Immunohistochemically examined, the tumor was stained positive for NSE, PLAP, CD117, OCT3/4, vimentin, positive for focally CKAE1/CKAE3 and CD56 but negative for alpha-inhibin, alpha-fetoprotein, synaptophysin, CD30 and CD99. Histopathologic and immunohistochemical examination of the specimen revealed pure dysgerminoma arising from the

left ovary and tuba with focal infiltration by neoplastic elements (Stage IIA). Extensive areas of necrosis are present.

Conclusion. Dysgerminoma in earlier stages (stage I/II) are associated with a favorable prognosis.

The survival rate of pure dysgerminoma is 95%.

According to recent data from the literature, NSE positive expression is closely related with hadvanced tumor stage (stage III/IV).

In our case (Stage IIA), the high levels of NSE serum corresponded to a immunohistochemical positivity for NSE on the neoplastic tissue. The elevated serum levels of tumor markers improved dramatically after the removal of the neoplasm and these were maintained until today.

These findings suggest that the tumor tissue are the main resources of serum NSE and that the serum NSE measurements can be considered as important marker for follow-up of resected dysgerminoma.

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Prostatic tissue in mature cystic teratoma of the ovary

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Introduction. Mature cystic teratoma is a benign germ cell tumor more frequent among women of childbearing age; it is composed of a variety of mature tissues derived from the three germ layers (endoderm, mesoderm and ectoderm).

Structures arising from the male urogenital sinus, in teratomas, are occasional findings: there are only 28 published cases with prostatic tissue.

We report the case of a nulliparous 31-year old woman underwent surgery for suspected ovarian neoplasm.

Methods used. The sample was fixed in 10% neutral buffered formalin, and representative samples were embedded in paraffin and stained with hematoxylin-eosin and with Mallory trichrome. Immunohistochemical analysis has been performed on paraffin inclusions, using the following antibodies: PSA, PSAP and CK 34βE12.

Results. The lesion was composed of solid and cystic areas, containing pilo-sebaceous material, lined by epithelium of epidermal and respiratory type, with relative skin appendages. There was also a residual ovarian tissue including a minute mucinous cystadenoma. A small not encapsulated nodular area, in the solid component of the tumor, showed glandular acini lined by a single layer of cubic or cylindrical epithelium, with clear or scant cytoplasm and small, round, basal or central nuclei, without nucleoli. There were also rare cells of the basal layer, sometimes hyperplastic.

These glandular acini were organized in lobules separated by a fibromuscular stroma.

Immunostains resulted as positive for PSA and PSAP, with uniform and regular pattern, in the 80% of the luminal glandular cells, and CK34βE12 stains the rare cells of the basal layer.

Conclusions. Microscopic aspects and immunophenotype confirmed that it was prostatic tissue.

Despite the wide variety of tissues found in mature cystic teratomas, the presence of prostatic tissue is occasional and probably underestimated.

Patologia mammaria

Correlation between Ki-67 and FISH testing of HER2 IHC 1+ invasive breast cancer

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Background. HER2 gene amplification or overexpression in invasive breast cancer (IBC) has been demonstrated to be a parameter for poor prognosis. Diagnostic assays for HER2 expression have a high predictive value because HER2 positive tumors can benefit from anti-HER2 targeted therapy.

In order to research patients suitable for target therapy, the aim of the present study was to analyze the incidence of HER2 gene amplification in selected tumors with adverse prognostic features scoring 1+ by immunohistochemistry (IHC).

Material and methods. In 2013, 331 consecutive IBC were tested by IHC for HER2 and 102 cases (31%) were scored 1+. Seventy-five cases out of 102 IBC in women who underwent elective surgery entered the study. According to the histotype, 61 tumors (81%) were classified as infiltrating ductal carcinoma (IDC) and 14 cases (19%) as infiltrating lobular carcinoma (ILC). Forty-eight IBC samples out of 75 (64%) (42 IDC and 6 ILC) were selected according to one or more unfavorable prognostic tumor characteristics (high histological grade, high proliferative index, absent hormone receptor expression, node positivity and vascular invasion) and tested by FISH. HER2 amplification was evaluated using the Vysis HER2/Cep17 probe (Path Vysion HER2 DNA Probe Kit®, Abbott Molecular, IL). In agreement with the ASCO/CAP/SIAPEC guidelines, HER2 ratio-based amplification was considered. Gene amplification was evaluated as present when the HER2/Cep17 ratio was 2 or more or when the mean HER2 copy number was more than 6.

Results. Forty-eight IBC samples scoring 1+ by IHC were included in the present study according to one or more unfavorable prognostic tumor characteristics; 22 out of 48 (46%) showed high histological grade (G3), 23 out of 48 (48%) had a high proliferative index (Ki-67≥30%), 32 out of 48 (67%) were node-positive and 27 out of 48 (56%) showed vascular invasion; regarding hormone receptor expression, 3 IDC (6%) showed no ER expression and 10 IDC (21%) showed no PgR expression. FISH was performed on 48 IBC scoring 1+ by IHC and 7 IDC out of 48 (14.6%) showed HER2 amplification; all 7 samples showed a high proliferative index. In this subgroup, the statistical analysis with Fisher's exact test evidenced a significant association between the presence of gene amplification and high proliferative index (P=0.0094). Results are shown in Table 1.

Conclusions. Our retrospective data suggest that IDC patients scoring HER2 1+ by IHC who show Ki-67≥30% must be tested by FISH because there is a significant association between HER2 amplification and high proliferative index. In this subgroup of patients Ki-67≥30% would represent a

predictive factor of HER2 amplification. The assessment of HER2 gene amplification tested by FISH could be clinically useful in order not to deny anti-HER2 targeted treatment to patients who are not eligible for this therapy according to the results of the IHC test.

Tab. I.

Evaluated cases (IHC 1+) n=48	IDC samples n=42		ILC samples n=6	
	FISH+	FISH-	FISH+	FISH-
Tumor characteristics				
histological grade G3	5	14	0	3
vascular invasion	3	20	0	4
node-positive	3	24	0	5
Ki-67≥30% *	7	16	0	1
ER negative	1	2	0	0
ER +	6	33	0	6
PgR negative	3	7	0	0
PgR +	4	28	0	6

7 IDC out of 48 show HER2 amplification

* statistical analysis with Fisher's exact test evidenced a significant association between the presence of gene amplification and high proliferative index (P=0.0094).

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A rare case of CD5 positive extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) arising in a female breast after surgical exeresis of an inflammatory pseudotumor

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Introduction. Primary lymphoma of the breast is extremely rare accounting for 0,04-0,5% of all breast malignancies. Up to 50% (56-84%) are diffuse large B-cell lymphomas, and indolent histological types such as extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphoma) occur rarely with a reported incidence variable between 0 and 64%, corresponding less than 0.5% of overall malignant neoplasms of breast. A 38-year-old caucasian woman was admitted to the surgery unit nine months after the exeresis of a pre-existing inflammatory pseudotumor of the breast because mammography investigation revealed asymmetrical, ill-defined parenchymal accumulation located in right axillary prolongation. The final diagnosis was obtained after surgical exeresis and pathological evaluation of the mass.

Materials and methods. The specimens were fixed in 4% formaldehyde for 24 hours, completely sampled, routinely

processed and paraffin-embedded at 56°C. 4 micron thick sections were cut and routinely stained with hematoxylin and eosin. Immunohistochemical stainings were performed using commercially obtained antibodies against: CD5 (clone CD5/54/F6; 1:50 working dilution - w.d.; DakoCytomation, Copenhagen, Denmark), CD3 (clone F7.2.38; 1:100 w.d.; DakoCytomation, Copenhagen, Denmark), CD20 (clone L26; 1:400 w.d.; DakoCytomation, Copenhagen, Denmark), CD15 (clone C3D-1; 1:50 w.d.; DakoCytomation, Copenhagen, Denmark), CD30 (clone BER-H2; 1:40 w.d.; DakoCytomation, Copenhagen, Denmark), CD10 (clone 56C6; 1:80 w.d. Novocastra Laboratories, New Castle, United Kingdom), CD138 (clone MI15; 1:50 w.d.; DakoCytomation, Copenhagen, Denmark), BCL-2 (clone 124; 1:100 w.d.; DakoCytomation, Copenhagen, Denmark), BCL-6 (clone PGB6P; 1:20 w.d.; DakoCytomation, Copenhagen, Denmark), Kappa chain (1:50000 w.d.; DakoCytomation, Copenhagen, Denmark), lambda chain (1:50000 w.d.; DakoCytomation, Copenhagen, Denmark) and Ki-67 (clone MIB-1; 1:100 w.d.; DakoCytomation, Copenhagen, Denmark).

Results. Histological examination revealed a diffuse proliferation of small to medium size lymphoid elements with cleaved centrocyte-like nuclei and moderate eosinophilic cytoplasmic amount commingled with a scattered rate of blast in the form of centroblastic-like cells and immunoblasts. Residual typical germinal centers were showed too. The immunohistochemical profile highlighted a diffuse, marked positivity for CD20, CD5, and BCL-2 but negativity for CD15, CD30, and Cyclin D1. Lambda light chain monoclonality was showed in contrast to negative staining for those Kappa. The labeling index with MIB-1 was evident in >30% of the neoplastic cells.

Conclusions. In conclusion, we described the rare transformation of a pseudolymphoma occurred in female breast toward a lymphoma. In particular, the clinical history, and the microscopical findings together with the immunohistochemical profile, might support the evolution of an inflammatory pseudo tumour versus a malignant lymphoid proliferation in the form of primary MALT lymphoma with an unusual CD5 staining of neoplastic cells.

Immunoprofile to stratify familial breast cancer patients

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Introduction. Familial breast cancer (BC) represents a heterogeneous disease with variable prognosis. The identification of an immunoprofile is important to predict tumor behavior for the routine clinical management of patients.

Material and Methods. Using immunohistochemistry on tissue microarrays, we studied 45 familial BCs in order to analyze the expression of different biomarkers involved in progression (Na⁺/H⁺ exchanger regulatory factor 1 (NHERF1), TWIST1, Claudin 1), DNA repair mechanisms [BRCT-repeat inhibitor of hTERT expression (BRIT1), SWItch 5 (SWI5), breast cancer susceptibility gene-1 (BRCA1) and Poly [ADP-ribose] polymerase 1 (PARP1)], angiogenesis [vascular endothelial growth factor receptor 1 (VEGFR1), vascular endothelial growth factor (VEGF), hypoxia inducible factor-1 alpha (HIF-1 α) and microvessel

density (MVD)] and breast staminal cell markers (CD44 and CD24). We considered also the expression of diagnostic biomarkers [estrogen receptor (ER), progesterone receptor (PR), human hepidermal growth factor receptor 2 (HER2) and proliferative activity (MIB1)]. Unsupervised hierarchical clustering analysis (HCA) was performed using the immunohistochemical score data.

Results. HCA revealed two biomarker and two patient clusters. We identified a group of patients characterized by the low expression of ER (P=0.009), PR (P<0.001), BRCA1 (P=0.005), nuclear NHERF1 (P=0.026) and HIF-1 α (P<0.001) and the higher expression of MIB1 (P=0.043), cytoplasmic NHERF1 (P=0.004), cytoplasmic BRIT1 (P=0.001), VEGF (P=0.024) and VEGFR1 (P=0.029). This immunoprofile identified those patients with a more aggressive phenotype, associated with a larger tumor size (P=0.012) and G3 grade (P=0.006) confirmed also by univariate and multivariate analyses. Although statistical significance was not reached between the two groups, the median DFS of the group with the more aggressive phenotype was 110 months compared to 137 of the other group.

Conclusion. The clinical application of HCA of immunohistochemical data in familial BC could allow the assessment of prognostic biomarkers to be used simultaneously. The 10 protein expression panel might be used to classify patients into different prognostic groups in order to guide them towards a different clinical therapy.

Immunohistochemical expression of fascin and androgen receptors in a comprehensive cohort of Triple-negative breast cancers

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Background. Fascin is an actin-bundling cytoskeletal protein normally expressed in mesenchymal tissues and the nervous system. This protein plays a critical role in cell migration, motility, adhesion and cellular interactions.

Fascin is upregulated in many carcinomas and overexpression of the gene may play a role in the metastasis of multiple types of cancer by increasing cell motility. Expression of this gene is also a marker for Reed-Sternberg cells in Hodgkin's lymphoma.

Recent studies have highlighted that fascin correlates with the aggressiveness of tumors and is involved in the chemotherapeutic resistance of breast cancer cells.

The androgen receptor (AR) is widely expressed in breast cancers and has been proposed as a therapeutic target in estrogen receptor alpha (ER) negative breast cancers that retain AR.

In breast cancers, androgen receptor (AR) is more widely expressed than estrogen receptor alpha (ER) or progesterone receptor (PR), and AR has recently emerged as a useful marker for the further refinement of breast cancer subtype classification

A little less than 1/3 of triple-negative breast cancers (ER-/PR-/HER2-) are positive for AR expression by immunohistochemistry (molecular apocrine or luminal androgen

receptor) and have a gene expression pattern that closely resembles that of ER+ breast cancers. Androgen Receptors (ARs) have been demonstrated to play an important role in the development of breast cancer, although their prognostic role in TNBC is still debated. AR has been considered a potential therapeutic target in ER-/AR+ breast cancers: the anti-androgen bicalutamide, enzalutamide and another new generation anti-androgen, ARN-509, inhibit the growth of molecular apocrine cell lines in vitro and in vivo, supporting the hypothesis that anti-androgens may be useful targeted therapies for such tumors.

In the present study, we evaluated the immunohistochemical expression of fascin and ARs expression and their correlations.

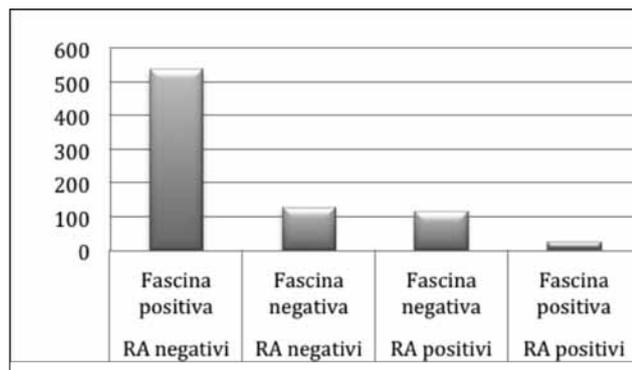
Methods. Between January 2000 and December 2014, data from consecutive patients diagnosed and treated of invasive TNBC followed at the reference oncological hospital of the Sardinia region (Cancer Hospital Regional Reference "Armando Businco" – Cagliari) were considered eligible.

The evaluation of fascin and AR immunohistochemical staining considered either the percentage of positive tumor cells (range 0-100%) and staining intensity (range 0-2+); then a global score was performed and patients were further divided into four groups (fascin positive vs AR positive, fascin negative vs AR negative, fascin positive vs AR negative, fascin negative vs AR positive).

Results. 807 TNBC patients were included.

In our dataset 70% (n=564) of cases revealed fascin positivity; 17% (n=139) of cases revealed AR positivity.

