Ospedali Galliera di Genova

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# PATHOLOGICA

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

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

## Congresso Annuale di Anatomia Patologica

## SIAPEC-IAP 2018

In this issue:



03

Vol. 110 September 2018

PACINI EDITORE MEDICINA

SIAPEC-IAP
Società Italiana di Anatomia Patologica e Citopatologia Diagnostica,
Divisione Italiana della International Academy of Pathology

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SIAPEC-IAP
Società Italiana di Anatomia Patologica e Citopatologia Diagnostica, Divisione Italiana della International Academy of Pathology

PACINI EDITORE MEDICINA

# Updated information for Authors including editorial standards for the preparation of manuscripts

*Pathologica* is intended to provide a medium for the communication of results and ideas in the field of morphological research on human diseases in general and on human pathology in particular.

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.

**Authors are invited to submit manuscripts according to the instructions outlined below:**

by mail addressed to:

**Pathologica – pathologica@pacinieditore.it**

The manuscript must have the following enclosures:

1) The manuscript must be submitted by e-mail to the address: pathologica@pacinieditore.it

The files containing the article, illustrations and tables must be sent in attachment and the statements of the Authors indicated at the previous points 2 and 3 must also be enclosed or sent by air mail.

2) A separate covering letter, signed by every Author, must state that the material submitted has not been previously published, and is not under consideration (in whole or in part) elsewhere, and that it is conform with the regulations currently in force regarding research ethics. The Authors are solely responsible for the statements made in their paper, and must state that they have obtained the informed consent of patients for their participation in the experiments and for the reproduction of photographs. For studies performed on laboratory animals, the authors must state that the relevant national laws or institutional guidelines have been adhered to.

Only papers that have been prepared in strict conformity with the editorial norms outlined herein will be considered for publication. Their eventual acceptance is conditional upon a critical assessment by experts in the field, the implementation of any changes requested, and the final decision of the Editor-in-Chief.

3) Conflict of Interests. in the letter accompanying the article, Authors must declare if they got funds, or other forms of personal or institutional financing – or even if they are under contract – from Companies whose products are mentioned in the article. This declaration will be treated by the Editor-in-Chief as confidential, and will not be sent to the referees. Accepted works will be published accompanied by a suitable declaration, stating the source and nature of the financing.

**Editorial standards for the preparation of manuscripts:**

Pathologica will accept for publication only manuscript in English.

The article, in English, should be written in Microsoft Word™ preferably, saving files in .RTF, .DOC or .DOCX format. Any other programme can be used, including open source programmes: please always save files in .RTF, .DOC or .DOCX format.

Do not use, under any circumstances, graphical layout programmes such as Publisher™, Pacemaker™, Quark X-press™, Adobe Indesign™. Do not format the text in any way (avoid styles, borders, shading …); use only character styles such as italics, bold, underlined.

Do not send the text in PDF.

Text and individual tables must be stored in separate files.

The article must include:

(1) a title (in English);

(2) an abstract (in English);

(3) a set of key words (in English);

(4) titles and legends for all of the tables and figures.

The Authors are required to correct and return (within 48 hours of their being sent) the first set of galley proofs of their paper.

On the first page of the manuscript should appear:

A concise *title*; a set of *key words* (no more than 5); the *names* of the authors and the *institution* or *organisation* to which each author is affiliated; the category under which the authors intend the work to be published (although the final decision here rests with the Editor-in- Chief); and the *name*, *mailing address*, and *telephone* and *fax numbers* of the author to whom correspondence and the galley proofs should be sent.

The second page should contain the abstract. At the end of the text should appear the bibliography, the legends to the tables and figures, and specification (where applicable) of the congress at which all or part of the data in the paper may have already been presented.

**Tables**

Must be limited in number (the same data should not be presented twice, in both the text and tables), typewritten one to a page, and numbered consecutively with Roman numbers. In the text and legend of the tables, Authors must use, in the exact order, the following symbols: *, †, ‡, ¶, **, ††, ‡‡ …

**Figures**

Send pictures in separate files from text and tables.

- Software and format: preferably send images in .TIFF or .JPEG format, resolution at least 300 dpi (100 x 150 mm). Will not be accepted for publication manuscript with images of bad quality.

The references must be limited to the most essential and relevant citations, identified in the text by Arabic numbers and listed at the end of the manuscript in the order in which they are cited. The format of the references in the bibliography section should conform with the examples provided in N Engl J Med 1997;336:309-15. The first six Authors must be indicated, followed by et al. Journals should be cited according to the abbreviations reported on Index Medicus.

**Examples of the correct format for bibliographic citations:**

*Journal articles:* Jones SJ, Boyede A. *Some morphological observations on osteoclasts*. Cell Tissue Res 1977;185:387-97.

*Books:* Taussig MJ. *Processes in pathology and microbiology*. Oxford: Blackwell 1984.

*Chapters from books:* Vaughan MK. *Pineal peptides: an overview*. In Reiter RJ (ed.). *The pineal gland*. New York: Raven 1984:39-81.

**Acknowledgements and information** on grants or any other forms of financial support must be cited at the end of the references.

Notes to the text, indicated by an asterisk or similar symbol, should be shown at the bottom of the page.

**Mathematical terms, formulae, abbreviations, units and measures** should conform to the standards set out in Science 1954;120:1078.

*Drugs* should be referred to by their chemical name; the commercial name should be used only when absolutely unavoidable (capitalizing the first letter of the product name and giving the name of the pharmaceutical firm manufacturing the drug, town and country).

The editorial office accepts only papers that have been prepared in strict conformity with the general and specific editorial norms for each survey.

The acceptance of the papers is subject to a critical revision by experts in the field, to the implementation of any changes requested, and to the final decision of the Editor in Chief.

The Authors are required to correct and return (within 3 days of their mailing) only the first set of galley proofs of their paper.

Authors may order reprints, at the moment they return the corrected proofs by filling in the reprint order form enclosed with the proofs.

The Publisher remains at the complete disposal of those with rights whom it was impossible to contact, and for any omissions.

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PATHOLOGICA 2018;110:123-124

EDITORIALE

# *Pathologica*: un tesoro antico da proiettare nel futuro

*Pathologica* è una rivista storica nel panorama internazionale: nasce infatti il 15 novembre del 1908 ad opera del Dott. Mario Segale, annoverando nel suo comitato di direzione, personaggi del calibro di Camillo Golgi. Da una analisi delle principali riviste del nostro settore, risulta fra le più antiche ancora in essere: più antiche di *Pathologica* vi sono solo *Virchows Archives* del 1847, *Journal of Pathology* del 1892 e *Americal Journal of Pathology* del 1896. Dal 1927 la proprietà della rivista è sempre stata degli Ospedali Galliera di Genova, e dal 2002 *Pathologica* è la rivista ufficiale della Società Italiana di Anatomia Patologica e di Citopatologia Diagnostica - Sezione Italiana della *International Academy of Patholog*y. Nel tempo i numeri annuali della rivista editi a stampa hanno subito molti cambiamenti, sia come numero di fascicoli che come numero di pagine, ed attualmente la rivista è esclusivamente on line. Oggi ci chiediamo, assieme all'intero Board scientifico della rivista, come riuscire a dare un nuovo impulso alla nostra rivista ed in quali termini ciò sia realizzabile. Riteniamo che sia compito e dovere di tutti noi dare un contributo perché ciò avvenga? Siamo convinti che tale rivista debba sopravvivere e nel contempo possa rappresentare un utile e quotidiano strumento di lavoro per noi patologi? Come ciò può accadere?

L'ipotesi che noi auspichiamo è che la rivista possa da un lato continuare ad essere uno strumento di divulgazione scientifica, con lavori originali, reviews, position papers, ecc. e dall'altro possa diventare sempre più anche uno strumento di comunicazione all'interno della nostra comunità professionale, con la pubblicazione di linee guida e una particolare attenzione a problematiche gestionali e legali. Per fare ciò abbiamo bisogno del forte sostegno della nostra Società, che come ben sappiamo, dal punto di vista organizzativo, ha due colonne portanti, senza le quali non potrebbe né esistere, né essere utile: i Gruppi di Studio e le Sezioni Regionali. A loro chiediamo uno slancio di entusiasmo per rilanciare la nostra rivista.

I *Gruppi di Studio* raggruppano prevalentemente patologi esperti in singoli campi di patologia (patologia mammaria, uropatologia, patologia polmonare, ginecopatologia, ecc.) o patologi che si occupano di particolari aspetti della nostra professione (qualità e sicurezza, paleopatologia, *digital pathology*, patologia sperimentale). In tutto attualmente i gruppi attivi sono 20. Per tutti il compito è quello di divulgare la conoscenza nel singolo settore di patologia, con riunioni, incontri, seminari e convegni di stendere linee guida e di organizzare nell'ambito dei Congressi Nazionali sessioni di aggiornamento delle classificazioni e di approfondimento in campo diagnostico. Da tali attività sono già scaturiti vari articoli e numeri monografici di *Pathologica* di elevato significato scientifico e pratico. Ci auspichiamo che nel prossimo futuro tale attività possa essere ulteriormente sviluppata, e che i gruppi si adoperino per produrre sia nuovi lavori scientifici che altri articoli di approfondimento organizzativo e gestionale.

Le *Sezioni Regionali* coordinate da un Segretario regionale, si occupano, sul territorio di loro competenza, di favorire lo scambio di esperienze sia organizzative che scientifiche tra le varie Strutture di Anatomia patologica, di instaurare rapporti con gli Assessorati della Salute e di promuovere incontri nelle opportune sedi per cercare di valorizzare la nostra attività al fine di avere organici sufficienti per supportare il carico diagnostico relativo alle richieste cliniche, strutture adeguate ed a norma ed apparecchiature moderne. Alcune Sezioni Regionali hanno prodotto documenti importanti estremamente utili a fornire alle istituzioni competenti informazioni necessarie per esempio alla corretta riorganizzazione delle diverse strutture presenti sul territorio. Riteniamo che *Pathologica* possa rappresentare un utile strumento di divulgazione anche di questi contenuti in campo organizzativo, gestionale, normativo e legale ed essere pertanto di aiuto concreto per il trasferimento di tali conoscenze a tutti i patologi. Alcuni articoli su argomenti organizzativi e gestionali potrebbero essere accompagnati da un breve sondaggio (due o tre domande massimo) per avere una fotografia di quanto succede nel nostro mondo, accessibile sul web.