Interestingly, in 67% (n=539) of cases, fascin positivity was associated with negative AR expression, in 16% (n=129) of cases both fascin and AR were not expressed, while AR positive expression and fascin negative expression was observed in 14% (n=114) of cases, while both were expressed in 3% (n=25) of triple-negative cancers.



Conclusions. Accumulating evidence indicates that the cell migration promoting protein fascin is a useful prognostic marker for multiple cancer types.

Our preliminary results revealed that:

- 1) a little less than three quarters of TNBC showed a high activation of fascin protein and no AR expression, suggesting that fascin expression in TNBC is predominantly associated to others subtype than Luminal AR;
 - 2) in our dataset the Luminal AR subtype represent the 17% of TNBC, less than described in the literature
 - 3) the combination of AR and fascin expression might be useful prognostic markers in TNBC.
- These IHC results will be compared to clinical data (DFS, OS

etc) to allow a better understanding of the clinical outcomes of the different triple negative subtypes.

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P16INK4a and fascin protein as biomarkers in triple negative breast cancer

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Background. Triple Negative Breast Cancer (TNBCs), which accounts for 12-24% of all breast carcinomas, is defined by the lack of expression of estrogen, progesterone receptors and HER2. TNBCs are a heterogeneous group of tumors with different clinical-pathologic features, genetic-molecular alterations and treatment responsiveness. Specifically, molecular profiling studies demonstrated that a high percentage of TNBCs showed basal-like features, whereas the remainder are biologically and genetically different subtypes. Due to their Triple Negative nature, chemotherapy is currently the mainstay of systemic treatment for patients with TNBC; therefore, developing a novel treatment strategy is crucial for improving the prognosis.

The cell-cycle is monitored by checkpoints that ensure the genome integrity and the fidelity of chromosomes separation, the deregulation of cell-cycle can lead to uncontrolled growth and contribute to tumor formation. Deregulated cell proliferation is common event in tumor cells, and gene aberrations affecting control of the cell cycle are extremely common in human cancers, including breast cancer. The p16INK4a and p53 pathways are the two control pathways frequently targeted in tumorigenesis.

Moreover, recent studies have highlighted that fascin correlates with the aggressiveness of tumors and is involved in the chemotherapeutic resistance of breast cancer cells; fascin is an actin-bundling cytoskeletal protein exhibited upregulated in many carcinomas; also, gene overexpression may play a role in the metastasis of multiple types of cancer by increasing cell motility.

The aim of this study was to characterize the immunohistochemical (IHC) expression of p16INK4a, p53, fascin in TNBC and analyze their correlations. moreover, basal markers such as CK5/6, CK14 and EGFR was evaluated to define TNBC with and without basal-like features (BL).

Methods. Tumor samples were selected from the Histopathology Departments archives of Cagliari (Italy). The immunohis-

tochemistry was performed using specific antibodies against CK5/6, CK14, EGFR, p16INK4a, p53, and fascin. Results were scored semi-quantitatively including intensity and estimated percentages of labeled cells, with subcellular localization. Data were analyzed using the Mann-Whitney U test and Chi2 or Fisher's Exact Test when appropriated, respectively. The statistical significance was set-up at <0.05 .

Results. One hundred and thirty-five consecutive primary TNBC samples were included in this study. Samples were characterized by ER, PR, and HER2 negativity, with ki67 proliferation index ranging from 15% to 95% of neoplastic cells, with a strong prevalence of grade 3 (90.4%). Patients' age ranged from 27 to 91 years (median age: 53).

In our dataset 58% of TNBC showed positivity for basal markers; but any significant association was identified between BL features and p16INK4a, p53 and fascin expression. In our dataset 74.8% of cases revealed p16INK4a positivity; and 83% of cases revealed fascin positivity.

Interestingly, in 67.4% of cases, p16INK4a positivity was associated with positive fascin expression, in 5.9% of cases both p16INK4a and fascin were not expressed, while p16INK4a positive expression and fascin negative expression was observed in 4.4% of cases, finally p16INK4a negative expression and fascin positive expression was observed in 15.5% of triple-negative.

A statistically significant association was obtained between protein expression of p16INK4a and fascin ($p=0.001$). No statistical differences were observed between protein expression of p16INK4a and p53, and between protein expression of fascin and p53.

Conclusions. Our preliminary results revealed that TNBCs were characterized by a more strongly expression of p16INK4a and p53 protein (71.8% of cases), although there isn't a significant association between the two markers, their expression might be important for the development of these tumors.

The strong association between p16INK4a and fascin expression suggest that they might be useful prognostic markers in TNBCs. These molecular alterations might be related to the aggressiveness of TNBC, increasing the proliferation and the migration of neoplastic cells.

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Patologia molecolare

OSNA: a molecular assay for detecting lymph node metastasis in breast cancer

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Introduction. On December 4th2014, the Department of Pathology at Moscati Hospital in Avellino started using the

RD-100i machine. It employs the OSNA (One Step Nucleic Acid Amplification) method, a procedure enabling intraoperative analysis of the whole sentinel lymph node in breast cancer patients.

The OSNA assay is an automated diagnostic examination with a high degree of specificity and sensitivity. It allows the evaluation of neoplastic cells in the lymph node closest to the tumor using a molecular biological technique, as it is able to detect the level of CK19 mRNA expression. CK19 is an epithelial cell marker, normally not present in lymph node tissue.

Intraoperative diagnosis is based on the quantitative evaluation of CK19 mRNA copy numbers following an isothermal amplification reaction.

According to strict quantitative criteria, it is possible to distinguish micrometastases from macrometastases, thus reducing the surgical phase to only one session.

Sentinel lymph nodes examined with OSNA are classified as: Negative for metastasis: CK19 copy numbers < 250 ;

Positive for micrometastasis: CK19 copy numbers between 250 and 5000;

Positive for macrometastasis: CK19 copy numbers > 5000 .

Materials and methods. Since December 4th 2014 we have evaluated 47 breast cancer patients, who had previously tested positive for CK19 performing immunohistochemistry on fine needle aspiration cytology (FNAC) of mammary nodes.

Every OSNA session involves surgery lists of a maximum of 3 patients.

Samples are processed as follows:

- Lymph node specimen transport on ice;
- Tissue evaluation by the pathologist (weight range: 50-600 mg, with adipose tissue removal);
- Lymph node sectioning and imprint cytology: immediate H&E staining
- Homogenization;
- Centrifugation;
- Sample dilution;
- OSNA assay: RD100i assessment of the sample.

CLINICOPATHOLOGICAL DATA:

- Patients: 47
- Average age: 55 yo (31-84)
- Sentinel lymph nodes (SLN): 83
- Average ratio: 1.8 SLN/patient

Results.

- SLN negative for metastasis: 59/83 (71.1%) detected in 36/47 patients (76.6%);
- SLN positive for micrometastasis: 15/83 (18.1%) detected in 15/47 patients (31.9%);
- SLN positive for macrometastasis: 9/83 (10.8%) detected in 8/47 patients (17%).

Conclusions. From a diagnostic point of view, the OSNA method:

shows a higher degree of sensitivity in the detection of lymph node metastases with respect to the traditional histopathological method; this is especially true for micrometastases diagnosis.

benefits the patient by sparing the discomfort and possible complications of a second surgical intervention guarantees a reduction of workload and time saving for the Pathology Department.

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Immunohistochemical Investigation Of Lactoferrin In Human Bone Primary And Metastatic Tumours

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Background. Lactoferrin (LF), an iron-binding glycoprotein, has a multifunctional role in humans, specifically in the regulation of iron homeostasis, host defense against infections and inflammations, even if some experimental studies attributed others activities to LF, such as cellular differentiation, regulation growth, protection against cancer development and metastases.

Methods. We performed herein an immunohistochemical analysis of LF in a cohort of primary and metastatic neoplasms occurred in the bone by using a monoclonal specific antibody; in detail, one hundred-twelve neoplastic specimens were studied, 82 of which were human primary bone and cartilage neoplasms and 30 bone metastatic lesions obtained through curettage or surgery from an equal number of patients (59 males, 53 females; age range 9-92 years; mean age 41.02 years). The cohort was represent by 10 giant cell tumours (GCT), 7 osteoid osteomas (OO), 6 ossifying fibromas (OF), 34 enchondromas (EC), 6 osteochondromas (OC), 5 chondroblastomas (CBL), 5 chondrosarcomas (CS) (three chondroblastic type and two chondroid type), 3 chondromixoyd fibromas (CMF), 3 osteosarcomas (OS), 1 myeloma and 2 adamantinomas; moreover, surgical bone metastases were obtained from files of our Department, the primary carcinomas of which included breast (10 cases), prostate (6 cases), kidney (4 cases), lung (4 cases), colon (2 cases) and uterus (4 cases). LF reactivity was quantified using an intensity-distribution (ID) score, as elsewhere reported.

Results. In primary bone tumours, LF immunostaining, as whole, was evident in 21/82 cases (25.60%), either benign or malignant. LF immunolocalization was encountered in 10/10 GCT, 5/7 OO, 0/6 OF, 0/34 EC, 0/6 OC, 3/5 CBL, 0/5 CS, 3/3 CMF, 0/3 OS, 1/1 myeloma and 2/2 adamantinomas. About bone metastatic lesions, LF immunopositivity was encountered in 14/30 cases (46.6%), mainly due to prostatic, renal, uterine and colonic carcinomas, while the positivity was reduced in metastases from breast carcinomas and it was completely absent in lung cancer.

Conclusion. On the light of these results, we suggest that neoplastic elements might produce LF in order to make a greater amount of iron available for their turnover. Additional analyses are needed concerning new applications of LF in clinical oncology either for its nutraceutical function either for its capability to potentiate chemotherapy.

The role of BRAF in thyroid cytology: preliminary study

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Background. BRAF V600E is the most frequent genetic alteration in thyroid papillary carcinoma (CPT) (1,2). Literature data indicate how extremely useful diagnostic role and prognostic of BRAF oncogene, in particular, because associated with papillary Carcinoma with specificity of 100%.

The aim of this study is to determine the rate of mutation of the BRAF gene in thyroid nodules with indeterminate cytology or suspected, and investigate whether adding molecular testing of BRAF improves diagnostic accuracy.

Methods. By a total of 270 cases of thyroid surgical resections using performed at unit operational of pathological anatomy of the P.O. "I. Bonomo of Andria, from January 2009 to December 2014, were considered only cases diagnosed with Papillary Carcinoma (Fig.1), 34 in all.

Of these 34 cases, 20 have previous cytological material. Of the remaining 14 cases it was tested only on histological material of surgical resection. Of these cases, we analyzed the gene mutation BRAF-V600E

The analysis of BRAF-V600E mutation was conducted using the pirosequencing technique.

DNA amplification was performed with the theascreen® BRAF QMO PCR kit through the polymerase chain reaction (Polymerase Chain Reaction, PCR) in RealTime on the Rotor-Gene instrument (Qiagen).

All target were analysed by pirosequencing (PiroMark-Q24 MDX; QIAGEN) (Fig. 2).

Results. Analysis of the results related to cytological reports showed that the BRAF-V600E oncogene was wilde-type (WT) in 2 cases of TIR1 cytology (100%) and mutated in both cases histological fees (100%).

In 8 cases TIR 2, 6 were BRAF WT (75%) and 2 mutated BRAF (25%), and similarly in histological fees.

In only one case the TIR3 the BRAF is mutated (100%) and also the corresponding histological findings.

In 7 cases of TIR4 with cytological diagnosis of atypia suspected for CPT, 4 were found to be mutated BRAF (57%) and 3 WT BRAF (43%), and the fees were all histological BRAF mutated (100 %).

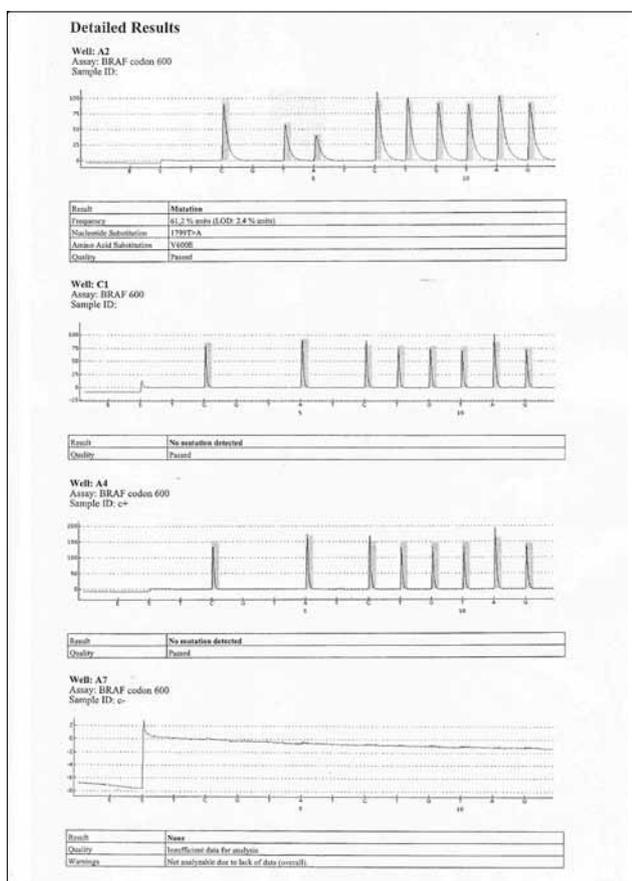
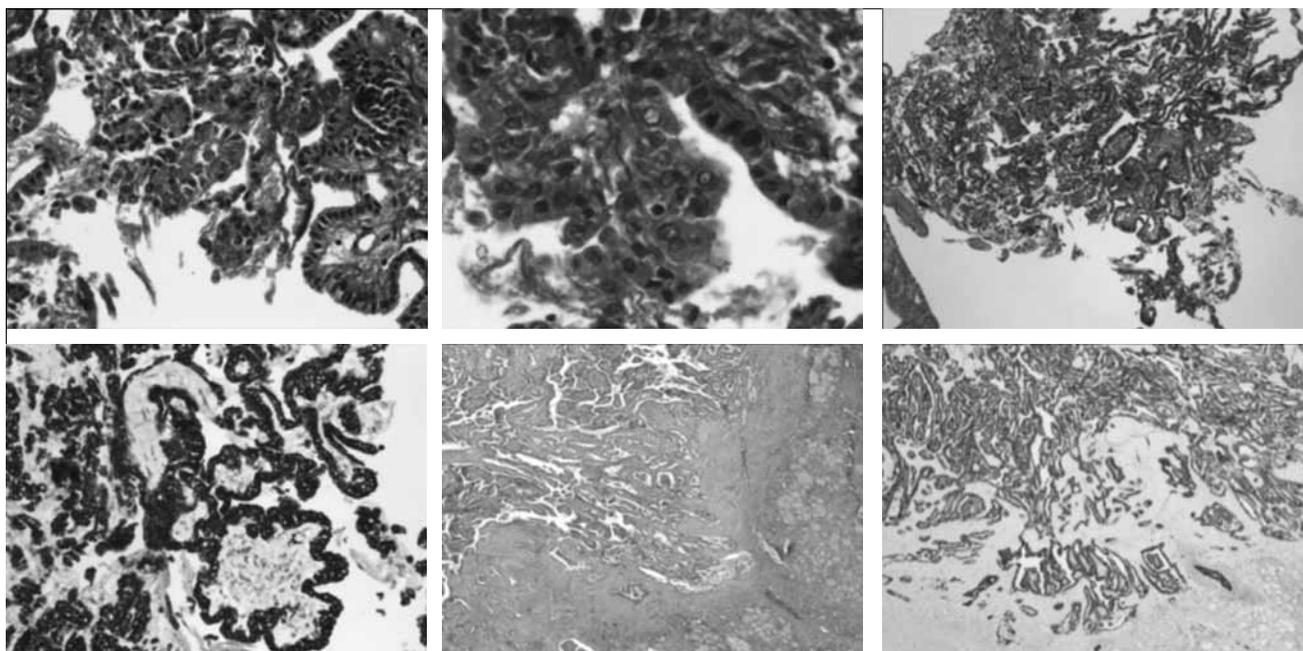
Finally, 2 cases of TIR5 were mutated BRAF (100%) and also the histological corresponding.