Attualmente *Pathologica* è una rivista *open access*, peer reviewed ed è stata prevalentemente caratterizzata da numerosi "case reports", e da un minor numero di editoriali, updates e articoli originali. *Pathologica* è edita in lingua inglese e questa resta la lingua ufficiale della rivista, ma alcuni contributi, su tematiche particolari quali quelle di tipo legale, potranno essere in italiano con un *extended abstract* in inglese.

Per facilitare il rinnovamento e accrescere la vitalità della rivista, è in corso il passaggio alla piena fruibilità online con passaggio a PubMed Central e attribuzione del *Digital Object Identifier* (acronimo DOI, in italiano "Identificatore digitale di un oggetto") agli articoli. Il DOI permette l'identificazione duratura e univoca di oggetti di qualsiasi tipo all'interno del web consentendo l'associazione ad essi dei relativi dati di riferimento – i metadati – secondo uno schema strutturato ed estensibile. In tal modo sarà possibile anche la anticipazione testi in e-pub. Saranno implementati anche un sistema per l'invio in formato elettronico dei lavori, nuovi standard di qualità per le immagini, un timing per il referaggio e per la ricezione alle modifiche che vengono richieste agli autori. L'obiettivo, sicuramente ambizioso, che ci piacerebbe perseguire è infine la attribuzione di un *impact factor* alla nostra rivista. È un percorso non facile che

ci auguriamo possa essere condiviso da tutta la nostra comunità. Per ottenere un *impact factor* occorre essere indicizzati da *Clarivate Analytics* nel database bibliografico "*Web of Science Core Collection*" che permette poi di calcolare ed attribuire un valore di *impact factor* attraverso il *citation index*. Per essere indicizzate le riviste devono essere prima valutate da *Clarivate Analytics* in base a una serie di parametri che cercheremo di perseguire. Oltre ai miglioramenti di fruibilità digitale suddetti, è necessario promuovere la qualità e originalità dei contenuti, mantenere una costanza molto stringente di periodicità della rivista e far sì che gli articoli pubblicati siano essi stessi oggetto di citazione. La qualità dei contenuti dipende anche dal nostro comune impegno a condividere su *Pathologica* i nostri lavori e dalla nostra capacità di stimolare altri colleghi a partecipare a questo nostro progetto con i loro contributi. Riteniamo inoltre auspicabile una sempre maggiore apertura della rivista verso il mondo scientifico internazionale, anche invitando personalmente alcuni prestigiosi colleghi stranieri a pubblicare sulla nostra rivista.

Per vivacizzare *Pathologica* abbiamo anche deciso di implementare le "*special sections*" aggiungendo alcune rubriche tematiche. "***Pearls in diagnostic***" potrà contenere brevi sintesi diagnostiche, gallerie di immagini di rilevante significato diagnostico, presentazioni sintetiche di nuovi marcatori immunoistochimici o molecolari. La rubrica, "***Selected readings in …***" sarà dedicata a brevi commenti a 4/5 articoli rilevanti apparsi in letteratura, focalizzata a rotazione sugli argomenti dei vari gruppi di studio: lo sforzo sarebbe di identificare dei lavori veramente significativi e di sintetizzarli in venti righe al massimo per ciascun articolo, facendo emergere perché possa essere interessante e meriti di essere letto. Entrambe le suddette rubriche potranno essere affidate a rotazione ai referenti dei Gruppi di Studio. La sezione "***Extraordinary cases***" riporterà casi di particolare interesse presentati con 4-6 immagini, una brevissima storia clinica e una altrettanto rapida e incisiva discussione; il tutto dovrebbe stare in una pagine della rivista. È una sezione pensata soprattutto aperta ai contributi dei Colleghi più giovani. Possibilmente tali casi potrebbero poi essere disponibili come "vetrini virtuali" sul sito della nostra società.. La rubrica "***Guidelines of the Italian Society of Pathology - SIAPEC***" sarà il veicolo per pubblicare le linee guida ufficiali della nostra Società che andranno a rappresentare e costituire indicazioni ufficiali di matrice istituzionale della SIAPEC-IAP. La sezione "***Digital pathology*"** sarà dedicata a alla presentazione sia delle tematiche oramai classiche di patologia digitale che in ambito di intelligenza artificiale applicata alla anatomia patologica. A sottolineare il retaggio storico della nostra rivista avremo anche la rubrica "***Historica***"avrà l'obiettivo di approfondire aspetti storici della nostra professione e rivisitare alcuni articoli comparsi sui più antici volumi di *Pathologica*, sia commentandoli che ripubblicandone parti rilevanti. Una rubrica, "***Pathology and law***" sarà rivolta alle tematiche di anatomia patologica e diritto, ove potranno trovare spazio sia commenti sulla giurisprudenza in relazione a nuove normative sia in relazione a casi reali che hanno coinvolto patologi. **"News and reports"** potrà riportare notizie dalle segreterie regionali, report di corsi e convegni o da enti che pubblicano notizie di interesse generale (es.: nuove linee guida CAP, RCPAth, NCCN…), attività dei patologi oltrefrontiera…

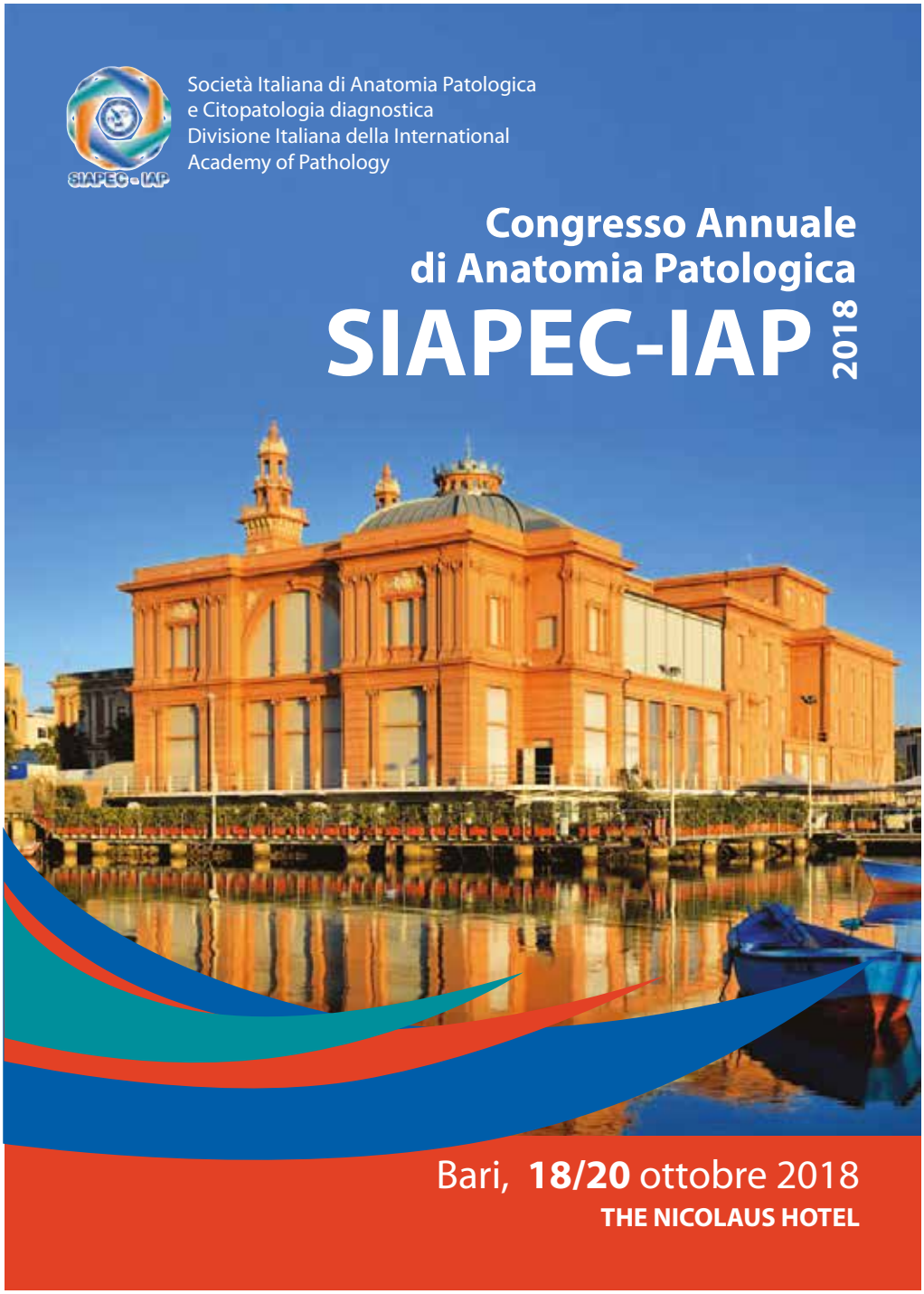
Questo anno il 15 novembre *Pathologica* avrà 110 anni di storia quindi "da adesso a…" come potrà cambiare e migliorare? Ci auspichiamo che le proposte fatte stimolino sempre più tutti noi Soci Siapec-IAP, con le rispettive competenze e conoscenze sia nel campo scientifico che organizzativo e normativo, a dare un apporto vivace e fecondo alla rivista. Chiediamo una volontà ed uno sforzo a ciascuno di noi per permettere che ciò avvenga e che la nostra rivista possa essere uno strumento utile e vitale per la nostra comunità scientifica.

Mauro Truini
*Presidente SIAPEC*

Mattia Barbareschi
*Direttore Scientifico*

PATHOLOGICA 2018;110:125-286

# ATTI



# Giovedì, 18 ottobre 2018

Sala Nico 1-2 – 12:30 - 15:00

## SESSIONE PLENARIA

Moderatori: M. Truini, G. De Rosa

### LE NUOVE PROSPETTIVE DI PATHOLOGICA

M. Barbareschi

*Unità Operativa Multizonale Anatomia Patologica, Azienda Provinciale per i Servizi Sanitari, Trento*

*Pathologica* nasce il 15 novembre del 1908 e dal 2002 è la rivista ufficiale della Società Italiana di Anatomia Patologica e di Citopatologia Diagnostica - Sezione Italiana della *International Academy of Pathology*. Oggi vogliamo dare un nuovo impulso alla nostra rivista facendo sì che possa essere uno strumento sempre più valido di divulgazione scientifica e possa diventare sempre più anche uno strumento di comunicazione all'interno della nostra comunità professionale. Per fare ciò abbiamo bisogno del forte sostegno di tutti noi patologi, della nostra Società, dei Gruppi di Studio e delle Sezioni Regionali.
Attualmente *Pathologica* è una rivista open access, *peer reviewed* ed è stata prevalentemente caratterizzata da numerosi "case reports", e da un minor numero di editoriali, updates e articoli originali. *Pathologica* è edita in lingua inglese e questa resta la lingua ufficiale della rivista, ma alcuni contributi, su tematiche particolari quali quelle di tipo legale, potranno essere in italiano con un *extended abstract* in inglese. Per facilitare il rinnovamento e accrescere la vitalità della rivista, è in corso il passaggio alla piena fruibilità online con passaggio a PubMed Central e attribuzione del *Digital Object Identifier* (acronimo DOI, in italiano "Identificatore digitale di un oggetto") agli articoli. Saranno implementati anche un sistema per l'invio in formato elettronico dei lavori, nuovi standard di qualità per le immagini, un timing per il referaggio e per la ricezione alle modifiche che vengono richieste agli autori.
L'obiettivo, sicuramente ambizioso, che ci piacerebbe perseguire è infine la attribuzione di un *impact factor* alla nostra rivista. È un percorso non facile che ci auguriamo possa essere condiviso da tutta la nostra comunità. Per ottenere un *impact factor* occorre essere indicizzati da *Clarivate Analytics* (https://clarivate.com/) nel database bibliografico "*Web of Science Core Collection*" che permette poi di calcolare ed attribuire un valore di *impact factor* attraverso il *citation index*. L'*impact factor* viene infatti calcolato ogni anno sulla base del rapporto tra articoli pubblicati nei due anni precedenti e numero complessivo delle citazioni dei lavori pubblicati: in altre parole è definito infatti come il numero complessivo di citazioni ricevute durante l'anno analizzato riferite agli articoli citabili durante i due anni precedenti (il numeratore), diviso il numero totale degli articoli citabili pubblicati nei due anni precedenti (il denominatore). Ottenere un *impact factor* è quindi un percorso di circa tre anni, da perseguire con costanza e determinazione. Il primo passo in tale processo è la domanda di indicizzazione a *Clarivate Analytics*, che valuta le riviste in base a una serie di parametri, quali le caratteristiche di *peer review*, la piena fruibilità digitale, la composizione dell'*Editorial Board* (possibilmente internazionale), la costanza molto stringente di periodicità della rivista, la rilevanza e qualità dei contenuti, l'originalità della rivista stessa.
La qualità dei contenuti dipende da nostro comune impegno a condividere su *Pathologica* i nostri lavori e dalla nostra capacità di stimolare altri colleghi, manche di altre nazioni, a partecipare a questo nostro progetto con i loro contributi. È infatti auspicabile una sempre maggiore apertura della rivista verso il mondo scientifico.
Per vivacizzare *Pathologica* abbiamo anche deciso di implementare le "*special sections*" aggiungendo alcune rubriche tematiche: "*Pearls in diagnostic*" con brevi sintesi diagnostiche, gallerie di immagini di rilevante significato diagnostico, presentazioni sintetiche di nuovi marcatori immunoistochimici o molecolari; "*Selected readings in …*" dedicata a brevi commenti a 4/5 articoli rilevanti apparsi in letteratura, focalizzata a rotazione sugli argomenti dei vari gruppi di studio; "*Extraordinary cases*" selezione di casi presentati con 4-6 immagini, una brevissima storia clinica e una altrettanto rapida e incisiva discussione; "*Guidelines of the Italian Society of Pathology - SIAPEC*" veicolo per pubblicare le linee guida ufficiali della nostra Società che andranno a rappresentare e costituire indicazioni ufficiali di matrice istituzionale della SIAPEC-IAP; "*Digital pathology*" dedicata a alla presentazione sia delle tematiche classiche di patologia digitale che in ambito di intelligenza artificiale applicata alla anatomia patologica; "*Historica*" per approfondire aspetti storici della nostra professione; "*Pathology and law*" rivolta alle tematiche di anatomia patologica e diritto; "N*ews and reports*" con notizie dalle segreterie regionali, report di corsi e convegni o da enti che pubblicano notizie di interesse generale (es.: nuove linee guida CAP, RCPAth, NCCN…), attività dei patologi oltrefrontiera…
Per un vero rinnovamento della rivista è indispensabile una volontà ed uno sforzo a ciascuno di noi: solo così potremo far sì che la nostra rivista sia uno strumento utile e vitale per la nostra comunità scientifica.

### LA RC IN ANATOMIA PATOLOGICA

G. Marzo

La Legge n. 24/2017, legge Gelli, ha:
- tipizzato il c.d. rapporto di cura;
- affermato il principio secondo cui la sicurezza delle cure è diritto del paziente (nel quadro della tutela costituzionale del diritto alla salute);
- regolamentato il processo civile per malpratica medica;
- istituito un reato speciale per omicidio colposo e lesioni colpose in ambito sanitario.

Per quanto di interesse, la Legge Gelli cristallizza le regole in tema di responsabilità civile sanitaria, alcune delle quali già affermate dalla Giurisprudenza. Precisamente:

a) viene confermata la responsabilità civile della Struttura, anche per l'attività prestata dagli esercenti la professione sanitaria, chiarendo trattarsi di responsabilità di natura contrattuale;
b) viene confermata la responsabilità dell'esercente la professione sanitaria, chiarendo che tale responsabilità nei confronti del paziente ha natura extracontrattuale, tranne nei casi in cui tra l'esercente la professione sanitaria e il paziente intercorra un rapporto contrattuale. Trattasi di innovazione, solo in parte già presente nella Legge Balduzzi, che appare destinata a ridurre il contenzioso nei confronti del medico (tradizionalmente si ritiene che in caso di esercizio dell'azione di risarcimento per la responsabilità extracontrattuale il paziente sia posto in particolari difficoltà probatorie, che invece non sono presenti in caso di esercizio dell'azione di risarcimento per inadempimento contrattuale);
c) viene disciplinata l'azione di rivalsa da parte della Strut-

tura privata e della compagnia di assicurazione verso l'esercente che, con colpa grave o dolo, abbia cagionato al paziente il danno che Struttura e/o Compagnia abbiano risarcito;

d) viene disciplinata l'azione per danno erariale (innanzi alla Corte dei Conti) nel caso di cui sopra relativamente alle Strutture pubbliche;

e) viene imposto l'obbligo di assicurazione (o altra analoga misura) per le Strutture e per il singolo esercente la professione sanitaria.

La posizione dell'ANATOMOPATOLOGO è, dunque, per qualche aspetto identica a quella di ogni altro esercente la prestazione sanitaria (egli risponde cioè del danno cagionato sia nei confronti del paziente, sia nei confronti della struttura in cui opera, ed è altresì soggetto a responsabilità per danno erariale o a responsabilità per rivalsa).

Per altri aspetti la posizione dell'ANATOMOPATOLOGO è, invece, assai più delicata che per altre specialità:

a) il mancato rapporto diretto con il paziente importa l'impossibilità di creare una relazione empatica (che sovente riduce il rischio di richieste risarcitorie);

b) la prestazione sanitaria viene eseguita su materiale raccolto da altro esercente la professione sanitaria (il vetrino è confezionato da un tecnico);

c) la rapidità in cui la prestazione sanitaria deve essere resa (se l'intervento è in corso l'anatomopatologo deve provvedere immediatamente a fornire il responso).

L'aspetto più interessante a fini giuridici, al quale occorre prestare particolare attenzione, è quello sopra richiamato sub b). Lo spirito con cui la Legge n.24/2017 affronta il cd "rapporto di cura" coinvolge e accomuna – quali responsabili verso il paziente – tutti gli esercenti la prestazione sanitaria anche quando la prestazione di ciascuno interviene non simultaneamente.

# Giovedì, 18 ottobre 2018

Sala Nico 2 – 16:00 - 19:00

## PATOLOGIA APPARATO DIGERENTE

## CRC: Histology & Molecular Pathology

Moderatore: M. Guido

### TUMOR BUDDING IN COLORECTAL CANCER: WHEN AND HOW

F. Galuppini

*Pathology Unit, Department of Medicine (DIMED), Padova, Italy*

Tumor budding is considered an independent prognostic factor in many gastrointestinal neoplasia, especially in colorectal cancer (CRC) [1]. Despite this, tumor budding has not yet been addicted in the TNM staging system and in many routine clinical reports. The reason is partly due to the lack of a shared definition, as over the years numerous formulations for the concept of "bud" have been going on, and to the difficulties in assessment techniques and in stratification in risk-based classes [2].

The most widely accepted definition of tumor budding was coined by Ueno et al. who identified buds as isolated malignant cells or up to 4 cell clusters in the stroma at the invasive front of the neoplasia [3].

An effort in the direction of a shared scoring method was been conducted in 2016 at the University of Bern where a group of international experts met in the International Tumor Budding Consensus Conference (ITBCC) to draw up a series of evidence on colorectal tumor budding [4]. The proposed scoring assessment for the most accurate prognostic risk stratification consisted in a three-tier system (low, intermediate and high budding) evaluated in a tumoral "hotspot" stained with hematoxylin and eosin.