While the 14 cases of surgical resection, 4 cases diagnosed with papillary Microcarcinoma are BRAF-V600E mutated results (67%) and 2 WT BRAF (33%); of 8 cases diagnosed with Papillary Carcinoma 7 have given result of mutated BRAF-V600E (87.5%) and a case BRAF WT (12.5 %).

Conclusions. According to our preliminary study, although limited to one case contained, the search for mutations in BRAF V600E-improves the sensitivity of Cytology alone investigation, and is useful especially in nodules that for cytological aspects can be inconclusive diagnosis.

Particularly in cases with negative cytology is useful mutational analysis to avoid false negatives, and in those cases with indeterminate cytology, where with the cytologic examination there are sufficient elements for a definite diagnosis.

In conclusion we believe useful to propose the mutation V600E of the BRAF gene as a molecular marker in diagnosis "preoperative" thyroid Papillary Carcinoma of as the thyroid, which is complementary to traditional cytological diagnosis and prognostic marker in risk stratification, useful both in planning of surgical strategy that in the clinical management of the patient.



Molecular profile of colorectal cancers with poorly differentiated clusters of neoplastic cells at the invasive margin

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Background. The presence of poorly differentiated clusters (PDC) of neoplastic cells at the invasive margin and in the tumor stroma has been recently introduced among the histological parameters predictive of adverse clinical outcome in colorectal cancer (CRC). The highest PDC count under the objective lens of a x20 microscopic field in a tumor mass defines PDC grade. PDC counts <5, 5 to 9 and ≥10 correspond to PDC grade 1 (G1), grade 2 (G2) and grade 3 (G3), respectively. Few data have been reported on the molecular pathways underlying the PDC development.

Material and methods. Aim of the study was to correlate the presence of PDC with the mutational status of KRAS, NRAS and BRAF genes in 175 CRC. Histological parameters including WHO grading, pTNM stage, lymph-vascular invasion and tumor budding were gathered for each tumors. Hotspots mutations were detected in genome-amplified DNA by using a Mass Spectrometry-based single base extension technique (Mass Array Sequenom Platform - Sequenom, San Diego, CA).

Results. Forty-two tumors out of 175 (24%) were found to exhibit mutations in the genes examined. Mutational status was significantly associated with PDC G3 (P<0,05), high pT stage (P<0,05), N+ status (P<0,05) and tumor budding (P<0,05). In particular, KRAS mutations were significantly associated with PDC G3 (P<0,05). BRAF mutations were associated with PDC-G3 although statistical significance was not reached. No significant associations were found between NRAS mutations and PDC's.

Conclusion. In CRC, the significant association between mutated KRAS-status and PDC grade suggests that KRAS mutations may be involved in the formation of PDC's.

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Muscle biopsy suggests a case of infantile neuroaxonal dystrophy

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Introduction. Classic Infantile neuroaxonal dystrophy (INAD) is a severe early-onset neurodegenerative disorder characterized by psychomotor regression and hypotonia. Onset is between 6 months and 3 year of age and the clinical course is rapidly progressive with a mean age of death at 9 years. Gait ataxia, ophthalmologic abnormalities (optic atrophy, nystagmus, and strabismus) and dystonia are frequent; seizures are rare.

INAD is an autosomal recessive disease caused by mutations in the PLAG2G6 gene (22q12-q13), encoding iPLA2-VI, a calcium-independent phospholipase which catalyzes hydrolysis of the sn-2 acyl-ester bonds in phospholipids. PLAG2G6 seems to have a role in phospholipid remodelling and in leukotriene and prostaglandin synthesis and apoptosis [1-2]. Characteristic neurophysiologic findings in INAD patients include EMG evidence of denervation, fast rhythms on EEG and abnormal visual evoked potentials. Almost all children have cerebellar atrophy on MRI.

We report the case of a 4 years old male, born from cousin-cousin marriage, in which muscle and skin biopsy suggested the diagnosis of INAD. The diagnosis was confirmed by PLA2G6 screening mutation analysis in the family.

Materials And Methods. Patient, muscle and skin specimens. A 4 years old male patient, born from cousin-cousin marriage, was recruited from U.O. of Infantile Neuropsychiatry of Sant'Orsola-Malpighi Hospital in Bologna. The child regularly acquired stages of psychomotor development until 18 months of life, when he presented regression of obtained skills, with loss of language capacity, ability to maintain upright posture and head control. Moreover, he developed progressive cerebellar atrophy, sensorineural deafness and absence of voluntary activity of lower limbs on EMG. Muscle and skin biopsies were performed under local anesthesia in consenting patient. Histologic, histochemical and morphometric analysis

Frozen cross-sections were routinely stained with hematoxylin and eosin (H&E), modified Gomori Trichrome (GO), reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR), oil red o (ORO), combined cytochrome c oxidase (COX), succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) at 9.4 pH. Morphometric analysis was performed on 9.4 ATPase stained sections using a computer software (ImagePro Plus) to measure lesser diameter of each fiber type. A total of at least 100 fibers of each type is measured and a histogram of the diameters of each fiber type plotted. A mean fibre diameter and standard deviation was calculated and compared with normal values.

Electron Microscopy Fresh tissues were fixed in 2,5% glutaraldehyde in cacodylate buffer, post fixed in 1% OsO₄ in the same buffer, dehydrated in graded ethanol, and embedded in Araldite. Thin sections, stained with uranyl acetate and lead citrate, were examined with Philips 400T Transmission Electron Microscope.

Genetic analysis Genomic DNA of all patients was extracted by peripheral blood with standard method and after PCR the purified products were analysed on 3500 Genetec Analyzer.

Results. **MUSCLE BIOPSY.** Histology and histochemical analysis of muscle biopsy revealed mild perimysial connec-

tive tissue, focal peripheral aggregation of mitochondria, mild accumulation of lipid droplets and grouping of fibers of the same type. These findings suggested a neurogenic damage. At morphometric analysis both fibre types had mean diameter lesser than normal, as expected in neurogenic muscle disorders. **SKYN BIOPSY:** Electronic microscopy showed myelinated and unmyelinated axons, surrounded by layers of perineurial cells and basal lamina. The founding of a dystrophic myelinated axon with accumulation of randomly disposed mitochondria, neuro-granules, filaments and phago-lysosomes led us to the diagnostic hypothesis of neuroaxonal dystrophy.

GENETIC ANALYSIS: Upon PLA2G6 mutation screening we identified a new nonsense mutation (c.1483C>T) in homozygous form in the proband. We found the same mutation in heterozygous form in his parents. This mutation was not found in a 150 healthy control population and in Exome Variant Server.

Conclusions. In this complex clinical case histology and histochemical analysis of muscle biopsy suggested a neurogenic damage. Subsequently, skin biopsy took us to the hypothesis of infantile neuroaxonal dystrophy and the diagnosis was confirmed by genetic analysis. The homozygous nonsense variant that we found in exon 11 introduces a previous stop codon at position 495 (p.Q495*). It occurs at amino acid position that are highly conserved and is probably damaging (non-sense mutation). In the nervous system, the phospholipase iPLA2 is essential for membrane homeostasis so lipid and mitochondrial alterations, seen in muscle biopsy, may be interpreted as secondary damage.

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MGMT promoter methylation status in different portions of surgically resected glioblastomas

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Introduction. O6-methylguanine-DNA methyltransferase (MGMT) gene encodes a ubiquitously expressed DNA repair enzyme that counteracts the normally lethal effects of alkylating agents by removing alkyl adducts from the O6-position of guanine. Due to this DNA repair activity, the MGMT protein is believed to provide resistance against cytotoxic effects of alkylating agents. Therefore assessment of the methylation status of the O6-methylguanine-DNA is routinely assessed in malignant gliomas as a predictive factor for response to chemotherapy with alkylating drugs as well as a prognostic parameter.

The aim of the present study was to analyze MGMT promoter methylation status in different portions of surgically resected glioblastomas (GBM) in order to verify its homogeneity across the surgical specimen.

Materials and methods. Thirteen patients with GBM submitted to surgical resection between 2012 and 2015 were selected

from the files of the Department of Human Pathology of the University of Messina, Italy, and included in the study.

Only those patients with at least two paraffin blocks corresponding to different topographical portions of the tumors were considered. One of the GBM had arisen as the progression of a grade II diffuse astrocytoma (DA), while two of the cases developed a recurrent GBM. Formalin fixed and paraffin embedded tumor tissue was available for DA as well as for the recurrent GBM.

Finally a total of sixteen tumors (1 DA, 13 GBM and 2 recurrent GBM) were analyzed for MGMT promoter methylation status. All samples were microdissected to select exclusively tumor cells and for each sample DNA was extracted by FFPE extraction kit. Methylation status was investigated by Real Time PCR utilizing AlphaReal MGMT kit (Alphagenics Biotechnologies).

Immunohistochemical data on isocitrate dehydrogenase 1 (IDH1) mutated (R132H) protein, p53 and Ki-67 expression were available in all of the cases.

Results. MGMT promoter methylation status was unmethylated in all of the analyzed tissue blocks in 6 GBM. On the other hand, 7 GBM were MGMT promoter methylated. Of these, two cases showed different and not-uniform pattern of methylation. In detail, one GBM displayed MGMT promoter methylation in the central, but not in the superficial portion of the tumor and one GBM showed MGMT promoter methylation in the central and intermediate portions but not in the superficial part of the tumor. Of these cases, one had R132H mutant IDH1, and one IDH1 wild type.

One of the GBM with heterogeneous MGMT promoter methylation status represented the progression of DA, which was MGMT unmethylated and had IDH1 R132H expression.

MGMT methylation status was unchanged in the recurrences of the GBM. In detail, one of the recurrent GBM was methylated and one unmethylated as were the primitive tumors.

Conclusions. Our data suggest that MGMT promoter methylation may be heterogeneous through GBM and may depend on the site of surgical sample collection. We may speculate that methylated phenotype might be acquired during tumor progression from low grade astrocytomas.

Does MYC protein expression correlate with MYC gene translocation in Burkitt lymphoma? The role of MYCN

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Background. MYC is a transcription factor forming heterodimers with the related protein MAX that binds to promoter regions of target genes. The transcriptional program regulated by MYC includes 10% to 15% of all human genes. The main cell functions and pathways under MYC control are cell proliferation and growth, DNA replication, protein biosynthesis, and regulation of metabolism and energy. MYC promotes the transition from the G0/1 phase to the S phase, activating

directly and indirectly the expression of CCND2 and CDKs and down-regulating cell cycle inhibitors. Perturbation of MYC regulation, through constitutive or deregulated expression of the MYC protein, in the appropriate context, leads to the oncogenesis. MYC was initially identified as the target oncogene dysregulated by the t(8;14)(q24;q32) translocation in Burkitt lymphoma (BL). MYC rearrangements involving the heavy- and light-chain immunoglobulin (IGL) loci and different non-IG genes were subsequently detected in other lymphoid neoplasms usually associated with very aggressive clinical behavior. However, Burkitt lymphoma cases with no detectable MYC rearrangement but expressing MYC at protein level have been identified, and alternative mechanisms can be involved in MYC dysregulation in these cases. Moreover, MYC dysregulation alone cannot cause lymphoma, indicating that this genetic alteration per se is not sufficient to trigger lymphomagenesis. In fact, in sporadic Burkitt lymphoma cases a number of other alterations affecting key cellular genes has been identified. Finally, MYC protein expression may occur in tumours without apparent genetic alterations. The relevant oncogenic role of MYC has stimulated the search for therapeutic strategies that may counteract its damaging functions. MYC protein itself has generally been considered “undruggable” and the potential approaches have been directed at reducing its expression. However, most of these strategies have been difficult to apply in in vivo models. Accordingly, there is the need to identify all the possible mechanisms involved in MYC protein over-expression that might represent target for therapies. In this study we aimed to assess the correlation of MYC protein and MYC-translocation in Burkitt lymphoma.

Methods. To determine whether MYC protein expression correlated with MYC gene rearrangement, we reviewed a total of 119 clinical, morphological and immunophenotypical typical BL cases and we checked the expression of MYC at both mRNA and protein level by respectively RT-PCR and immunohistochemistry. In addition, FISH analysis for MYC-translocation was also performed by using the available probes (dual-color break-apart probe).

Results. Different patterns of MYC protein/MYC-translocation were identified:

- 99 specimens were positive for both MYC-translocation and MYC protein, thus fitting to what is already known in hematopathology field

- 10 samples showed MYC protein expression but were negative for MYC-translocation: in these cases we studied their microRNA profiling by comparing with the MYC translocation-positive ones in order to uncover possible differences at the molecular level. We identified four microRNAs differentially expressed between the two groups (hsa-miR-29a and miR-29b down regulated in MYC translocation-negative cases and hsa-miR-513a-5p, and hsa-miR-628-3p up-regulated). The impact of these microRNAs on the expression of selected genes was then investigated by prediction algorithm. We found 64 genes of particular interest. Among all these genes we focused on DNA methyltransferases family members (DNMTs) and MYCN genes as a direct regulation by the hsa-miR29 family member has been previously demonstrated. In particular, in MYC translocation-negative cases we found over-expression of DNMT1, DNMT3a and DNMT3b (at both mRNA and protein level), consistent to hypo-expression of the hsa-miR-29 family. This finding suggests an alternative way for the activation of lymphomagenesis, based on global changes in methylation landscape, aberrant DNA hypermethylation, lack of epigenetic control on transcription of targeted genes,

and increase of genomic instability. In addition, we observed an over-expression of another MYC family gene member, MYCN (at both mRNA and protein level) that may therefore represent a cooperating mechanism of MYC in driving the malignant transformation in those cases lacking an identifiable MYC translocation but expressing the gene at the mRNA and protein levels

- 10 cases did not express MYC at protein level but demonstrated a translocation involving MYC gene: we were very intrigued by these cases and to evaluate whether they missed protein or mRNA, RT-PCR for MYC mRNA was performed. We found that only 5 cases expressed MYC mRNA. In these cases we are carrying out sequence analysis of MYC by Illumina platform and Sanger to detect possible mutations of the gene impairing the protein synthesis or involving the epitope identified by the commercial available antibody. In those cases (n=5) lacking both protein and mRNA, we hypothesized a defect in MYC transcription. Previous reports assessed that enhanced level of N-MYC could be involved in abolishing MYC transcription. Therefore, N-MYC expression was analyzed at mRNA (by RT-PCR) and protein (by immunohistochemistry) level. An almost exclusivity between MYC and MYCN expression was identified, with cases expressing N-MYC, lacking MYC. MYCN is an oncogene frequently over-expressed in pediatric solid tumours, whereas few evidences suggest his involvement in the pathogenesis of haematologic malignancies (i.e. T-cell acute lymphoblastic leukemia and B-cell chronic lymphocytic leukemia). To address how MYCN could be amplified in our samples, FISH analysis of chromosome 22q11.2/CEP2 (Vysis, Abbott) was performed. However, no amplification was detected. The next step will be to sequence MYCN gene to identify activating mutations or a possible proviral insertion that could explain an enhanced MYCN transcription in the absence of increased copy number, as it has been previously demonstrated for neuroblastoma cell line. This hypothesis sounds very fascinating considering the polymicrobial nature of BL.

Conclusion. Collectively, our results showed that MYC translocation-positive and MYC translocation-negative Burkitt lymphoma cases are slightly different in terms of microRNA and gene expression. MYC translocation-negative Burkitt lymphoma, similarly to other aggressive B-cell non Hodgkin lymphoma, may represent an important model disease and the source of many intriguing insights into the biology of cancer, providing the opportunity of discovering new options for the diagnosis and treatment of tumours.

Patologia polmonare

A case of concomitant EGFR mutation and ALK rearrangement. Are these double mutations so rare?

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Background. Non small cell lung cancer (NSCLC) is the leading cause of cancer mortality worldwide. Genetic aberrations that drive human malignancies can be used as therapeutic targets for specific drugs. EGFR gene mutations in

NSCLC are a strong predictive factor of response to EGFR-tyrosine-kinase inhibitors (EGFR TKI) gefitinib and erlotinib that induce tumor responses and improve survival. The mutation rate of EGFR in the Caucasian population is around 10%. ALK gene rearrangements have been more recently identified in a subset of 1-7% of NSCLCs and good outcomes have been reported in patients who underwent a crizotinib-based therapy. EGFR mutations and ALK rearrangements and other oncogenic driver mutations such as KRAS are conventionally considered mutually exclusive. The coexistence of ALK rearrangements and EGFR mutations in a subset of NSCLCs has been reported in few cases (>1%) suggesting different therapeutic opportunities.

Case report. In October 2014, a 76-year-old Caucasian woman, never-smoker, referred to our Institution due to left pleural effusion and multiple bilateral pulmonary nodules together with enlargement of mediastinal lymph nodes. After a video-assisted thoracentesis and pleural biopsy, a diagnosis of poorly differentiated carcinoma with solid and adenomorph aspects was made. At immunohistochemistry (IHC), tumor cells were positive with TTF-1, CAM 5.2 and negative for CD56, chromogranin and synaptophysin. The baseline staging showed extrapulmonary bone lesions. Due to symptomatic disease (bone pain and dyspnea), the patient started first-line chemotherapy with cisplatin plus pemetrexed, and a bone radiotherapy was planned, while the biomolecular assessment was ongoing. The biomolecular assessment of EGFR mutations revealed exon 19 deletion. Thus, the patient underwent targeted treatment with the EGFR TKI gefitinib (250 mg/daily), maintaining a good clinical response. The CT-scan performed on May 2015 showed a new hepatic lesion with diameter of 1 cm, whereas the other metastatic sites were stable. This status was judged as oligoprogressive disease.

The first biomolecular assessment included the immunohistochemical analysis by using the D5F3 monoclonal antibody and no expression of ALK protein was detected.

In addition, the assessment of ALK gene rearrangement was requested and tested by FISH using the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe®; (Abbott Molecular, Abbott Park, IL). Surprisingly ALK rearrangement was detected in 35% of the tumor cells; the sample was considered positive in agreement with the AIOM/SIAPEC guidelines.

This finding would allow for the choice of a second-line therapy with ALK-inhibitors, even if the status of oligoprogressive disease could support the decision of continuing EGFR-TKI beyond progression while integrating a locoregional ablative therapy due to the global good Performance Status of the patient.

Conclusions. The case reported here highlights the importance of testing EGFR mutations and ALK rearrangements as first assessment although the detection of simultaneous presence of both the genetic alterations is very rarely reported. In conclusion, the possibility of finding a double driver oncogenic alteration is not so anecdotal, as reported in recent papers, and could make the treatment choice more complex. However, in these cases the treatment strategy could become more tailored for the patients.

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Metachronous occurrence of two primary sporadic lung adenocarcinoma (ADKL) and solid endometrioid adenocarcinoma (EAC) of the uterus: immunohistochemical and mutational analysis and role of ultrasound-guided fine needle aspiration cytology (FNAB) in diagnosis by cell block

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Metachronous occurrence of two primary sporadic lung adenocarcinoma (ADKL) and solid endometrioid adenocarcinoma (EAC) of the uterus: immunohistochemical and mutational analysis and role of ultrasound-guided fine needle aspiration cytology (FNAB) in diagnosis by cell block

Background. Fine Needle Aspiration Biopsy (FNAB), CT-ECO guided as tool for diagnosing benign and malignant neoplasia and/or staging tumors, is becoming more frequent. Its usefulness, safety and accuracy have been repeatedly proved¹. Of note FNA samples have also been shown to be adequate for molecular testing². As the majority of patients with lung cancer have a locally advanced disease, cytological material in the form of FNAB is the most accurate method to reach a precise diagnosis of malignancy and to ensure the patient receives a timely and appropriate treatment. Material obtained should be preserved as cell blocks so that the tumor should be used for immunohistochemistry (IHC) and molecular studies³⁻⁴. Current guidelines recommend initial EGFR testing and ALK testing in patients with adenocarcinoma⁵. Early mutational profiling of EGFR directs clinicians in treatment selection.

We report the case of a 71-year-old woman never smoker affected by a locally advanced ADKL who, 12 months after diagnosis, developed a uterine mass. The aim of the current study is also to explore the potential of cell blocks to increase the specificity of diagnosis and to assess EGFR mutations and ALK in ADKL.

Materials and methods. In September 2013, the patient, received diagnosis of ADKL on pleural effusion and bronchial biopsies. In December 2014, a pelvic TC revealed a 7x5 cm uterine mass. FNAB on the lung and uterine mass were performed using a 21G needle and the samples obtained were preserved as cell blocks. Parallel serial sections were obtained from the cell blocks and routinely stained by H&E. Other sections were mounted on poly-lisine-coated glasses and submitted to immunocytochemical analysis utilizing CK7, TTF1, p63, ALK (Ab anti-ALK 5A4) and estrogen receptor antisera for lung and uterine cancer. Moreover, progesteron receptor, CD10, ACML, synaptophysin, vimentin, and WT1 were also tested on the uterine cancer. The growth fraction was recorded

as Ki-67. The morphological and immunohistochemical characteristics of both tumors were examined. EGFR mutations were tested by RT-PCR on histological bronchial samples previously obtained. ALK rearrangement was detected by FISH using the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe®; (Abbott Molecular, Abbott Park, IL) on cytological sample (cytological cell block) of ADKL.

Results. Cell block sections from the lung lesion showed a haemorrhagic background with architectural patterns including strips of cells, acinar and nests of cells correlated with ADKL. Instead, the uterine mass exhibited aggregates of small cells, with hyperchromic nuclei and scarcely represented cytoplasm, indicating thus a different histogenesis. The immunohistochemical markers confirmed the malignant nature of neoplastic lesions and their primitivity of arising. Moreover the architectural and cytomorphological characters and immunohistochemical profile of the uterine mass correlated with the histopathological patterns observed in the corresponding surgical samples and a diagnosis of solid endometrioid adenocarcinoma (EAC), grade 3, was made on the radical hysterectomy⁶.

ADKL showed immunohistochemical cytoplasmatic expression for Ab anti-ALK 5A4 and it was uniformly ALK-rearranged on cell block sample. It was associated with acinar, cribriform pattern and with low grade tumor according to the SICA method⁷. EGFR wild type was found on the first bronchial biopsy of ADKL. Our case suggests the use of IHC as first level screening test to detect ALK rearrangement. Negative cases would be then tested by molecular techniques in order to identify possible ALK rearranged cases. We demonstrate that IHC could be used when dealing with small biopsy samples or scarce cytology when DNA content may be unsuitable for molecular analysis. Molecular techniques are sensitive and reliable, but they require a significant amount of biological material. Furthermore, they are not always possible in diagnostic pathology laboratories because the only available material are small biopsies, not effective for EGFR wild type patients eligible for ALK screening.

Conclusion. In conclusion, cell blocks provide a differential diagnosis of neoplasms and are an important staging tool for patients with locally advanced lung cancer. The major change is the need for close cooperation between pathologists and oncologists. It is essential to maximize the diagnostic sensitivity and to develop a tissue management strategy in which the pathologist not only makes a diagnosis but also preserves as much tissue as possible to be submitted for molecular testing.

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Fine-needle aspiration diagnosis of sclerosing hemangioma of the lung: a cytological challenge?

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Introduction. Sclerosing hemangioma (SH) is an uncommon but distinctive benign lung tumor, rarely diagnosed on cytology. The cytology of SH has been defined in less than 20 cases so its features are still relatively unclear. Herein we describe a case of SH that was diagnosed at first on cytological grounds and then treated by an atypical pulmonary resection histologically confirming the previous diagnosis.

Materials and methods. A 70-ys old smoker man, complaining dyspnea, discovered pulmonary nodule. The lesion was located in the periphery of the right middle lobe, presented no contrast enhancement at Computed Tomography (CT) scan but was positive at Positron Emission Tomography (PET) scan. He underwent Fine Needle Aspiration (FNA) under CT-scan guidance with a 22G needle. The cytological examination documented a predominant population of round, epithelioid elements with inconspicuous nucleoli, with abundant eosinophilic cytoplasm and occasional, scattered binucleated cells. A lesser population of large cells with foamy cytoplasm and evident intranuclear inclusions was present. Besides these findings, thin-walled vascular structures and small fragments of hypercellular, sclerosing stroma were detected. Neither mitoses nor necrosis were seen. On these bases, a diagnosis of sclerosing hemangioma of the lung was favored. Histological confirmation was then suggested and performed a month later. At the last follow-up, the patient is alive and well.

Discussion. SH is a rare tumor of the lung mainly affecting middle-aged adults with a striking preponderance in females. Due to the rarity of cytological diagnoses, cytology of SH has not been widely investigated. There are very few reports of FNA cytological features of SH in the worldwide literature. In our experience, this diagnosis can be very challenging and problematic since this entity can be confused with other malignant pulmonary neoplasms both primary and secondary. In our opinion, the preoperative cytological approach was of paramount importance in the correct treatment planning: since SH usually behaves in a benign manner, this procedure allowed the patient to avoid excessive surgical treatment (i. e. lobectomy). However, further studies are needed to investigate cytological issues of SH in order to establish a general diagnostic algorithm.

Asbestos bodies: establishing their exact composition to understand their cytotoxicity

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Introduction. Asbestos bodies are the histological hallmark of exposure to the asbestos. These structures are golden brown, beaded or segmented dumbbell-shaped, with a characteristic microscopic appearance that is readily recognized by the pathologist. Their identification in histological sections is an important component of pathologic diagnosis of fibrosis of the lung induced by inhalation of asbestos fibers.

Asbestos Bodies (AB) are formed in human lungs by deposition of iron-proteins (ferritin or hemosiderin) and mucopolysaccharide on the asbestos fibers. This process can occur inside macrophages or in extracellular matrix. There are also some asbestos bodies c.d. "enrobant" forms by Le Bouffante & al, in which the coating is made of oxalate crystals, especially in patients with longstanding renal failure.

Recent studies using synchrotron radiation techniques suggested that magnesium also participates, along with iron (Fe), in the coating process, and that Fe in the coating is mainly in the form of ferritinferrihydrite. The Fe-coating developed around the asbestos fibers after long permanence in lung tissue is believed to enhance the cytotoxic properties of asbestos. Nevertheless, since its exact composition is still unclear, it is not possible to formulate solid hypothesis on its carcinogenicity.

Material and methods. AB from lung tissue of two individuals subjected to long term occupational exposure to asbestos were investigated combining synchrotron radiation fluorescence and absorption techniques. Spatially resolved elemental quantification of single AB and the distribution of elements higher than Fe, is presented for the first time.

Results and conclusions. The results confirm that the AB are highly enriched in Fe (~20%), supporting the presence of iron-storage proteins such as ferritin or hemosiderin. The analysis of the x-ray absorption spectra of single AB confirmed that Fe is in the 3+ oxidation state and, which means, that it is mainly present in the form of ferritin/ferrihydrite. The comparison of the results obtained on AB studied after the removal of the organic matrix and those studied in histological sections suggested that species previously reported to be associated with the AB, probably migrate in the AB from the organic tissue during its dissolution by chemical treatments. The detection of high levels of metals and semi-metals, such as Cu, Zn, and As, and of Ba, on the AB, suggested that the coating is a host for potentially toxic species. The distribution of some of the detected elements also suggested a possible formation model for the AB.

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Patologia testa e collo

Immunohistochemical evaluation of odontogenic epithelial lesions and usefulness of CD56, bcl-2 and CD99 in differential diagnosis: preliminary report

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Background. Odontogenic epithelial lesions are a heterogeneous group of pathologies, ranging from benign cystic lesions (dentigerous cyst, radicular cyst) to benign locally aggressive lesions (ameloblastoma, keratocystic odontogenic tumor) and malignant lesions (ameloblastic carcinoma). In many cases, these neoplasms can be differentiated from each other on histologic grounds, but distinction may sometimes be challenging, particularly in case of unicystic lesions or small

bioptic specimens. The studies reported in literature about this diagnostic challenge are few and with discordant results, so the aim of this study is to establish the utility of immunohistochemical markers in differential diagnosis of these lesions.

Materials and methods. Immunohistochemical expression of different markers (CD99, CD56, bcl-2, Pan-Cytokeratin, type IV Collagen, Chromogranin A) was evaluated in 90 odontogenic lesions including ameloblastomas, keratocystic odontogenic tumor and radicular cysts.

Results. CD56, bcl-2 and CD99 showed different pattern of staining in ameloblastomas compared to other odontogenic epithelial lesions. Instead Pan-Cytokeratin, type IV Collagen and Chromogranin A gave no significant result to the aim of differential diagnosis.

Conclusion. Our preliminary results suggest a significant role of CD56, bcl-2 and CD99 in differential diagnosis of odontogenic epithelial lesions. This study will proceed including cases of dentigerous cysts and conducting a statistic analysis of the Results.

Adipose and glial choristoma of the tongue

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Introduction. Choristoma is a benign tumor, histologically characterized by an island of normal tissue that occurs in an abnormal anatomical location. This term may be used synonymously with heterotopia. It has been described in the head and neck. Lingual localization occurs more frequently and the lesion is generally localized on the dorsum of the tongue. Several different tissues can occur like cartilage, bone, glial tissue and thyroid tissue. All lesions are treated by surgical excision. Here we report a case of adipose and glial choristoma in the base of the tongue in a 65-year-old female.