Budding is a morphologically visible sign of tumor dissemination and has been linked to epithelial to-mesenchymal (EMT)-like processes. Most recently, transcriptome profiling studies have linked tumor buds with the mesenchymal type of CRC [5]. In fact, we could not speak about budding without considering the fundamental role of the surrounding microenvironment in its development and modulation. The budding phenome could be considered as an interaction between neoplastic cells and stromal and inflammatory elements at the invasive front of the tumor [6 7]. In the last decade, numerous studies have shown that tumor-infiltrating lymphocytes hinder the development of budding and above all positively affect the prognosis [8 9].

It is therefore not surprising that large bodies of data have consistently demonstrated tumor budding to be an independent adverse prognostic marker in CRC and associated with unfavourable clinico-pathological features, nodal and distant metastases.

The budding prognostic value was been established particularly in pT1 and in stage II CRCs. In the former, it was been shown that high budding values correlate with a greater probability of lymph node metastasis, while in the latter with a poorer overall survival rate [10].

Tumor budding in CRC is a robust biomarker which is simple to use and can be assessed using routine light microscopy on H&E stained slides. The standardized method as proposed by the ITBCC consensus recommendations will hopefully pave the way of integrating tumor budding in reporting protocols as has recently been the case for the latest update of the CAP CRC checklist. Further validation studies such as we present here aim to promote more widespread integration of the ITBCC consensus method in standardized reporting of tumor budding in CRC.

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## THE PATHOLOGIST'S ROLE IN THE TREATMENT OF RECTAL CARCINOMA

L. Saragoni

*Department of Pathology, G.B.Morgagni-L.Pierantoni Hospital of Forlì*

Quality of rectal cancer care is highly variable. The vast majority of surgery for rectal cancer is performed by non specialists in low-volume hospitals. Moreover, there is a sub-optimal adherence to evidence-based guidelines. So, there is the need of accreditated rectal cancer centres. They should satisfy the following parameters :

- high volume of the activity and specialization of the surgical team
- dedicated imaging service
- systematic pathological assessment of CRM/margins/lymph nodes retrieval
- neoadjuvant/adjuvant treatments
- multidisciplinary team (MDT)

The role of MDT is pivotal : it allows to improve the selection of patients candidated to the neoadjuvant therapy, compare pathology to pre-operative staging and give to the surgeons the feedback on the quality of TME through demonstrating mesorectal grade and CRM.

When the plane of surgery reaches the muscularis propria the risk of local recurrences is about three times in comparison with patients with mesorectal integrity. Factors associated with a positive CRM are advanced TN, large tumor size, infiltrative margin, poor differentiation and vascular invasion.

Another important pathological parameter is represented by the distal tumor spread (DTS). It defines the microscopic tumor spread distal to the main mass. DTS can be intramural or extramural by the way of mesorectum and it can be continous or discontinous. In the last case the invasion can be lymphatic, perineural and/or by satellite nodules. More extensive DTS is associated with higher T stage, higher N stage, poor tumor differentiation and vascular invasion. Apart from CRM and DTS, the number of retrieved lymph nodes from rectal specimens influences the prognosis.It has been demonstrated that more are the examined lymph nodes, better are the outcomes. Under 12 lymph nodes, which is the minimum number accepted, the prognosis is significantly worse. Anyway, it is demonstrated that neoadjuvant chemo-radiotherapy decreases the number of lymph nodes harvested from rectal cancer specimens. In particular, the number of lymph nodes is lower in ypT0-1 patients (11.0 +/- 3.0). Lympho/vascular invasion has been associated with a significantly higher risk of lymph node metastases, whereas perineural invasion (PNI) decreases both overall and disease free survival. Stages I-II with PNI+ have worse prognosis than stage III without PNI.

Moreover, PNI is associated with a higher local recurrence (RR = 3.32), decreased disease free survival (RR = 2.35) and cancer related survival (RR = 3.61).

Evaluation of tumor response to neoadjuvant chemo-radiotherapy (CRT)

CRT decreases local recurrence and good tumor response correlates with better overall survival and lower incidence of lymph node metastases. Tumor regression and tumor down-staging are not equivalent. Tumor regression equals tumor down-staging when there is complete tumor effacement. Pathological complete response (pCR) leads to T0. Marked tumor regression can be observed without tumor down-staging. Tumor regression grade is an independent prognostic indicator of disease free survival for ypN0 patients. Moreover, TRG influences significantly overall survival, disease free survival and cumulative recurrence. Tumor regression grade remains an independent prognostic factor also after adjusting for pTN stage, lymphovascular invasion and margin status.

**Pathological Report**

It should include the evaluation of mesorectum, pathological staging, all the histopathological prognostic factors, margins of resection (CRM and distal) and grading of tumor regression if the patient underwent adjuvant therapy.

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# Giovedì, 18 ottobre 2018

Sala Cigno – 16:00 - 19:00

## NEUROPATOLOGIA E PATOLOGIA PEDIATRICA

Moderatori: P. Collini, G. Magro

## UPDATE ON PERIPHERAL NERVOUS SYSTEM TUMORS

P. Collini

*Soft Tissue and Bone Pathology, Histopathology and Pediatric Pathology Unit IRCCS Istituto Nazionale dei Tumori of Milan – Milan, Italy*

Peripheral nerves tumors represent the most frequent peripheral nervous system tumors (PNST). They are now includes in the last WHO Classification of soft tissue and bone tumors. Respect to the other soft tissue tumors they are characterized by a frequent association with a genetic disorder (i.e.,

Tab. I.

| Diagnosis | H3K27me3 IHC Loss/Total Cases |
|---|---|
| Cutaneous melanoma | |
| Pure desmoplastic melanoma | 0/37 |
| Mixed desmoplastic melanoma | 0/11 |
| Spindle cell melanoma | 0/5 |
| Synovial sarcoma (MF, BF, and PD) | 0/113 |
| GIST | |
| *KIT*/*PDGFRA* mutant | 0/109 |
| SDHB-deficient WT pediatric and adult | 0/13 |
| WT dedifferentiated GIST | 0/1 |
| Liposarcoma | |
| Well differentiated | 0/31 |

NF1) and the possible origin from a benign precursor (i.e., neurofibroma). They are formed by Schwann cells, perineural cells, fibroblasts, vessels, inflammatory cells, fibrofatty tissue, represented in vary percentages. Neurofibroma (NF) is the post frequent benign PNST. It is formed by all the previous components, with sparse axons. The most part are sporadic and solitary, and multiple in NF1. Four types are recognized: localized cutaneous NF, diffuse cutaneous NF, intraneural (solitary, plexiform) NF, and massive NF of soft tissue. Localized and plexiform intraneural NFs are precursors of MPNST, with a risk of 5-10% in NF1. MPNST arises in extremities, trunk, and head and neck. In NF1 it occurs at earlier ages, even in children and young adults, and has a worse outcome.
The differential diagnosis of MPNST among spindle cell sarcomas can be difficult due to the lack of a diagnostic marker, S100 protein included, that is typically negative in MPNST. The loss of H3K27me3 can be of aid.
Another debated issue are the criteria to define malignancy in MPNST associated with NF in NF1 patients. Updated criteria have been recently published.
In pediatric ages the incidence is reported lower than adults. Localized and resectable tumors have a better survival. Unresectable tumors have a very poor prognosis. The application of a multidisciplinary approach and multimodal therapy (CT+/-RT and surgery) seem to give an advantage along time.

# Slide Seminar

# Diagnosi inaspettate: a case-based update

Moderatori: E. D'Amore, M. Gardiman

## WHEN MOLECULAR PROFILE IS CONTRAST WITH HISTOPATHOLOGY: AN INTRIGUING CASE OF GLIOMA

V. Barresi

*Dipartimento di Patologia Umana dell'Adulto e dell'Età Evolutiva, Università di Messina; * Dipartimento di Diagnostica e Sanità Pubblica, Università di Verona*

Gliomas are tumors which are supposed to derive from glial cells. They represent the most common primary neoplasias of the central nervous system (CNS). According to the most recent World Health Organization (WHO) Classification, they are subdivided into two main groups: astrocytomas and oligodendrogliomas. Astrocytomas are composed of cells with oval nuclei and nuclear atypia (which progressively increase with the histological grade). Oligodendrogliomas are formed by cells with rounded nuclei and clear peri-nuclear halo.
On the basis of Isocitrate Dehydrogenase 1 and 2 (IDH1/2) gene mutations, astrocytomas are further sub-grouped in astrocytomas IDH wild type and astrocytomas IDH mutant. On the other hand, oligodendrogliomas are defined by the presence of IDH mutation and 1p/19q codeletion. It is estimated that 100% tumors with classical oligodendroglial morphology (round nuclei and clear peri-nuclear halo) are IDH mutant and 1p/19q codeleted. Among those categories, oligodendroglioma shave the best prognosis, while astrocytomas IDH wild type have the worst.
Due to their different prognosis, these tumors also receive different treatments after surgery.
Herein, we describe a frontal lobe glial tumor, which was incidentally discovered after a trauma in a 44-year old man. It had classical histopathological features of oligodendroglioma, immunohistochemical positivity for GFAP, olig-2 and ATRX, but absence of IDH mutation and 1p/19q coledetion. Ki-67 labeling index was 1%. At immunohistochemistry, expression of PTEN was lost. According to 2016 WHO Classification, the tumor should be classified as diffuse astrocytoma, IDH wild type, grade II, which carry an adverse prognosis. The patient did not receive any adjuvant treatment after complete surgical resection. No recurrence has been observed after 8 months follow-up. Since pediatric oligodendrogliomas are IDH wild type and non-codeleted, we wonder whether this tumor could represent an oligodendroglioma with pediatric molecular phenotype arisen in an adult subject.

## CORDOMA SCARSAMENTE DIFFERENZIATO CON PERDITA DI ESPRESSIONE DI SMARCB1/INI1: PRESENTAZIONE DI UN CASO

D. Bifano

Il Cordoma è una rara neoplasia ossea maligna che origina dai resti della notocorda fetale pertanto insorge quasi esclusivamente nello scheletro assiale; il Cordoma scarsamente differenziato rappresenta un ancor più raro sottotipo che colpisce primariamente i bambini ed è associato ad un decorso clinico aggressivo.
Descriviamo un caso del 2013, di una bambina di 3 anni con atteggiamento coatto del capo da alcuni mesi. Alla RMN encefalo-cervicale si evidenziava un processo espansivo del clivus e della giunzione cranio-cervicale, solido con disomogenea impregnazione dopo m.d.c. La paziente veniva sottoposta ad intervento di asportazione subtotale del tumore e a successiva radioterapia.
Istologicamente si osservava una neoplasia costituita da grandi cellule epitelioidi con moderato pleomorfismo nucleare, coese in "sheets" e nidi immersi in una matrice fibrosa. All'indagine immunoistochimica le cellule neoplastiche rivelavano diffusa positività per panCK, EMA, focale per S100 e negatività per INI-1/SMARCB1; non è stata effettuata l'immunocolorazione per brachyury perché non disponibile presso il nostro servizio. Veniva posta diagnosi di Cordoma Scarsamente Differenziato. Il caso è stato sottoposto a varie revisioni istologiche che, pur

senza l'immunocolorazione per brachyury, confermavano la suddetta diagnosi. In un solo caso, sulla scorta del risultato dell'analisi FISH che evidenziava riarrangiamento del gene EWSR1, veniva posta diagnosi di carcinoma mioepiteliale. Recentemente è stata eseguita l'immunocolorazione per brachyury risultata diffusamente ed intensamente positiva.

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## PEDIATRIC SOFT-TISSUE PERINEURIOMA, AN UNDERESTIMATE NEOPLASM


M. Montella[1], A. Ronchi[1], I. Cozzolino[1], I. Panarese[1], G. Roccuzzo[1], G. Toni[1], R. Franco[1], A. De Chiara[2]

*[1] Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università della Campania "Luigi Vanvitelli", Naples, Italy; [2] Pathology Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori, "Fondazione G. Pascale", IRCCS, Naples, Italy*


**Objective**

Peripheral nerve sheath tumors (PNSTs) are common soft tissue neoplasms encountered in the surgical pathology practice, most of which have specific histologic and immunohistochemical features. Much less common than schwannomas and neurofibromas, perineuriomas are benign PNST composed by perineurial cells. Despite being a neoplasm with very specific characteristics, perineuriomas can be sometimes a diagnostic challenge for surgical pathologists.

**Materials and methods**

We present a case of a 19-year-old male patient with a nodular mass in the right arm. An ultrasonography was performed and detected a sub-cutaneous hypoechoic nodule, measuring 4,5x2 cm, with sharp border, firm and painless. The patient underwent to surgical excision. Grossly the nodular mass was well circumscribed, encapsulated, with a yellowish-white fibrous cut surface. Microscopically, the proliferation was composed by a moderately cellular mesenchymal proliferation of spindle cells, arranged in vague storiform pattern and also forming whorls, with no/very slight atypia. Focally, were detected formation of tiny "onion bulbs". On the basis of the pure morphology, the most important differential diagnosis included: solitary fibrous tumor (SFT), low grade fibro-myxoid sarcoma (LGFMS), perineurioma, and dermatofibrosarcoma protuberans (DFSP). SFT usually display an encapsulated/well demarcated proliferation of spindle cell with both hypocellular and hypercellular areas, light-mild atypia and thin, dilated, branching "staghorn" blood vessels. SFT characteristically shows nuclear staining for STAT6 and also positivity for bcl2, CD34. LGFMS is a low-grade malignancy soft-tissue neoplasm composed by hypocellular-moderately cellular proliferation, sharply demarcated, showing alternating fibrous and myxoid areas containing monomorphic spindled to ovoid tumor cells, arranged in a fascicular, storiform, or whorled growth pattern. LGFMS commonly express MUC4 and EMA, rarely CD34 and bcl2. Perineurioma is a quite rare PNST, usually encapsulated, with a whorled or storiform architecture, composed by a spindle cell proliferation with characteristic thin, delicate bipolar cytoplasmic processes. By definition, perineuriomas show immunohistochemical staining positive for EMA, GLUT1 and claudin and negativity for S100. DFSP is a locally aggressive neoplasm with significant potential for local recurrence, but without metastatic risk. In its classical form DFSP has ill-defined and diffusely infiltrative border. The neoplasm is composed by a hypercellular population of slender spindle cells, disposed in a monotonous storiform pattern. Typically tumor cells are strongly and diffusely positive for CD34. At immunohistochemistry the neoplasm showed positivity for EMA, Glut-1, claudin, focally positive for CD34 and negativity for S100, MUC4 and STAT6. On the basis of microscopic features, in association with the immunohistochemical and molecular findings, a final histological diagnosis of soft tissue perineurioma was performed.

**Results and conclusion**

We report a case of a young male patient affected by Perineurioma. This neoplasm is quite rare, especially in pediatric patients. In their cohort of 81cases, Hornick et al. reported only 4 cases in children (4.9%). There are four main clinicpathologic forms of perineurioma: soft tissue perineurioma, intraneural perineurioma, sclerosing perineurioma and mucosal perineurioma. Perineuriomas have several mimicking neoplasms from which it must be distinguished for proper clinical practice.

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# Giovedì, 18 ottobre 2018

Sala Orione – 16:00 - 19:00

## PATOLOGIA GINECOLOGICA

## Lesioni placentari: patologia o adattamento?

Moderatori: G. Bulfamante, E. Fulcheri

### THE PLACENTAL SURFACE VESSELS: A STILL LITTLE EXPLORED SECTOR IN PRENATAL DIAGNOSTICS AND HISTOPATHOLOGY


F. Buffelli, M. Lituania


There are numerous abnormalities of the umbilical cord and of the amniochorial vessels and the pathological conditions associated with them; although known for many years in pathological anatomy, they have not aroused as much diagnostic interest in the obstetric-gynaecological field, despite being frequently related to fatal deaths, intra- or peri-partum deaths, neonatal neurological damage or states of disability. The thrombotic and haemorrhagic pathologies of the cord are often related to several conditions: the morphology, structure,

length and thickness of the umbilical cord; anomalies in the number of vessels, placental insertion, the presence of vasa previa, endouterin distortion processes, primitive or secondary vascular pathologies, hematoma, oedema, cysts and tumour masses of the funiculus.

Haemorrhagic and thrombotic pathology can occur along the entire course of the arteries and the umbilical vein. In order to correctly frame the vascular pathology, it is necessary to distinguish the portions of the vascular tree, in order to allow topographically targeted and codified instrumental investigation. The course of the umbilical arteries consists of two portions: the intra-abdominal tract and the intra-amniotic tract. The umbilical arteries originate from the subdivision of the internal iliac arteries, surround the bladder, and converge forward and rostrally up to the umbilical ring. From the umbilical ring, together with the umbilical vein, they run into the cord (intra-amniotic portion).

The umbilical arteries reach the placenta, forming anastomoses in 95% of cases, generally within 0.5-1.5 cm from placental insertion (equalizing the values of flow and pressure between the two umbilical arteries in order to uniform the distribution of blood between the placental lobes). Foetal blood reaches the placenta through the umbilical arteries; each branch directed towards a main cotyledon is subdivided into intra-placental arteries to form the capillaries of the chorionic villi. The oxygenated blood returns from the capillary loops of the villi to the umbilical cord through the veins that flow first into the amniochorial vessels and then into the umbilical vein. The course of the umbilical vein consists of two portions: the intra-amniotic tract and the intra-abdominal tract. The first tract, from the placenta to the umbilical ring, runs in the funiculus together with the two umbilical arteries, immersed in the Wharton's jelly. The intra-abdominal tract runs from the umbilical ring to the portal sinus. The umbilical vein enters the foetal abdomen at the level of the umbilical ring, heads upward into the falciform ligament, until it reaches the lower margin of the liver (extra-hepatic portion). It then rises rapidly until it penetrates into the liver parenchyma (intra-hepatic portion), where it anastomoses with the portal vein at the portal sinus.

A simple classification allows us to nosographically schematize and distinguish different pictures responsible for haemorrhagic and thrombotic pathologies that affect the intra-amniotic portion of the umbilical cord and, at the same time, to justify the flow variations, which range from transitory or intermittent to complete block. The main abnormalities of the cord and of the placenta related to pathological conditions that could potentially cause thrombotic-haemorrhagic events of the funiculus and of the amniotic cation vessels are summarized in Table I.

A complete study of the foetus should always include the evaluation of the umbilical cord and of the amniochorial vessels in search of possible anomalies. Pathologies of the cord and of the amniochorial vessels may be related to abnormalities of the placenta, or of the foetus or to chromosomal aberrations. When a pathology of the funiculus is detected, it is mandatory to perform careful foetal re-evaluation with the aim of completing the diagnostic procedure and planning the ultrasound follow-up, the management of the pregnancy, the timing and the modalities of delivery. In particular, color Doppler and fluximetric study are an indispensable means of evaluating and diagnosing vascular anomalies of the cord and of the first arterial or venous branches, and of establishing the onset of haemodynamic changes associated with alterations of foetal growth. 3D and 4D Doppler ultrasounds of the funicular also allow more accurate diagnosis of vascular abnormalities. The early diagnosis of cord abnormalities and serial follow-up should be able to reduce the risk of perinatal mortality and morbidity by favoring a decision-making process to protect the mother and the fetus.