Materials and methods. A 65-year-old female was admitted to our hospital with a swelling at the base of the tongue. A clinical examination revealed a firm, white, tender and well circumscribed swelling, located at the posterior zone of the tongue. MRI revealed an oval, uniform area without contrast enhancement, measuring 25 mm in its great diameter with indistinct borders. Signs of invasion were observed. The patient underwent a surgical complete excision of the lesion and grossly, the specimen consisted of a gray-white mass measuring 25 mm in its great diameter. The mass was tender in consistency and had a smooth surface. Microscopically the unencapsulated mass showed a globular appearance and infiltrated the skeletal muscle. Its surface was lined by stratified squamous epithelium. The underlying fibrocollagenous stroma contained mature glial elements formed by astrocytes with round to oval basophilic nuclei, showing strong expression of glial fibrillary acidic protein (GFAP). They were intermingled with mature adipose tissue showing positivity for S100 protein and negativity for MDM2. In the absence of cellular atypia, mitoses and necrosis a diagnosis of adipose and glial choristoma was performed. The postoperative course was favourable and there was no recurrence at 1 year postoperatively.

Conclusions. The pathogenesis of lingual choristoma is still uncertain. Three main theories could explain the pathogenesis of these lesions: the developmental malformation theory (branchial arch persistence theory), the reactive one (post-traumatic). Moreover lingual glial choristomas might develop from a nest of pluripotent cells. Although these lesions are

very rare it is important to make the correct histopathological diagnosis including the differential diagnosis of congenital masses. The wrong diagnosis might lead the patient to an incorrect surgical overtreatment. In conclusion, adipose and glial choristomas have an excellent prognosis and the surgical conservative excision is adequate and curative.

Disappearance of submandibular gland parenchyma related to chronic obstructive sialoadenitis

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Introduction. Major salivary gland atrophy without development anomalies is a still unclear, rare disorder. The submandibular gland is usually affected and the few studies published favored chronic obstructive sialoadenitis as the main etiopathogenetic risk factor.

We report a rare case of complete atrophy and total replacement of the gland with adipose and fibrous tissue.

Materials and Methods. A 50-ys-old man affected by recurrent, chronic, obstructive sialoadenitis complained swelling of the left oral floor. An ultrasound scan revealed hypoechoic salivary gland parenchyma and a dilated Warthon duct containing a hyperechoic calculus of 2 cm in its greater diameter. He underwent surgical removal of both the gland and the calculus. The histological examination of the specimen underlined scattered chronic inflammatory infiltrate and atrophy of salivary gland, that were replaced by adipose and dense fibrous tissue. The remaining excretory ductal structures were ectatic with squamous metaplasia. Now the patient is healthy after 6 months follow-up.

Conclusions. We describe, to the best of our knowledge, the third report of submandibular salivary gland total atrophy and its etiopathogenesis probably related to chronic obstructive sialoadenitis. Previous experimental studies on rats described that extended ligation of excretory ducts induced a massive glandular atrophy beginning with acute inflammatory infiltrates and leading to severe atrophy of glandular acini.

In conclusion, this case provided us the opportunity to stress further the fundamental importance of clinic-pathological correlations. In fact, histopathological findings highlighted by this entity could be surprising, as well as misleading, for pathologists. In our experience, a multidisciplinary approach including radiological and clinical features is essential to diagnose correctly this unusual and unexpected lesion.

Seromucinous Hamartoma

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Introduction. Serous hamartomas (or glandular hamartoma or microglandular adenosis) are rare lesions of the nose and sinus tract.

22 cases are reported in the literature, the first was described in 1974.

Methods. A 47 year old female, with polypoid lesion of the left nasal cavity was observed. Macroscopically, a polypoid brownish lesion, of 2,4cm in diameter of apparent inflammatory pattern is evident.

Microscopically the polyp was covered by respiratory type

epithelium, with multiple small ducts and tubules, lined by monolayer of cuboidal cells, with eosinophilic cytoplasm and dark nuclei or sometimes flattened cells.

Immunohistochemical study: S-100e CK-7 positive in the 100% of the neoplastic cells.

P63 and CK34βE12 are negative in neoplastic tubular component.

Conclusion. The seromucinous hamartoma is a rare benign lesion.

A main differential diagnosis is with: LGSNA (low grade nasosinusoidal adenocarcinomas) and with others hamartomatous lesions of the nose-sinusal tract.

The LGSNA non-intestinal type are lesions of presumed seromucinous gland origin with different growth patterns some are constituted: uniform tubules, with small micropapillae, epithelial tufts or delicate papillae with fibrovascular cores.

Clear signs of invasion in LGSNA are often absent and immunohistochemistry is not helpful.

Patologie apparato digerente e pancreas

A morphologic and molecular approach to Attenuated Polyposis

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Background and methods. Attenuated Polyposis can tentatively be defined by the presence of a number of synchronous polyps of the large bowel ranging between 10 and 99. The disease is usually sporadic, but in some cases may segregate in families as Classical Familial Polyposis (FAP, > 100 adenomatous polyps). Its frequency is difficult to determine, but current screening procedures might lead to a sharp increase of diagnosis. We purposed to subdivide and classify attenuated polyposis adopting a clinical, morphologic and molecular approach. A total of 74 patients (in 72 different families) were diagnosed with attenuated polyposis between 1984 and 2015, during clinical setting for excluding or confirming FAP. Besides clinical and morphologic data, molecular studies (APC and MutYH constitutional mutations) were carried out in 63 out of 74 patients (85%).

Results. The study group included 74 patients (40 males, 34 females), with a mean age at diagnosis of 44.0 ± 12 years, without significant differences between sexes. The average number of polyps was 30.4 ± 20 (range 10-98), predominantly distributed in the whole large bowel (46%); the disease was detected because of screening (45%), rectal bleeding (18%), abdominal pain (13.5%) or other symptoms. In the entire study group, colorectal surgery was carried out in 45 patients (61%), colectomy or hemicolectomy being the most frequent operations. Colorectal cancer developed in 28 patients (38%), and 11 individuals (14%) died of the disease.

A total of 2,129 polyps were described at endoscopy and 1,784 (84%) were resected and analyzed. Adenomatous morphology was found in 1,227 lesions (69%), hyperplastic polyps in 450 (25.2%), serrated lesions in 60 (3.5%). Adenomas were prevalent (> 50% of the lesions) in 56 patients (76%), hyperplastic polyps in 17 patients (23%), while 1 patient showed features of inflammatory polyposis. Mutations were

found in 16 patients (22%) and only over a background of adenomatous features. There were 8 patients with APC gene truncating mutations and 8 patients with homozygous or compounds heterozygous MutYH mutations. No mutations were detected in patients with a predominance of hyperplastic/serrated polyposis.

Conclusions. Attenuated polyposis is a heterogeneous disease in which adenomatous features predominate but are commonly associated with hyperplastic/serrated polyps. In an attempt to classify the disease, approximately 2/3, with prevalence of adenomas, could be defined as AFAP (Attenuated familial adenomatous polyposis), though familial aggregation is rare. Mutation can be detected in approximately 25-30% of these patients (22% of the whole study group, 29% of those showing predominance of adenomas). APC and MutYH contribute equally as genetic markers of the syndrome. Other genes could be taken into consideration for hyperplastic/serrated polyposis.

Intestinal occlusion determined by an unusual intra-abdominal desmoid tumour

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Background. Desmoid-type fibromatosis is an infiltrating neoplastic proliferation of soft tissues, defined as locally aggressive without potential for metastasis. It represents 3% of all soft tissue tumors with a higher incidence in third and fourth decades of life; although typically is a sporadic disease, it can be associated with familial adenomatous polyposis (FAP/Gardner syndrome), pregnancy and surgical trauma. According to the anatomical site, desmoid-type fibromatosis can be divided into extra-abdominal fibromatosis (60%), fibromatosis of the abdominal wall (25%) and intra-abdominal fibromatosis (8-15%). This latter one, usually arising in the mesentery, in the retroperitoneum or in the omentum, is the most biologically aggressive type owing to its capability of infiltration of both pelvic and abdominal organs.

Materials & Methods. Surgical specimen was obtained from a 72 years old man admitted to the Unit of General Surgery for a painless mass in the anterolateral abdomen determining intestinal occlusion. Formalin-fixed paraffin-embedded tissue blocks were cut to obtain 5 mm sections, routinely stained with hematoxylin and eosin (H&E). On serial consecutive sections, an immunohistochemical analysis was performed using an automated immunostainer with the following antibodies: vimentin; -SMA, desmin, h-caldesmon, -catenin, S-100 protein, CD31, CD34, CD117, cytokeratins (AE1/AE3), epithelial membrane antigen (EMA).

Histologically, the prominent morphological feature was a proliferation of bland-looking spindle-shaped cells, arranged in long sweeping fascicles and set in a finely collagenous stroma. The majority of neoplasm were composed of haphazardly arranged spindle, to stellate, to polygonal cells set in a prominent myxoid stroma containing interspersed keloid-like collagen fibers. Tumor borders were of the infiltrative-type with subserosal and muscle wall invasion of the small intestine. All surgical resection margins were tumor-free. Immunohistochemically, the neoplastic cells were diffusely positive for vimentin and focally positive for -smooth muscle actin. Nuclear immunoreactivity for -catenin was observed in 90% of neoplastic cells. No immunostaining was obtained with antibodies against CD31, CD34, CD117, desmin, h-caldesmon, pan-cytokeratins, epithelial membrane antigen (EMA) and S-100 protein.

Conclusions. The mesenteric intra-abdominal desmoid-type fibromatosis is not easy to diagnose preoperatively due to its non-specific clinical signs and imaging features; the definitive diagnosis is based on histological evaluation of surgical specimen, mainly associated with immunohistochemical profile in to differentiate from other solid tumors, such as fibromyomas, leiomyomas, GIST, neurofibromas, inflammatory myofibroblastic tumour, finally, sclerosing mesenteritis and idiopathic retroperitoneal fibrosis should be excluded.

Jejunum obstruction due to multiple polyps: an unexpected diagnosis of occult renal cell carcinoma metastasis

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The most common tumors metastasizing to the jejunum are malignant melanoma, carcinoma from lung, breast, ovary and choriocarcinoma. Renal cell carcinoma (RCC) may metastasize to almost any organ, but metastasis to the small bowel is very rare and its diagnosis is very difficult. Clinical presentation includes obstruction, bleeding, intussusception and rarely perforation. We report the case of 76 year old patient admitted to our hospital with acute abdominal pain. We diagnosed the patient with gastrointestinal obstruction, and an abdominal computed tomography (CT) scan showed thickened jejunum wall and a renal mass measuring 3cm in the maximum diameter. He had indication for surgery and a jejunum segmental resection was performed. The gross examination demonstrated the presence of 23 polypoid masses which at histological evaluation presented morphological and immunohistochemical features consistent with the diagnosis of clear cells RCC. We present this article as an exceptional example of multiple metastasis of the jejunum originated from a small asymptomatic RCC united to a detailed review of the literature.

Mismatch repair proteins detection in colorectal cancer

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Background. The recently reported European society for Medical Oncology guidelines suggest that Microsatellite Instability (MSI) should be evaluated in stage II colorectal cancer (CRC) patients in order to contribute in treatment decision-making regarding chemotherapy administration^{1,2}. MSI is defined by changes of microsatellite length resulting from deficient mismatch repair (dMMR) during DNA replication³. The protein complex responsible for mismatch repair function is a tetramer composed of 2 hetero-dimers: MLH1/PMS2 and MSH2/MSH6. For instance, PMS2 can be replaced by their homologous, PMS1 and MLH3, while MSH6 can be replaced by MSH3^{10,11}. CRC results from both genetic and environmental factors and the most common genetic susceptibility for CRC is Lynch Syndrome (LS), formerly known as hereditary non-polyposis colorectal cancer (HNPCC). LS accounts for approximately 3%

of all CRCs⁵ and also for 2% of all endometrial cancers⁶. LS is indeed caused by germline mutations in mismatch repair genes and the definitive diagnosis is currently made by identification of an inactivating germline mutation in one of the MMR genes MLH1, MSH2, MSH6 or PMS2⁷. Therefore, the detection of mismatch repair deficiency in colorectal cancer by immunohistochemistry could potentially serve three important roles: as a screening tool for HNPCC; as a prognostic marker of survival; and as a predictive marker of response to chemotherapy, identifying those individuals who will not benefit from treatment^{8,9}.

Aims and Methods. To investigate the accuracy of immunohistochemical staining (IHC) in the identification of patients with dMMR system and to evaluate the impact of this information on survival and response of patients to 5-fluorouracil based chemotherapy we randomly selected 33 patients with colorectal cancer who underwent curative surgical resection between January 2005 to December 2011 and we assessed the expression of MLH1, MSH2 and MSH6 on Formalin Fixed Paraffin Embedded (FFPE) tumor tissues of these patients using immunohistochemical techniques.

Loss of MMR protein was defined as complete absence of nuclear staining within the tumor, while MMR protein expression was defined as the presence of nuclear staining within the tumor cells, regardless its intensity or the number of positive nuclei, as mentioned in previous studies. Nuclear immunostaining of normal epithelial cells, lymphocytes, and stromal cells served as internal positive controls in each case^{12,13,14}. Within the cohort, 19 patients were diagnosed as CRC "Stage II" and 14 patients were diagnosed as CRC "Stage III". In Stage II patients, 17 received postoperative fluorouracil-based adjuvant chemotherapy (FU-AC), while in Stage III patients, 13 received postoperative FU-AC.

Results. Stage II patients who showed loss of MMR protein (MMRP-negative) were 37%, while Stage III patients with MMRP-negative were 14%. Loss of MLH1 was more frequently involved in both stage II and Stage III patients. In terms of "Overall Survival" (6-years survival rate), all patients (100% of patients) with MMRP-negative tumor survived, in both Stage II and Stage III patients while, 27% of Stage II MMRP-positive patients and 15% of Stage III MMRP-positive patients, died. Therefore, in our study, patients whose tumors showed loss of MMR protein expression had a better clinical outcome than patients with MMRP-positive tumors as in Stage II than in Stage III disease.

On the other hand, in terms of Progression Free Survival (PFS) during 6 years of survey, patients with MMRP-negative tumors showed a PFS of 50% in Stage II and 0% in Stage III when treated with FU-AC while patients with MMRP-positive tumors, treated with FU-AC, showed a better PFS (73% in Stage II and 54% in Stage III disease). These data suggests that patients with MMRP-negative tumors are more resistant to fluorouracil adjuvant therapy.

Instead, between Stage II patients who didn't received FU-AC, those with MMRP-negative tumors didn't developed distant or local metastasis while all patients with MMRP-positive tumors developed a distant or a local metastasis. One patient Stage III with MMRP-positive tumor who did not received FU-AC developed local and distant metastasis and died after 1 year. These data suggests that, at least Stage II patients with MMRP-negative tumors who don't receive FU-AC, get a clinical advantage, while Stage II and III patients with MMRP-positive tumor properly need a FU-AC.