Table I.

| Type of anomalies of the cord and amniochorial vessels | Associated pathological conditions |
|---|---|
| Morphological | Spiralization (hyper and hypospiralization), anomalies in the diameter of the cord (oedema, narrowing or coarctation), anomalies in the length of the cord (excessive length or absolute shortness) |
| Insertion | Marginal, velamentous, interposing velamentous, "furcate" insertion, vasa previa, angiodystopic arches on the free membranes angiodystopic arches of the amniotic cation vessels on the placental disk |
| Placentae variants | "Bi-lobate", "pluri-lobate", "succenturiate" |
| Abnormalities due to mechanical causes | Cord twisting, true knot, cord entanglement in monochorionic mono-somatic twin pregnancies, nuchal cord, bandolier wraps around the thorax, loop wraps around the limbs, amniotic bands, funicular rupture, funicular prolapse |
| Vascular | Cord thrombosis, haematoma, varicose vein, aneurysm, anomalies in the number of vessels (SUA), thrombosis of the amniochorial vessels, fractures of the amniochorial vessels ("Breus pseudomola"), phlebectasis of the amniotic corium vessels, aneurysms or angiodysplasias of the amniochorial vessels |
| Masses of the cord | Tumours: haemangioma, teratoma<br>Cord cyst |

## MONOCHORION TWIN PLACENTAS HISTOLOGICAL EVALUATION AFTER FETOSCOPIC LASER PHOTOCOAGULATION: THE PATHOLOGIST ANSWERS CLINICAL QUESTIONS

V. Toto[1*], M.A. Rustico[2*], G.P. Bulfamante[3]

*[1] Division of Human Pathology, ASST Santi Paolo e Carlo, Milan, Italy; [2] Fetal Therapy Unit "U.Nicolini", Vittore Buzzi Children's Hospital, Università degli Studi di Milano, Milan, Italy; [3] Unit of Human Pathology and Developmental Pathology, Department of Health Sciences, Università degli Studi di Milano, ASST Santi Paolo e Carlo, Milan, Italy; *equal contributors*

Twin to twin transfusion syndrome (TTTS) is a severe complication of monochorionic (MC) twin pregnancy. Refinements in fetoscopic laser photocoagulation technique, in particular the introduction of sequential selective ablation, "Solomon technique", have led to significant improvements in pregnancy outcomes, expecially regarding the most relevant complications, recurrent TTTS and twin anemia

polycythemia sequence (TAPS). Solomonization has been seen to decrease the number of residual anastomoses; however, questions remain regarding the depth of damage to the chorionic plate and potential clinical effects such as hemorrhage, membrane rupture, placental abruption or acute twin to twin transfusion.
Few studies have been made since now to understand the histological aspects of placenta after Solomon photocoagulation and to investigate the pathogenesis of the pregnancy complication related to this new technique.
The strict cooperation between our Pathology Department and the Fetal Therapy Unit has led us to analyze a large number of monochorionic twin placentas after Solomon photocoagulation. The aim of histological evaluation, besides finding a reasonable pathogenesis to fetal endouterine demise or to severe intrauterine growth restriction, is to analyse the parenchymal changes after photocoagulation and to explain the related pregnancy complication, expecially concerning placental abruption. Furthermore, a deep analysis has been done to understand histological changes in TAPS, which remains a severe pregnancy and perinatal complication.

### PLACENTAL PATHOLOGY AND FOETAL OUTCOME: WHICH VALUE FOR THE PLACENTAL HISTOLOGICAL "LESIONS"?

A. Marzullo, L. Resta, N. Laforgia*

*Anatomia patologica, UOC di Neonatologia* – Azienda Ospedaliero-Universitaria Policlinico – Università degli Studi di Bari*

Results of a study targeted to the detection of the weight that different pathological categories of the evaluation of the placental injure have regarding placental functionality and fetal outcome has been presented. Basis of the research was the consideration that it is still unknown if a certain pathological condition histologically observed is able, and to what extent, to alter the physiological development of the foetus. 364 Placentas and 392 new-borns were evaluated. All the cases were divided in 8 groups: pre-term, SGA<2000 g, perinatal asphyxia, malformed foetuses, jaundice, sepsis, perinatal death and foetuses with physiological outcome. The histopathological diagnosis were reviewed according the parameters of the Amsterdam Consensus Conference held in 2015 (Khong TY et al. Arch Pathol lab Med 2016; 140 (7): 698-713). The correspondence between placental pathology and perinatal disease was elevated, as expected; particularly meaningful was the correlation between early maternal mal-perfusion and the two groups of SGA and pre-term newborns. Surprisingly a high percentage of placental abnormalities was observed in new-born with favourable outcome (60%). Some considerations are made including the possible legal implications of such results.

## Pitfalls in patologia ginecologica

Moderatori: G. Negri, G.F. Zannoni

### PITFALLS IN PATOLOGIA MESENCHIMALE UTERINA

E. Bragantini, A. Pesci

*APSS Ospedale Santa Chiara, Anatomia Patologica Trento; IRCCS Ospedale Sacro Cuore Don Calabria, Negrar, Verona*

Tra le neoplasie mesenchimali dell'utero i tumori muscolari rappresentano l'entità più frequente. Le caratteristiche citomorfologiche, il tipo di cellularità, la presenza e numero di mitosi ed il tipo di necrosi, sono i criteri utilizzati per la classificazione di questi tumori in benigni, ad incerto potenziale maligno e maligni.
Sia nelle forme benigne che in quelle maligne esistono pattern morfologici simili che rendono complessa la definizione del comportamento biologico della neoplasia.
Inoltre l'influenza di ormoni endogeni o da eventuale terapia alterare i quadri morfologici ponendo dubbi diagnostici.
Nella definizione dell'istotipo e nella corretta classificazione della lesione è molto importante pertanto riconoscere il pattern morfologico e confermarlo con l'immunoistochimica. E' sempre utile, pertanto, utilizzare un panel di marcatori che contemplano le diagnosi differenziali ipotizzate all'attento esame morfologico.
Nella valutazione morfologica della lesione che stiamo diagnosticando è importante riconoscere pattern ed il tipo di cellula (fusata e/o epiteliode, eosinofila e/o chiara), il background in cui si sviluppa, le caratteristiche dello stroma (mixoide, sclerotico o idropico), la presenza, tipologia e distribuzione dei vasi nel contesto della lesione.
Esistono inoltre entità rare che devono essere considerate nella diagnosi
differenziale.
Il PEComa è tra le più interessanti per istogenesi e per implicazioni cliniche. La cellula PEC è stata descritta da Bonetti et al nel 1992 per indicare una cellula a morfologia epitelioide caratterizzata da un citoplasma chiaro e/o eosinofilo, con un'origine perivascolare e che esprime marcatori di tipo melanocitario (HMB45) e muscolare. La WHO dei tessuti molli del 2002 introduce la definizione di cellula PEC e di PEComa, identificando una famiglia di tumori a cui appartengono l'angiomiolipoma, il clear cell sugar tumor, linfangioleiomiomatosi, clear cell myomelanocitic tumor del legamento falciforme ed i clear cell sugar tumor di pancreas, retto, utero e cervice.
L'eterogeneità di questa lesione e la sua rarità nel tratto ginecologico la rende un importante pitfall diagnostico. In letteratura veniva messa in discussione l'esistenza del PEComa come entità in sede genitale femminile: alcuni autori sostengono che il PEComa sia una lesione leiomuscolare di tipo epitelioide con una variabile espressione di markers melanocitari. Recenti recenti studi hanno precisato e definito i criteri morfologici e molecolari stabilendo inoltre il cut-off di malignità. La corretta diagnosi di PEComa è importante per una serie di implicazione clinico-terapeutiche e prognostiche.

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### PITFALL IN PATOLOGIA OVARICA

A. Santoro, G.F. Zannoni

*[1] Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS – Università Cattolica del Sacro Cuore, Rome, Italy*

Diffuse Yolk Sac Tumor patterns in a peritoneal carcinomato-

sis in an elderly woman following chemotherapy for Ovarian High Grade Serous Carcinoma.

**Background**
Yolk Sac Tumor (YST) is the second commonest (20–25%) subtype of Ovarian Germ Cell Tumor (OGCT). Usually it occurs in young women as pure form or associated with other germ cell components. Rarely it has been reported in postmenopausal patients with an unusual association with Müllerian epithelial elements, for the most part malignant. The simultaneous presence of YST and ovarian malignant epithelial tumor may herald a more aggressive behavior regardless of the stage of presentation and the mixed tumors may be less responsive to traditional germ-cell tumor chemotherapy.

**Case report**
In 2014 our patient underwent hysterectomy with bilateral salpingo-oophorectomy followed by adjuvant chemotherapy for ovarian high grade serous cancer (HGSC). Two years later the patient developed peritoneal carcinomatosis and multiple intra-abdominal recurrences. High serum levels of Alpha-Fetoprotein (AFP) were documented. Then, a secondary cytoreductive surgery comprehensive of omentum, peritoneal nodules, spleen and ileum, was performed.
The pathological examination revealed a pure glandular YST component in the omentum, in peritoneal nodules, in pelvic lymph nodes, in the round ligament and in the spleen. In the recurrence located in the ileum the neoplasm maintained the same morphology of the antecedent tumor, without a detectable YST component. The morphological tumoral heterogeneity has also been confirmed by immunohistochemistry (IHC).
The YST component was positive for AFP, Vimentin and CAM5.2; negative for CK7, EMA, PAX8, WT1, ER and PR. The component with an epithelial serous morphology showed positivity for CK7, PAX8, WT1, ER and PR and negative imunostain for AFP.

**Discussion**
The retrodifferentiation-neometaplasia theory has been postulated in order to explain the exceedingly rare association between a somatic carcinoma and a YST component.
The Müllerian neoplasia is rich of cancer-derived induced pluripotent stem cells (iPSC) able to form tumors with components resembling pediatric GCTs. In this way the YST component could be considered a somatically derived neoplasia. This theory is supported by some reports describing the origin of these types of tumors from endometrioid carcinoma or endometriotic deposits.
Scientific literature reports biphasic ovarian tumors composed of a mixture of serous epithelial carcinoma and YST. Our case showed a pure somatic morphology in the ovary and in the ileum while all the other recurrences consisted exclusively of a pure YST component.
At present, all the reported cases of mixed neoplasms regard women which received the first diagnosis following a cytoreductive surgery. Our case represents the first evidence in the literature of YST developing after adjuvant chemotherapy for HGSC, in this way providing another possible explanation of this unusual association: the neo-metaplasia possibly induced by chemotherapy.

**Conclusions**
Somatically derived YSTs are rare in post-menopausal women and are described as peculiar entities with aggressive behavior. Pathologists and clinicians should always consider this diagnosis in patient with a pelvic-abdominal mass and raised AFP. Adequate pathological sampling is necessary to identify the tumoral morphological and immunophenotypical heterogeneity.
Our findings have hypothesized the role of chemotherapy as 'stressor event' in the phenomenon of retrodifferentiation/neo-metaplasia. Further studies should be performed on larger series of cases.

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# Giovedì, 18 ottobre 2018

Glass House – 16:00 - 19:00

## PATOLOGIA ENDOCRINA

## La patologia endocrina e l'immunoistochimica: un approccio integrato per l'innovazione

Moderatori: C. Doglioni, G. Pelosi

### MINENS VS MANECS VS COMBINED: THE ROLE OF IMMUNOHISTOCHEMISTRY

S. La Rosa[1], F. Sessa[2], S.Uccella[2]

*[1] Institute of Pathology, Lausanne University Hospital, Lausanne, Switzerland; [2] Department Surgery and Medicine, University of Insubria, Varese, Italy*

Neoplasms composed of both a neuroendocrine and non-neuroendocrine component represent a diagnostic and therapeutic challenge. The application of immunohistochemistry in diagnostic routine has showed that these tumors are not as rare as traditionally believed. They can be found in every organ of the body, although they are more frequent in the gastroenteropancreatic (GEP) system and in the lung. The combination of neuroendocrine and non-neuroendocrine cells shows a wide range of possibilities ranging from non-neuroendocrine carcinomas with interspersed neuroendocrine cells to neuroendocrine neoplasms with focal exocrine differentiation; however, true mixed neuroendocrine/non-neuroendocrine neoplasms are defined by the presence of two discrete, morphologically recognizable, components, each component representing at least 30% of the lesion. However, this cut-off has been proposed for GEP mixed neoplasms and it is not applied in other organs, including the lung. Indeed, the terminology

used to define mixed neoplasms has been a matter of debate for years and different terms have been used, creating some confusion among both clinicians and pathologists. The simple term "mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN)" has been recently proposed and introduced in the WHO classification of GEP neoplasms. It represents an umbrella category, which includes clinico-pathologic entities with different morphological, clinical, and prognostic features, ranging from indolent tumors composed of adenoma and neuroendocrine tumor (MANET) to aggressive mixed adenocarcinoma and neuroendocrine carcinoma. The old term "mixed adenoneuroendocrine carcinoma (MANEC)" proposed in the 2010 WHO classification can be used to define this latter category. In the lung, mixed neoplasms are defined "combined"; they include combined small cell carcinoma and combined large cell neuroendocrine carcinoma as subtypes of the relative pure forms (SmCC and LCNEC, respectively) and the WHO classification defines them as an admixture of neuroendocrine and non-neuroendocrine components. In addition, cases of SmCC admixed with a component of LCNEC accounting for at least 10% of the tumor mass are also defined as combined.

Immunohistochemistry plays a crucial role in the diagnostic work-up of MiNENs demonstrating the neuroendocrine component and characterizing the non-neuroendocrine one. The neuroendocrine component of MiNENs can be represented by either NET (NET G1, NET G2, and NETG3) or NEC. Synaptophysin and chromogranin A are expressed both in NETs and NEC components, although chromogranin A can be absent or focally expressed with a typical paranuclear dot-like pattern of immunoreactivity in NECs. The immunophenotype (hormone expression) of the NET component is variable and depends on the site of origin. The expression of transcription factors is generally site-related for NETs, but not for NECs. CD117 expression has been found to be associated with worse prognosis, whilst microsatellite instability is a good prognostic marker in high grade MiNENs (MANECs). The assessment of the Ki67 proliferative index is mandatory for the correct classification of the neuroendocrine component and it has recently been demonstrated that the Ki67 proliferative index of the neuroendocrine component >55% has an adverse prognostic role in MANECs, similarly to pure NECs.

The non-neuroendocrine component differs in relation to the site of origin and the morphological and immunohistochemical characteristics are extremely heterogeneous, depending on the type of the non-neuroendocrine neoplasm arising at the site of origin of each MINEN.

## MODIFICAZIONI MORFOLOGICHE ED IMMUNOFENOTIPICHE DEI TUMORI NEUROENDOCRINI PANCREATICI POST-TERAPIA

M. Schiavo Lena

La PRRT (peptide receptor radionuclide therapy) è una strategia terapeutica basata sull´utilizzo di analoghi della somatostatina marcati con radiouclidi (90Ittrio e/o 177Lutezio) che permette si somministrare le dosi del radiopeptide direttamente alle cellule che esprimono i recettori della somatostatina. La PRRT è una opzione terapeutica che si è dimostrata efficace nei tumori neuroendocrini ben differenziati G1-G2 del pancreas per i quali non è indicata la terapia chirurgica in quanto metastatici e/o non resecabili, con un buon profilo di tollerabilità e di qualità di vita dei pazienti.

L´esperienza nell´utilizzo della PRRT come approccio neoadiuvante nei tumori neuroendocrini ben differenziati del pancreas è invece limitata a singoli case reports o piccole serie, con incoraggianti risultati clinici.

Noi abbiamo confrontato retrospettivamente le caratteristiche patologiche di un gruppo di pazienti (n. 21) affetti da tumore neuroendocrino ben differenziato del pancreas potenzialmente operabile da subito ma considerato al alto rischio di recidiva e sottoposto a PRRT con intento neoadiuvante e successivamente a chirurgia, con un gruppo di controllo (n. 21) con analoghe caratteristiche anagrafiche e di stadiazione radiologica sottoposto subito a chirurgia.

Nel gruppo sottoposto a PRRT l'asse maggiore del tumore valutato radiologicamente si riduce mediamente di 11 mm dopo la terapia neoadiuvante (da 56 a 45 mm, P<0.0001).

Al momento dell´esame istologico il grado tumorale, lo stadio T, M, i margini di resezione, l´infiltrazione perineurale e l´invasione vascolare sono risultati parametri simili nei due gruppi. Il numero dei pazienti con linfonodi negativi (N0) è risultato maggiore nel gruppo PRRT (n=13 vs n=6, P=0.030), senza che vi fossero differenze significative nel numero totale di linfonodi isolati ed esaminati nei due gruppi (P=0.333).

Il rapporto stroma/tumore è risultato significativamente maggiore nel gruppo PRRT (40% vs 20%, P<0.0001). La presenza di presuntivi segni di risposta alla terapia quali edema stromale (P=0.005), aghi di colesterina (P=0.004), alterazioni strutturali della parete dei vasi intratumorali (P<0.0001), stravasi emorragici (P=0.002) e di siderofagi (P=0.004) è risultata di più frequente riscontro nei pazienti pre-trattati con PRRT.

La nostra casistica necessita di un periodo di follow-up più lungo per mettere in relazione i dati morfologici osservati con la sopravvivenza dei pazienti e possibilmente proporre un sistema di valutazione di risposta alla terapia neoadiuvante con valore prognostico.

**Domande Test ECM**

1) La più rilevante alterazione istologica riscontrata nei PanNET ben differenziati dopo terapia neoadiuvante con PRRT è:
- La presenza di emorragie.
- La presenza di raccolte di istiociti schiumosi.
- L´aumento del rapporto stroma/tumore.
- Nessuna delle precedenti.

2) Attualmente la maggiore esperienza clinica con la PRRT nei tumori neuroendocrini è:
- Come terapia neoadiuvante
- Come terapia per tumori inoperabili e/o metastatici.
- Come terapia adiuvante
- Nessuna delle precedenti.

3) Il radionuclide adesso maggiormente utilizzato per la PRRT è:
- 90Ittrio.
- 223Radio.
- 213Bismuto.
- 177Lutezio.

4) La tossicità più rilevante della PRRT è:
- Ematologica e renale.
- Stomatiti e altri disturbi infettivi.
- Gastro-enterica (nausea, diarrea, dolore addominale).
- Relativa alla funzione epatica.

# Slide Seminar

## NET POLMONARE: COMBINED SMALL CELL LUNG CANCER

F. Pezzuto, F. Fortarezza, F. Rea, F. Calabrese

*Department of Cardiac-Thoracic-Vascular Sciences and Public Health, University of Padova*

Small cell lung cancer (SCLC) accounts for 15-20% of all lung cancers. Among them, approximately 30% of cases are defined as combined tumours. Combined SCLCs (CSCLC) consist of a small cell carcinoma with an additional component of any non small cell lung cancer (NSCLC): adenocarcinoma, squamous cell carcinoma (SCC) and large cell neuroendocrine carcinoma (LCNEC). CSCLCs do not show significant differences in clinical characteristics and overall survival than pure SCLC but the response to chemotherapy is poorer. The pathogenesis of combined tumours is not completely understood. Nevertheless, several genetic mutations and chromosomal aberrations (e.g. Rb1 and p53) have been reported. A more in-depth knowledge on the biology and the relationships between the components is important for the treatment. While surgery remains a milestone for NSCLC, chemotherapy, surgery, radiotherapy and other strategies may be used for treating CSCLC.
We describe a case of a CSCLC (SCLC and SCC) whose previous biopsy showed only the non small cell component. The patient underwent radiotherapy with a quite complete regression of the squamous neoplasia and the spread of the neuroendocrine component.
The present case offers the opportunity to discuss on pitfalls and key-aspects for the differential diagnosis in neuroendocrine tumours.