Between MMRP-positive, five Stage II patients showed a percentage of positive tumor nuclei less than 10% or a "weak"

coloration intensity in some of MMRP. MSI analysis of ten microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, D17S250, NR21, NR24, BAT40, e TGF β R2, D18S58) conducted on paraffin embedded tumor tissue compared to normal mucosa of these patients, demonstrated a low MSI (MSI-L) defined previously as less than 4 markers unstable. Nowadays, no clear differences in clinical behavior between MSI-L and Microsatellite Stable (MSS) tumors are described¹⁰. Conclusions: Evaluation of MMRP/MSI is useful on Stage II patients with Colorectal cancer because of its important information about prognosis and response to chemotherapy so as suggested from ESMO guidelines. According to other studies¹²⁻¹⁵⁻¹⁹, our data confirm that Stage II patients with MMRP-negative tumor should not require any additional treatment after surgical resection and that only patients with MMRP-positive tumor are sensitive to FU-AC chemotherapy. Immunohistochemistry demonstrated to be an efficient method to investigate MMRP on paraffin embedded tumor tissues in CRC patients.

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Foveolar dysplasia: an interobserver Study

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Background. This study examined the strength of agreement between pathologists in foveolar dysplasia diagnosis.

Design. Whole-slide scanned images of H&E stained slides of 9 gastric biopsies and 2 resections were circulated and assessed by 13 gastrointestinal pathologists. Each pathologist recorded the type of lesion (reactive vs dysplastic), the degree of dysplasia (low-grade vs high-grade) and the type of dysplasia (foveolar vs adenomatous). Results were entered into a standardized data collection form and analyzed using k- statistics.

Results. The cases were selected by and the diagnoses agreed on by SS and RC. Four cases were reactive, 5 had low-grade dysplasia (4 foveolar, 1 adenomatous), and two had high-grade dysplasia (both foveolar). The results were as follows: reactive vs dysplastic and low-grade vs high dysplasia: slight to substantial agreement (K= 0.2 to 0.72) with slight-fair agreement for 7 pathologists and moderate agreement for 5 pathologists; agreement with respect to the type of lesion, foveolar vs adenomatous, ranged from less than chance to substantial (K=-0.08 to 0.67) with moderate agreement for 7 pathologists.

Conclusions. This study showed that there is poor to moderate agreement in separating reactive change from foveolar dysplasia, in grading dysplasia and in distinguishing between foveolar and adenomatous dysplasia.

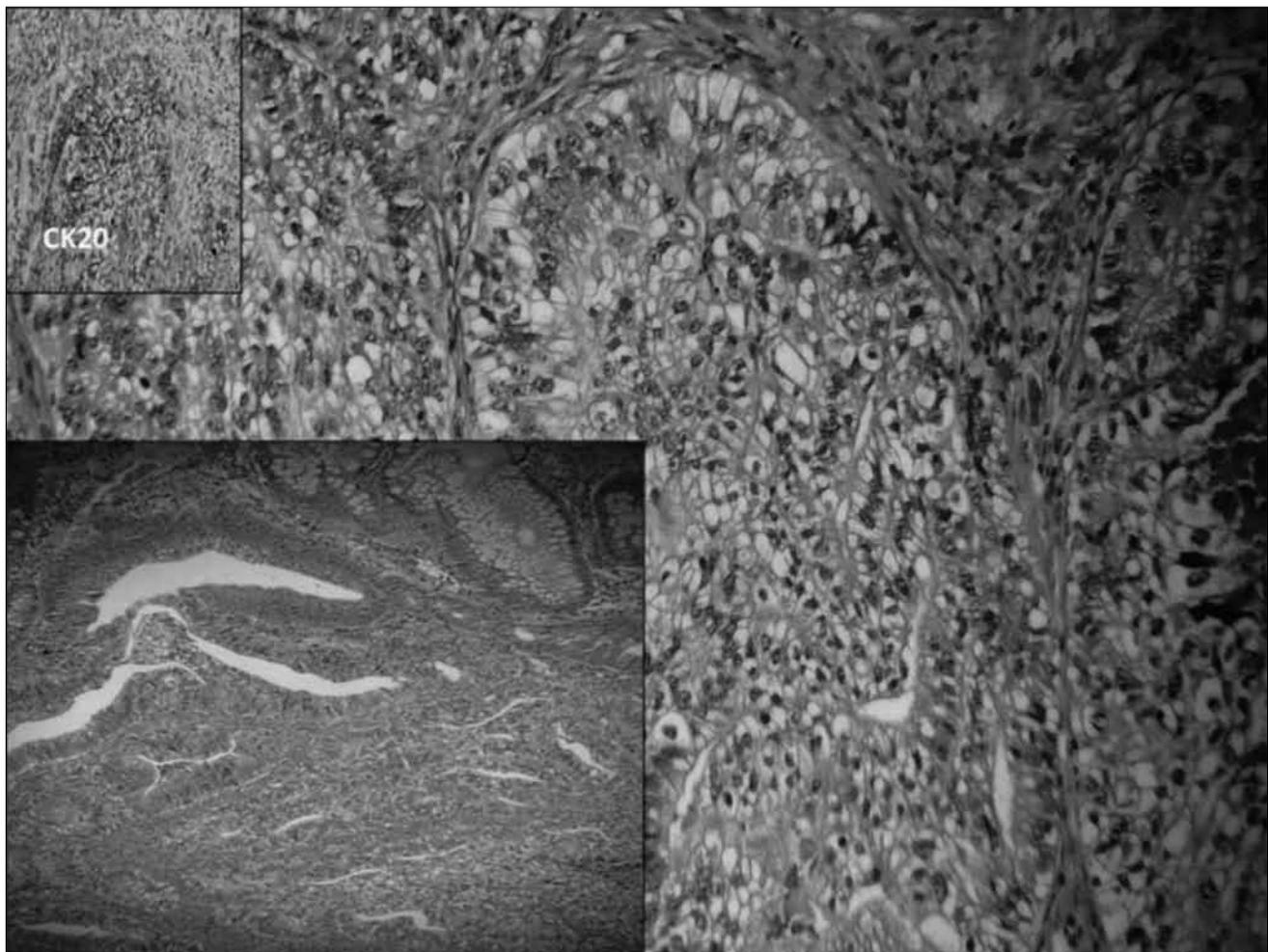
Extensive clear cell change in colonic adenocarcinoma: a case report

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Background. There is a conspicuous number of types of metaplasia or differentiation that have been encountered in colonic adenomas including squamous morules, a minor component of Paneth cells, focal stromal malakoplakia, and osseous metaplasia. Clear cell change is a rare finding and has been observed in a very small subset of patients. We describe a case of invasive colonic adenocarcinoma with extensive clear cell change within a tubulo-villous adenoma in the left colon and followed-up for more than 7 years.

Methods. A 66-year-old woman presented in October 2007 to our Endoscopic Division because of a positive finding on stool hematest in the context of a screening program. Endoscopy showed two polyps which were resected endoscopically and one of them in the rectum was classified as serrated sessile adenoma (SSA). The other lesion consisted of a 2,5x2x1,8 "berry-like" polypoid mass with a granular surface in the sigmoid colon. Then, the patient underwent left colon resection and omentectomy.

Results. At histology, the polyp was a tubulovillous adenoma with multiple foci of high-grade dysplasia also in clear cell areas and invasive clear cell adenocarcinoma in the submucosa focally extending to the cut margin. There was a focal sharp transition



between residual areas of conventional adenocarcinoma and the prevalent clear cell malignant epithelial component. The tumor cells were uniformly clear and grew in both glandular and solid-cribriform configurations. Columnar and polygonal shapes were frequently seen and the nuclei included one or more prominent vesicular and pleomorphic nucleoli. Microscopic examination of colonic sections at the level of the previous polypectomy showed no evidence of neoplastic disease. One lymph node was metastatic and was 1,4 cm in its largest diameter (9 lymph nodes assessed). An additional finding was detected, namely the presence, inside the metastatic lymphnodal growth of many clear cell cribriform areas with central dirty necrosis. Immunohistochemically neoplastic cells were positive for cytokeratin 20 and CDX2 and negative for cytokeratin 7, CD10 and PAX8. The immunohistochemical assays confirmed the colonic origin of the neoplasm and ruled out any suspicious of metastasis from kidneys, ovaries and lower genital tract. Ki-67 was positive in about 40-45% of all neoplastic cells. The clear cells showed granular PAS-reactive material in their cytoplasm that was lost with diastase. The patient did not show recurrence or distant metastasis at the last control in May 2015.

Conclusions. This case confirms that clear cell is a rare poorly understood pathologic variant of colonic adenocarcinoma, composed of neoplastic cells resembling the physaliferous cells of chordoma tumors. This type of cholic adenocarcinoma can arise in association with identifiable precursors¹. Awareness of them and their immunoprofile allows distinction from clear cell tumors from other sites¹.

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Clear cell sarcoma-like of the gastrointestinal tract: a case report of a patient 27 years after a neuroblastoma

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Introduction. Clear cell sarcoma like of the gastrointestinal tract (CCSLG) is a new entity firstly described by Zambrano et al in 2003. It is a soft tissue malignant tumor with morphologic features resembling malignant melanoma (MM) or clear cell sarcoma of soft tissues (CCS). However, it is distinguished from the listed neoplasms because of some morphological aspects, such as the lack of melanotic pigment or the presence of multinucleated non-neoplastic giant cells and because of the immunohistochemical profile. CCSLG shows distinctive positivity for protein S100 but does not express HMB45 or MelanA. Moreover, it often shows positivity for neuroendocrine markers, such as CD56 and Synaptophysin.

Diagnosis is confirmed by FISH analysis, which shows rearrangement of the EWSR1 gene, that is not shown in MM. Very rarely, this neoplasm has developed in patients many years after a different neoplasm, who received radiotherapy. Here we describe the case of a young female, who previously received the diagnosis of neuroblastoma.

Case Report. The patient is a 29 year old female, to whom at the age of 2 years old a diagnosis of adrenal neuroblastoma was performed. The patient was surgically treated, and after received high doses of radiation. After the treatment, the patient did not show any recurrence or notable pathological events. Twenty-seven years later, the patient caught the surgeons attention because of abdominal obstruction. A CT scan revealed an ileal mass of about 6 cm and loco-regional lymph nodes enlargement. The tumor was surgically removed and submitted to pathology. It was a circumferential hard whitish mass, protruding to the lumen. Microscopically it was composed of large nests of markedly atypical cells, which occasionally were disposed in a pseudoalveolar and pseudopapillary pattern. Mitoses were frequent, as well as cell cannibalism. A broad immunohistochemical panel was performed; neoplastic cells were: Cytokeratin, LCA, NSE, HMB45, Melan-A, Chromogranine, Bcl2, CD34, CD99, EMA, CD117, Actin, Desmin, CD68 negative, but showed positivity for protein S100, CD56, Synaptophysin and Vimentin. Neither melanotic pigment was seen, nor histiocytic giant cells. All lymph nodes were metastatic. A melanoma metastasis was suspected, but the patient had no history of cutaneous melanoma; a CCS was a more reasonable diagnosis. However, the immunohistochemical profile was slightly different. FISH analysis revealed the EWSR1 rearrangement. All the data suggested CCSLG.

Discussion. CCSLG is a rare entity recently described and therefore difficult to recognize. Some authors describe it not simply as a gastrointestinal variant of the known CCS but as a malignant neuroectodermal tumor, therefore appointing it as Gastrointestinal Neuroectodermal Malignant Tumor. Diagnosis is suspected on morphological grounds and confirmed by immunohistochemical data together with FISH analysis. Differential diagnoses are firstly MM, either primitive or metastatic, then CCS. Both diagnosis can be excluded only considering the global ground: clinically, mucosal MM are rare, most localizations of the gastrointestinal tract representing metastases, CCS is normally described in soft tissue and gastrointestinal localization is extremely rare. Morphologically, presence of melanin is typical of MM and CCS while presence of histiocytic multinucleated giant cells has been mostly described in CCSLG. The immunophenotype is of great help: S100 positivity together with lack of HMB45, Melan-A (both melanocyte-specific markers) and expression of neuroendocrine markers (CD56, Synaptophysin) is characteristic of CCSLG. FISH rearrangement of EWSR1 excludes MM, but is shared from CCS; gene fusion EWSR1-CREB1 is more often seen in CCSLG than CCS.

In our case we reached the diagnosis of CCSLG only matching clinical, morphological, immunohistochemical and FISH analysis data.

Here we described the occurrence of CCSLG in a patient with a history of malignant neuroectodermal tumor, a neuroblastoma. Moreover, the patient was treated with radiotherapy.

To the best of our knowledge, two reports have been made of CCSL in a patient with past history of neuroblastoma. Few reports exist of CCSL in patients who were treated with radiotherapy for different tumors, such as Ewing sarcoma and Hepatoblastoma. All cases show the occurrence of CCSLG between 20 and 30 years after the first tumor.

We suppose that such neoplasms are history related, either with the previous neuroectodermal malignant neoplasm or

with radiotherapy, and should be considered among the long term complications of these tumors.

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Lymphangioma of the jejunum: report of a case and brief review of the literature

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Introduction. Lymphangioma is a rare benign condition characterized by proliferation of lymphatic spaces ¹. It is believed to result from congenital lymphatic malformation rather than a true lymphatic neoplasm ^{2,3}. It is usually found in the head and neck of affected children ¹. Lymphangioma of the small bowel often presents with anaemia. As it has traditionally been difficult to investigate the ileum and jejunum, these lesions have been regarded as being rare, and often not considered in the differential diagnosis of patients with iron deficiency anaemia related to the gastrointestinal tract. With the advent of capsule endoscopy and enteroscopy, the incidence of reporting of cavernous lymphangiomas appears to be increasing ⁴. Importantly, it can cause fatal complications such as volvulus or acute bleeding, requiring emergency surgery ³. Herein, we describe a case of jejunal lymphangioma, making also a brief review of the literature.

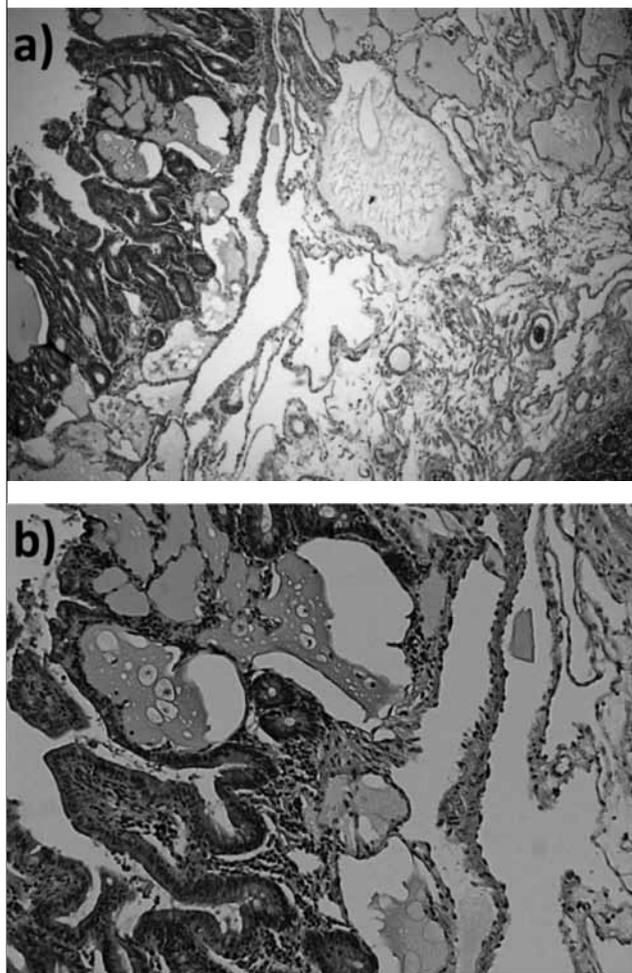
Materials and methods. A 48-year-old man was admitted to our hospital for abdominal pain associated with fatigue. Upon examination, the patient's abdomen was soft and lax with no tenderness, rigidity, or guarding noted. Since fecal occult blood was positive, upper and lower endoscopic procedures were done, but the endoscopic findings were non-significant. Abdominal computed tomography (CT) revealed a polypoid lesion in the distal jejunum. The lesion with adjacent jejuna tract were resected and a small bowel anastomosis was performed and the specimens were submitted to histologic examination.

Results. After formalin fixation, the resected specimen showed a bleeding, whitish, polypoid lesion of 3,5 cm in maximum diameter, with irregular borders. The cut surface of the mass revealed multicystic spaces of varying size. Significant samples of the lesion were taken and paraffin embedded. Sections of 4 µm of thickness were cut and stained with haematoxylin and eosin. Microscopic examination revealed that the mucosa and the submucosa of the lesion were markedly expanded and replaced by dilated lymphatic spaces, whereas the adjacent jejunum was normal. The lymphatic spaces were lined with flat endothelial cells and filled by lymphatic fluid (Figure 1a-b). The intermixed stroma contained smooth muscle bands and scattered lymphoid infiltrates. In conclusion, the diagnosis was jejunal lymphangioma.

Conclusions. Lymphangioma is a mass-forming lesion characterized by numerous thin-walled lymphatic spaces and usually manifests in the first few years of life ¹. The common sites are the head, neck, and axillary regions. Other locations such as the abdominal or mediastinal cavity are rare, account-

ing for approximately 5% of lymphangiomas². Among these, lymphangioma of the small bowel has been described in less than 1% of lymphangiomas¹. 60% of the tumors are present in patients under the age of 5 years, but a significant percentage of them does not manifest until adult life⁵. Lymphangioma appears to result from congenital malformation of lymphatic vessels rather than a true lymphatic tumor^{2,3}. The former causes sequestration of lymphatic vessels during the embryonic period¹. However, some data suggest that inflammation, abdominal trauma, abdominal surgery, radiation, or lymphatic obstruction may play a role in the genesis as a tumor⁶. Lymphangiomas are traditionally classified into three histologic types: capillary (simple), cavernous, and cystic¹. The capillary (simple) type usually originates in the skin and consists of uniform small thin-walled lymphatic spaces. The cavernous type is composed of various sizes of dilated lymphatic spaces associated with lymphoid stroma and shows a connection with the adjacent normal lymphatic spaces. The cystic type consists of dilated lymphatic spaces of various sizes associated with collagen and smooth muscle bundles in the stroma but lacks connection to the adjacent normal lymphatic spaces. Cystic lymphangioma findings are similar to cavernous lymphangioma findings in that dilated lymphatic spaces

Figure 1. a) the mucosa and the submucosa of the lesion were markedly expanded and replaced by dilated lymphatic spaces, whereas the adjacent jejunum was normal (40x; HE stain); b) the lymphatic spaces were lined with flat endothelial cells and filled by lymphatic fluid (100x; HE stain)



of variable size are seen for both⁶. Intra-abdominal lymphangioma usually presents as abdominal distension, a palpable abdominal mass, or acute intestinal obstruction². The latter is the most common presentation of mesenteric lymphangioma in the form of small bowel volvulus³. Small bowel volvulus is the rotation of the small bowel and its mesentery, usually complicated by acute intestinal obstruction. The precipitating factors to volvulus include postoperative adhesion bands, congenital bands, colostomy, ileostomy, fistula, tumors, omental defect, and Meckel's diverticulum⁶. Partial small-bowel obstruction induced by volvulus was responsible for the patient's first visit, as manifested by abdominal distension. In the past, an abdominal lymphangioma was seldom diagnosed preoperatively. Currently, the diagnosis can usually be suspected with a combination of radiologic studies⁵. Ultrasonography is useful for localizing and determining the cystic nature of the tumors. On CT scans, the tumors appear as homogeneous, non-enhancing lesions with variable attenuation values depending on whether the fluid is chylous or serous. So, the number of lymphangiomas reported has increased in recent literature⁴. This is likely due to improvements in diagnostic imaging with the advent of capsule endoscopy and double-balloon enteroscopy rather than a true increase in the prevalence of the tumour. The standard management of lymphangiomas until recently has been through surgical resection. However, with the advent of double-balloon enteroscopy, this modality may be able to treat small tumours⁴.

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Histological findings in a rare case of Italian acute gastric anisakiasis

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Introduction. Anisakiasis is a gastrointestinal parasitic disease caused by the ingestion of raw sea fish infected by third stage larvae of the nematode *Anisakis simplex* (AS)^{1,2}. This infection is more frequent in countries where the consumptions of raw or undercooked sea fish is widespread, such as Japan and North Europe. In Italy this infection is rare, although a higher incidence has been reported in these last years, partly due to the increased popularity of Asian cuisine. Anisakiasis has a wide range of clinical manifestations and may mimic several other pathologies, depending on where the larvae are located. The acute reaction involves the stomach, and is characterized

by abdominal pain, vomiting, and nausea within hours of the ingestion of contaminated food¹.

We present a rare case of acute gastric anisakiasis occurring in an Italian patient, shortly after she had eaten raw fish.

Materials and Methods. A 42-year-old woman presented to the emergency department of our hospital for the onset of epigastric pain, nausea and vomit. She referred that she had eaten raw fish (anchovies) the day before. CT scan and esophago-gastroduodenoscopy (EGDS) were performed.

Results. The CT scan revealed severe edema of the gastric mucosa and submucosa (figure 1A). In addition, the inflammatory process seemed to involve the perigastric adipose tissue (figure 1B).

The EGDS report confirmed the CT findings; Moreover, it revealed nematode larvae, attached to the gastric mucosa of the body and the antrum (figure 1C). The nematodes were removed by means of biopsy forceps.

Histological examination of the gastric biopsies revealed the presence of mucosal edema as well as intramucosal cysts (figure 1D).

Histological examination of the larva sent to our laboratory showed its various portions (figure 2) and characterized it *Anisakis simplex*.

Discussion. In Japan, gastric Anisakiasis is far more common than intestinal Anisakiasis, while in Europe, the intestinal form is more common^{1,3}.

The pathology of anisakiasis is due mainly to two mechanisms: allergic reaction, ranging from urticaria and angioedema to life-threatening anaphylactic shock associated with gastrointestinal symptoms, and direct tissue damage, due to invasion of the gut wall, development of eosinophilic granuloma or perforation³.

Most reports on Anisakiasis indicate that the most frequent parasitic location is in the great curvature, probably because this zone provides a good environment for AS larvae, due to the large number of folds and the more active mucus secretion in this region⁴.

Treatment of gastric Anisakiasis is hastened by removing larvae endoscopically, as no medical treatment is available

Figure 1A: The CT image shows severe edema of the gastric mucosa and submucosa (arrows).

Figure 1B: The CT image shows how the inflammatory process seemed to involve the perigastric adipose tissue (arrow).

Figure 1C: larva of AS strongly attached to the gastric mucosa in EGDS (arrow).

Figure 1D: edema of the gastric mucosa as well as intramucosal cysts in gastric body biopsy (H&E, 100X).

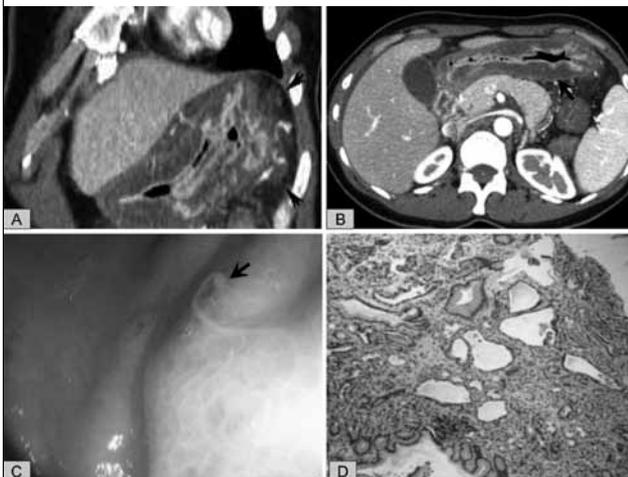
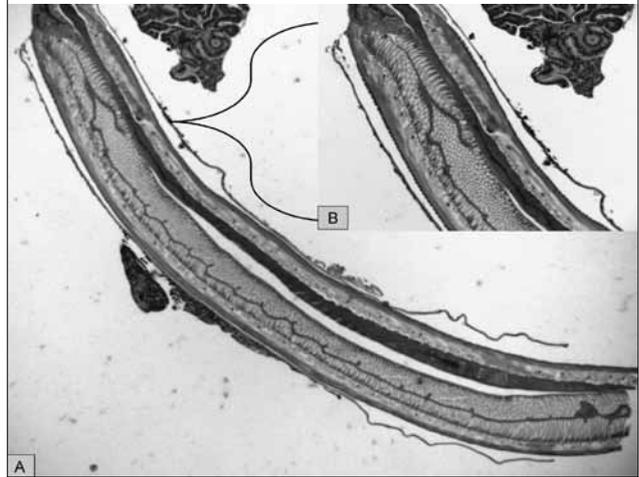


Figure 2A: microscopic images of AS from top to bottom: inferior end of the oesophagus, ventricle and intestine. Musculature is also observed (H&E, 100X).

Figure 2B: an insert at higher magnification shows the larva's structure in more detail (H&E, 400X).



to date^{1,3}.

Conclusion. Although rare in our country, Anisakiasis should be suspected when abdominal symptoms occur following raw fish ingestion. In such cases, an accurate clinical history is warranted, in order to correctly guide the diagnosis, alerting the endoscopist to search for the parasite.

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Uropatologia

Prognostic value of tumor volume on disease progression of patients treated with radical prostatectomy for node positive prostate cancer: results from a single tertiary care center

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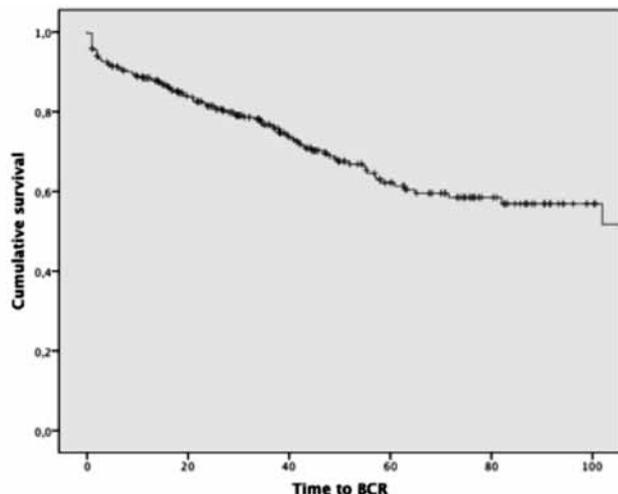
Introduction. Previous studies demonstrated the association between tumor volume (TV) oncological outcomes in patients treated with radical prostatectomy (RP) for prostate cancer (PCa). However, the potential prognostic value of TV in the context of node positive PCa is still unclear. Accordingly, the aim of test study was to test ability of pathological TV in predicting disease progression in patients with lymph node invasion (LNI) treated with RP and lymph-node dissection (PLND). **Materials and methods.** Overall, we identified 1004 patients treated with RP and PLND between 1991 and 2013 for node

positive PCa at a single tertiary referral center. Only patients after 2005 with available information about tumor volume were included in the study (n=328). All patients received adjuvant treatment. Complete clinical, pathological and follow-up data were available for all patients. Tumor volume was calculated by visual inspection, according to the College of American Pathologists guidelines. First Kaplan-Meier methodology was employed to assess the biochemical recurrence (BCR) and metastasis (MFS) free survival rates. Uni- and multivariable Cox regression analyses tested the impact of TV on the risk of BCR and clinical recurrence (CR). Covariates consisted of pathological stage, pathological Gleason Score (GS), number of positive nodes, positive surgical margins (SM), and adjuvant radiotherapy (aRT) receipt. The same analyses were repeated after stratification of patients according to number of positive node (1-2 vs. ≥ 3).

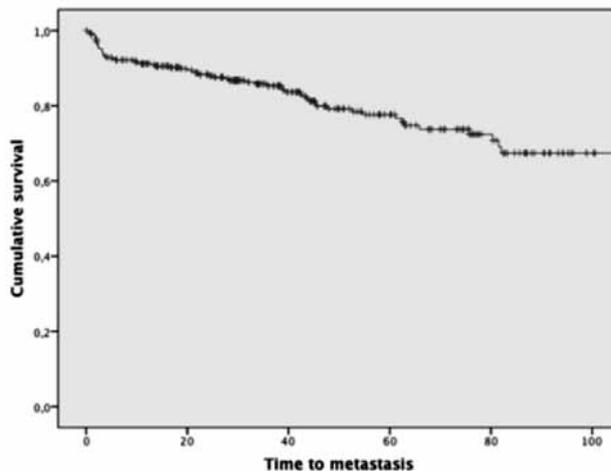
Results. Mean tumour volume was 15.6 ml (median: 10.8). Mean number of positive nodes was 4 (median 2). Overall, 123 (37.5%) patients had ≥ 3 positive nodes. Mean follow-up time was 45 months (median 39). The 1, 3 and 5 years BCR-free survival and MFS rates were 87.5, 75.3 and 59.7% vs. 90.4, 85.0 and 75.6%, respectively. At UVA analyses TV emerged as a significant predictor of BCR and metastasis (all HR=1.03; all $p < 0.001$). These results were confirmed at MVA, where TV was significantly associated with BCR (HR 1.03) and metastasis (HR 1.05; all $p \leq 0.001$). Number of positive nodes and aRT also emerged as predictors of BCR and metastasis (all $p \leq 0.005$). When assessing the aforementioned outcomes within patients with 1-2 positive nodes, TV remained the only significant predictor of BCR (HR 1.04) and metastases (HR 1.05; all $p \leq 0.001$). Conversely, no significant association between TV and oncological outcomes was observed (all $p \geq 0.1$). Only aRT was associated with BCR and metastases in the group with ≥ 3 positive lymphnodes (both HR=0.3; all $p \leq 0.001$).

Conclusion. Pathological TV represents an independent predictor of disease progression in patients treated with RP and PLND even in the context of node positive PCa. Specifically, TV should be included in predictive models when a low burden of nodal involvement is found, while in patients with ≥ 3 positive nodes its prognostic value loses its strength. Further studies with larger patients population are needed to properly evaluate the effect of TV on survival outcomes.

Kaplan-Meier depicting BCR free survival rates at 1, 3 and 5 years



Kaplan Meier depicting CR free survival rates at 1, 3 and 5 years



MVA predicting the impact of TV on BCR

Variabili nell'equazione						
	B	SE	Wald	df	Sig.	Exp(B)
psm	,280	,242	1,339	1	,247	1,323
aRT	-,840	,228	13,576	1	,000	,432
tumor_vol	,025	,007	13,699	1	,000	1,025
T.CAT	,042	,261	,026	1	,871	1,043
GS6vs710	,299	,231	1,678	1	,195	1,348
positive_nodes	,020	,006	12,019	1	,001	1,020

MVA predicting the impact of TV on CR

MVA predicting the impact of TV on CR

Variabili nell'equazione						
	B	SE	Wald	df	Sig.	Exp(B)
psm	-,010	,298	,001	1	,974	,990
aRT	-,796	,280	8,055	1	,005	,451
tumor_vol	,027	,008	12,036	1	,001	1,028
T.CAT	,692	,377	3,366	1	,067	1,999
GS6vs710	,262	,295	,789	1	,374	1,299
positive_nodes	,020	,007	9,486	1	,002	1,020

MVA predicting the impact of TV on BCR in patients with LN+ ≥ 3

MVA predicting the impact of TV on BCR in patients with LN+ ≥ 3

Variabili nell'equazione						
	B	SE	Wald	df	Sig.	Exp(B)
psm	,349	,388	,806	1	,369	1,417
aRT	-,1286	,322	16,002	1	,000	,276
tumor_vol	,016	,009	2,993	1	,084	1,016
T.CAT	,570	,577	,975	1	,323	1,767
GS6vs710	,147	,357	,169	1	,681	1,158

MVA predicting the impact of TV on CR in patients with LN+ ≥ 3

MVA predicting the impact of TV on CR in patients with LN+ >= 3

Variabili nell'equazione						
	B	SE	Wald	df	Sig.	Exp(B)
psm	,171	,421	,165	1	,684	1,187
aRT	-1,205	,361	11,137	1	,001	,300
tumor_vol	,013	,011	1,538	1	,215	1,013
T.CAT	,590	,656	,810	1	,368	1,804
GS6vs710	-,042	,411	,011	1	,918	,959
MVA predicting the impact of TV on BCR in patients with LN+ < 3						

MVA predicting the impact of TV on BCR in patients with LN+ < 3

Variabili nell'equazione						
	B	SE	Wald	Df	Sig.	Exp(B)
psm	,201	,316	,406	1	,524	1,223
aRT	-,550	,322	2,908	1	,088	,577
tumor_vol	,038	,011	11,924	1	,001	1,038
T.CAT	-,315	,316	,995	1	,319	,730
GS6vs710	,392	,304	1,667	1	,197	1,480
MVA predicting the impact of TV on CR in patients with LN+ < 3						

MVA predicting the impact of TV on CR in patients with LN+ < 3

Variabili nell'equazione						
	B	SE	Wald	df	Sig.	Exp(B)
psm	-,126	,426	,088	1	,767	,881
aRT	-,433	,450	,927	1	,336	,649
tumor_vol	,053	,013	16,199	1	,000	1,054
T.CAT	,364	,479	,576	1	,448	1,439
GS6vs710	,620	,411	2,272	1	,132	1,859

Is HOBX13 a valid marker to identify the prostatic origin of metastases?

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Introduction. HOBX13 is an important transcription factor that plays a fundamental role in the development of the posterior abdomen during vertebrate embryogenesis and especially in the prostatic formation. Several authors demonstrated that this protein is normally expressed in prostatic cells and its over-expression was found in prostate cancer. However, little is known on HOBX13 expression in the metastatic lesions from prostatic carcinoma. The aim of this study was to investigate the role of this protein as a marker of the prostatic origin of metastases.

Methods. 50 consecutive cases of metastatic tumors of known primary (age range of the patients: 51-87 yrs; median age: 66 yrs) were collected. In detail, the primitive tumor was: prostatic adenocarcinoma in 15 cases, lung carcinoma in 12, urinary bladder urothelial carcinoma in 12 and colorectal carcinoma in 11. The immunohistochemical

expression of HOXB13, prostatic specific antigen (PSA) and alpha-methylacyl-CoA racemase (AMACR) was investigated in all of the cases. Tumors were considered to be HOXB13 positive when staining was observed in more than 10% of the neoplastic cells. Intensity of the staining was graded as weak or strong. The sensitivity and specificity of HOXB13, PSA and AMACR in the recognition of prostatic origin were investigated.

Results. A strong staining for HOXB13 was observed in 15/15 (100%) metastases from prostate cancer and in 2/12 (17%) metastases from urothelial carcinoma. No HOBX13 immuno-expression was observed in the other cases. PSA immunostaining was detected in 6 (40%) cases of prostatic origin, while no staining was found in the other cases. AMACR positivity was observed in all of the metastases from prostate cancer (100%), in 4 from urinary bladder urothelial carcinoma, in 6 from lung cancer and in 9 from colorectal cancer. The sensitivity and specificity in the detection of prostatic origin were 100% and 94% for HOXB13, 40% and 100% for PSA and 100% and 46% for AMACR.

Conclusions. Our findings show that HOBX13 is a specific and sensitive marker to identify the prostatic origin of metastatic tumors. PSA had high specificity but low sensitivity, while AMACR was highly sensitive but no specific for the diagnosis of prostatic metastases. Hence HOBX13 seems to be a better marker than PSA and AMACR to identify the prostatic derivation of metastases, especially in doubtful cases when PSA analysis is not conclusive.

Epithelioid angiosarcoma arising in schwannoma of the kidney: a case report of a rare lesion in an extremely rare anatomic location and review of the literature

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Schwannoma and angiosarcoma are infrequent pathologies that have been rarely reported in the kidney. Angiosarcoma is an uncommon malignant tumor presenting a recognizable vascular differentiation. It can develop in any site but the most common locations include skin, soft tissues, breast, bone, liver and spleen while renal localization has been very rarely reported in the literature. Schwannoma is a benign peripheral nerve sheath tumor composed of cells with the immunophenotype and ultrastructural features of differentiated schwann cells. It has a wide anatomical distribution but the most frequent locations include subcutaneous tissues of the extremities and head and neck region, and the retroperitoneal and mediastinal soft tissues. The occurrence of an angiosarcoma in a pre-existing schwannoma is an extremely rare event. Microscopically, these tumors are composed of a mixture of a benign schwannoma and an epithelioid angiosarcoma. A case of angiosarcoma arising in a renal schwannoma is presented with a detailed review of the relevant literature. To the best of our knowledge the present is the first case of an angiosarcoma arising in a schwannoma of the kidney.

Burned out-vanishing tumor phenomenon of the testis with iliac lymph node metastasis of seminoma

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Introduction. The rare "burned out" phenomenon in germ cell tumors is known as the presence of an extragonadal germ cell tumor without evidence of neoplasm in the testis showing histological lesions suggestive for the earlier presence of a completely regressed testicular tumor.

About 5% of the malignant germ cell tumors originates in extragonadal sites. However metastatic deposit in lymph nodes without presence of any tumor in the testis is uncommon.

Materials and methods. We report a case of a burned out tumor of the testis in a 54-years old man presenting with scrotal discomfort and right iliac bulky lymphonodal swelling without other signs of malignancy. On clinical examination the right testis was firm and ultrasound analysis revealed a hypoechoic mass. Serological tumor markers for testicular neoplasms were normal. Subsequently, right orchiectomy was performed and the histological examination showed a distinctive constellation of findings (severe and diffuse tubular atrophy due to tubular sclerosis and associated to small areas of ischemic necrosis and to fibro-sclerotic stroma without Leydig cells). No diagnostic images for germinal cell neoplasia were found. In the adipose tissue of spermatic cord, heterotopic adrenal cortical tissue was detected. Then the patient underwent iliac lymph node excision. Histologically, the lymphoid parenchyma was widely replaced by clusters of epithelioid, cohesive cells with clear and abundant cytoplasm characterized by positivity for PLAP, CD117, cytokeratin CAM5.2 and negativity for CD30, α -fetoprotein, β -HCG. According to these histological and immunohistochemical findings, a diagnosis of lymph node metastasis from seminoma in the setting of a burned out testicular neoplasm was achieved.

Conclusions. We describe a very peculiar case of seminoma where spontaneous regression of a primary testicular tumor occurred after demonstration of iliac lymph node metastasis, a phenomenon known as "burned-out/vanishing" seminoma. Two other main features of our case are the unusual onset in iliac lymph nodes instead of retroperitoneal ones and the simultaneous presence of accessory adrenal cortical tissue in the spermatic cord.

It is of paramount importance to distinguish "burned-out" tumors of the testis from true extragonadal germ cell tumors. In conclusion, we describe this case to underline that pathologists must keep in mind the phenomenon of burned out tumors when observing, in the testicular parenchyma, a distinctive constellation of findings, suspicious for a completely regressed malignancy.

Cytological and histological changes produced in the urothelium by electromotive drug administration (EMDA) and the combination of intravesical hyperthermia and chemotherapy (thermochemotherapy)

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Introduction. Current standard therapy in case of high grade

non muscle invasive bladder carcinoma or CIS is intravesical Bacillus Calmette-Guerin (BCG). Although 30-40% of patients do not respond to one course of six instillations, half of these still achieve a complete response after an additional course of six instillations¹.

In case of BCG- refractory high grade non muscle invasive bladder carcinoma or CIS, cystectomy is the treatment of choice².

Device assisted strategies for high grade non muscle invasive bladder carcinoma or CIS include photodynamic therapy, electromotive drug administration (EMDA) and the combination of intravesical hyperthermia and chemotherapy (thermochemotherapy).

In our study we tried to describe cytological and histological changes in the urothelium after electromotive drug administration (EMDA) treatment and after thermochemotherapy in patients with BCG- refractory high grade non muscle invasive bladder carcinoma or CIS.

Material and methods. We investigated 71 patients collected at department of histopathology of Catholic University of Sacred Heart in Rome and at histopathology service of Synlab Italy in Brescia with diagnosis of high grade non muscle invasive bladder carcinoma or CIS from 2006 to 2014, 20 subjected to the EMDA treatment between 2012-2014 and 51 subjected to the thermochemotherapy treatment between 2006-2013^{3,4}. Cystoscopy and urine cytology were repeated every 3 months for a follow-up period of 24 months. Three different pathology (F.P, C.B, M.G) revalued first urine specimens bladder washing and biopsies taken during cystoscopy. All urine specimens were prepared with ThinPrep method (Hologic, Marlborough, MA). Numerous cytological and histopathologic parameters were evaluated.

Results. 8 out of 20 patients treated with EMDA and 17 out of 51 with thermochemotherapy had received cytological diagnosis of suspicious for malignancy at the first control (after 3 months from treatment) and a cystoscopy with bladder washing and biopsies were performed. In 5/8 cases treated with EMDA with cytologic diagnosis suspicious for malignancy and in 10/17 cases treated with thermochemotherapy, histopathological diagnosis of malignancy were performed, in the other cases a diagnosis of dysplasia were made.

Conclusions: EMDA treatment and thermochemotherapy produce numerous histological and cytological changes in normal urothelium. (Tab I. and Tab. II)

Cytological alterations produced by EMDA treatment or thermochemotherapy are very similar to those observed in low grade bladder tumors.

Markedly irregular nuclear borders, thickened chromatin and irregular nucleoli are the most important parameters identifying high grade urothelial tumor and CIS after EMDA or chemotherapy treatment.

Cells with spindle nuclei are frequently observed in EMDA, very rare in urine samples from patients subjected to thermochemotherapy treatment. In absence of nuclear alteration (irregular nuclear borders, thickened chromatin and irregular nucleoli) they should be considered always benign.

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Tab. I.

Cytological features associated with EMDA/termochemoterapy treatment and low/high grade urothelial tumours			
Feature	EMDA/termochemoterapy	LG Uroth Tumor	HG Uroth Tumor
Cellularity	High	High	High
Louse and dyscohesive cells	Abundant	Rare	Abundant
Clusters	Present	Abundant	Present
Cell size	Enlarged	Enlarged	Enlarged
N/C ratio	Increased	Increased	Increased
Staining	Hyperchromatic	Hyperchromatic	Hyperchromatic
Nuclear borders	Slightly Irregular	Slightly Irregular	Markedly Irregular
Chromatin	Fine, regular	Fine, regular	Thickened
Nucleoli	Variable	Variable	Multiple and irregular
Multinucleated cells and vacuolated cytoplasm	Common	Rare	Rare
Melamed-Wolinska bodies	Common	Rare	Present
Cells with spindle feature	Common (EMDA)	Rare	Present
Rare (termochemo-therapy)			

Tab. II. Pathological alterations associated with EMDA/termochemoterapy treatment

Denudation of the surface urothelium
Dyscohesive cells
"Atypical" changes in the superficial umbrella cells
Intermediate cells with nuclear enlargement, small nucleoli
Cells with spindle feature (EMDA)
Hyperchromatic nuclei
Lymphoid aggregates within lamina propria
Granulocyte within lamina propria and epithelial layer

Tiroide

A critical study concerning a diagnostic predictor model of malignancy for indeterminate thyroid FNC samples (Tir3)

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Background. The management of patients with indeterminate thyroid cytology specimens continues to be problematic especially with regard to the identification, between the indeterminate diseases, of nodules requiring surgical treatment and those benign nodules that can be only clinically observed. In this study we looked at a known diagnostic predictor model and the role of V600E BRAF that is a specific marker for Papillary Thyroid Cancer.

Methods. After an ultrasound-guided FNC, we classified the thyroid diseases in five cytological categories, according to Italian Consensus Conference Morphological Criteria of Italian Society of Pathology and Cytology (SIAPEC). A V600BRAF test was used according to our previous study. In addition, on Tir3, we applied a predictor model based on

atypia of indeterminate significance, follicular pattern with atypia, follicular pattern without atypia, obtaining twosub-groups with malignancy risk criteria:

- Tir3a: nodule with a low risk of malignancy;
- Tir3b: nodule with a moderate risk of malignancy.

Results. After FNAC cytological examination, we obtained these results: Tir2=103, Tir3a=6, Tir3b=8, Tir4=2; Tir5=5. The B-RAF mutation was found on 1 Tir3a, 2Tir3b. The final cytological classification showed: Tir2 =103, Tir3a=5 ; Tir3b= 6; Tir4=2, Tir5=8.

A Thyroidectomy was performed on 16 patients classified Tir3b, Tir 4 Tir5. For the calculation of diagnostic accuracy, the follicular adenomas diagnosed in histology were considered as true positive (Tp). Tir2 and Tir3a were followed up. Sensitivity = 100%; Specificity = 98.2%; Positive Predictive Value of malignancy (PPV) = 50%.

Conclusion. The comparison of our cytological data with a predictor model created by Banks et al., on 639 patients with cytological indeterminate diagnosis, has confirmed the necessity to subdivide AUS/FLUS category into two categories with a malignancy risk that in the future would not be calculated in empiric matter (29,31) but using more appropriate statistical analysis.

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