## LA PATOLOGIA ENDOCRINA E L'IMMUNOISTOCHIMICA: UN APPROCCIO INTEGRATO PER L'INNOVAZIONE: PATOLOGIA TIROIDEA

C. Ugolini

*Anatomia Patologica III Universitaria- Dipartimento di Medicina di Laboratorio, Ospedale S. Chiara, Azienda Ospedaliero-Universitaria Pisana, Pisa (Italy)*

**Objective**
Thyroid tumors are heterogeneous entities. Some of them show an indolent outcome and can be assimilated to "borderline" or "in situ" lesions. Others show a worse prognosis and lack the response to the conventional radio-iodine therapy. Immunohistochemistry, together with molecular tests, may be a valid help in different aspect of thyroid pathology: diagnosis, prognosis and management.
The diagnosis of thyroid follicular lesions can be extremely complex and subject to interobserver variability. In particular, the distinction between a benign from a malignant lesion. For this reason, recently, a group of endocrine pathologists reclassified encapsulated and well-delineated follicular lesions in NIFT-P (Non-invasive follicular thyroid neoplasm with papillary-like nuclei) based on nuclear morphological alterations. However, up to now, there is no a consensus based on morphological alterations. In the initial study, by Nikiforov et al., the presence of papillae was an exclusion criterion and the percentage of less than 1% was proposed. Very soon became evident that only one papilla could be sufficient to make the lesion a classical variant papillary carcinoma with predominant follicular architecture. In this landscape is necessary to identify an easy to use marker able to screen papillary from follicular neoplasm.
Although major thyroid cancer shows a good prognosis and a high probability of healing, a minor percentage (15– 30%) of patients will have recurrent disease, 5–10% will have distant metastasis, for whom 5-year survival is only about 50%. Genetic mutations may influence thyroid cancer progression and response to therapy, along with clinical features that portend a worse prognosis such as age, tumor invasiveness, and metastasis. The most note and the most important genetic alteration in thyroid cancer is V600E BRAF mutation. It is present in approximately 45% of papillary thyroid cancer and in part of anaplastic cancer and it has been associated with most of clinico-pathological features related to prognosis. The reasons of this observation are unknown. One of the mechanisms, proposed, by which BRAFV600E tumors may be more aggressive is the alteration of endogenous host immune surveillance and the promotion of tumor immune escape. Immune checkpoint inhibitor therapies targeting PD-L1/PD-1 have been shown to be effective in treating several types of human cancer (i.e. lung cancer or melanoma). In thyroid carcinoma little is known about the expression of PD-L1/PD-1 in the tumor microenvironment and the relationship between BRAFV600E status and known strategies of tumor-mediated immune suppression. The identification of a marker could be a valid help in therapy-resistant tumors.
The aim of this research is to identify some particular immunohistochemical biomarkers useful in distinction between papillary and follicular neoplasm and other markers useful in the management of radio-resistant thyroid cancer. For brevity reasons not all markers can be reviewed in this presentation and the most cited (BRAF, PD-1, PD-L1) were considered.

**Materials and methods**
PubMed literature search for "immunohistochemical biomarkers" in thyroid cancer and in thyroid lesions since 2008.

**Results**
The continuous changing in the morphological criteria of classification of NIFT-P category are developing a new entity called papillary carcinomas with predominant follicular architecture.
Classical PTC has a higher risk (i.e. local node metastasis) than a follicular neoplasm. For this reason, they must be recognized and diagnosed. On this basis additional studies, including the use of the VE1 antibody against the mutant BRAFV600E are recommended. Now a day, immunohistochemistry, using VE1 antibody is the most important ancillary test in the distinction of classical variant PTCs with predominant follicular growth as well as any BRAFV600E-mutant thyroid carcinoma. It is not clear the role of RAS mutation specific antibodies, while RET antibody is not usable.
Mutation of the BRAF gene, corresponds to the constitutively active BRAFV600E protein. It has been associated with worse clinical outcomes in thyroid cancer. The reasons of this observation are unknown. One of the mechanisms, already under study, by which BRAFV600E tumors may be more aggressive is the disruption of endogenous host immune surveillance and the promotion of tumor immune escape. BRAFV600E tumors show an immunosuppressive profile and an altered host tumor immune surveillance that may contribute to the poorer outcomes. Authors found BRAFV600E tumors more

often express high levels of PD-L1 1 (53% vs. 12.5%) and human leukocyte antigen G (41% vs. 12.5%) compared to BRAF wild-type tumors. Moreover, there was a significantly lower CD8+/FoxP3+ cell ratio in BRAFV600E compared to BRAF wild-type tumors, indicating relative increases in suppressive T cell. Recently, the expression of PD-L1, PD-1, and BRAF V600E have been studied by immunohistochemistry. Significant correlations were found between expression of BRAF V600E and expression of PD-L1 and PD-1 suggesting that immunotherapies targeting PD-L1/PD-1 might be effective for PTC patients with the BRAF V600E mutation, in particular in refractory to radioiodine therapy patients.

**Conclusions**

In the era of the next-generation sequencing, immunohistochemistry in thyroid pathology continues to have an important role. The importance of immunohistochemical markers in thyroid diagnosis is note. However, some biomarkers can be useful to give information about classification, prognosis, prediction, predisposition and target therapy.

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## CASE 4-WELL DIFFERENTIATED NEUROENDOCRINE TUMOR (NET G3) OF THE RECTUM


S. Uccella

*Dept. of Medicine and Surgery, Unit of Pathology, University of Insubria, Varese, Italy*


A 78-year-old man with no significant clinical history underwent screening colonscopy for occult fecal blood and a 1.8 cm sessile polyp was found in the rectal ampulla. The endoscopist tried an en-bloc resection and the polyp was sent for pathological examination.

The histological slides showed a subepithelial proliferation of medium-sized, elongated cells, arranged in intersecting trabeculae and, focally, in rosettes, with clear eosinophilic, granular cytoplasms and round nuclei with well dispersed, salt-and-pepper, chromatin. The neoplastic proliferation was present on the resection margin. No images of angioinvasion or neuroinvasion were found. The suspected diagnosis was a well differentiated neuroendocrine tumor of the rectum, so an immunohistochemical panel including general neuroendocrine markers, glucagon, glicentin, PYY, prostatic acid phosphatase, serotonin, substance P and Ki67 was employed to better define the lesion.

Tumor cells were strongly and diffusely positive for synaptophysin and chromogranin A. The immunohistochemical expression prostatic acid phosphatase was present and, focally, of glucagon-like peptides, pointing to an L-cell rectal NET, albeit also serotonin and substance P were focally expressed, indicating the presence of an EC-cell component. The Ki67-related proliferation index was 35%, and 9 mitoses per 10 HPF (x400) were counted.

According to the 2010 WHO classification of gastrointestinal neuroendocrine neoplasms (NENs), a proliferation index above 20% shifts the diagnosis towards a poorly differentiated neuroendocrine carcinoma. However, lessons from pancreatic NENs have changed this approach, as it has been recognized that morphologically well-differentiated NETs may present a Ki67 proliferation index higher than 20% without behaving as aggressively as NECs. Importantly, in pancreatic NENs, it has been demonstrated that these highly proliferating well-differentiated NETs did not respond well to platinum-based chemotherapeutic regimens adopted for NECs, whereas m-TOR inhibitors and somatostatin analogues were efficient in the control of metastatic disease. Further studies demonstrated that NECs and highly proliferating NETs has two different molecular profiles. For all these reasons, the term NET G3 was introduced to designate this new category of NENs, ad it was included in the 2017 WHO classification of pancreatic NENs. In the last few years, a number of highly proliferating well-differentiated NETs have been described in the stomach, in the small intestine and, outside the gastroenteropancreatic system, in the lung. The forthcoming WHO classification of the digestive NENs will thus include gastrointestinal NET G3. This is the first reported case of NET G3 of the rectum, and represent a further confirmation that both morphological differentiation and proliferative index are important in stratifying the patients' risk and in establishing a correct therapy for advanced disease.

# Venerdì 19 ottobre 2018

Sala Nico 1 – 08:00 - 10:30

## PATOLOGIA PLEUROPOLMONARE

## Update in Patologia Toraco-Polmonare

Moderatori: G. Rossi, O. Nappi

## Slide Seminar

### THE DISGUISE UNDER UNUSUAL CONDITIONS: PRIMARY LUNG LYMPHOID DISORDERS

S. Campione

*AORN A Cardarelli Napoli*

In this slide seminar two lung lymphoid disorders are presented: nodular lymphoid hyperplasia, which is a reactive condition and BALT lymphoma, a malignant disease.
Both conditions were unexpected by clinicians and came to surgery for other suspicion.
Pathological examination revealed their nature.
Histopathological features of both conditions are here presented and discussed. The differential diagnosis between the two conditions may be extremely difficult, as a pulmonary pathologist may not be hardened enough in the recognition of these rare conditions.
Molecular biology analysis can be helpful in determining either the clonal or reactive nature of the lesion.

# Venerdì 19 ottobre 2018

Sala Nico 1 – 11:30 - 12:30

## EMATOPATOLOGIA

Moderatori: M. Paulli, E. Sabattini

### LINFOMA DI HODGKIN: AGGIORNAMENTI CLINICO-TERAPEUTICI

M. Gotti

*S.C. Ematologia, Fondazione IRCCS Policlinico San Matteo, Pavia*

Il Linfoma di Hodgkin (LH) è una patologia linfoproliferativa della linea B linfocitaria relativamente rara con una prevalenza di 3 casi ogni 100.000 abitanti/anno nei paesi industrializzati. Secondo la classificazione WHO (World Health Organization) esistono due principali entità di LH: il LH classico, a sua volta suddiviso in sclerosi nodulare, cellularità mista, deplezione linfocitaria e ricco in linfociti, e il LH a predominanza linfocitaria nodulare (NLPHL).
La stadiazione iniziale riveste un ruolo fondamentale non solo nello stabilire la diffusione di malattia, ma anche nell'individuare eventuali fattori di rischio che ci guidino nella scelta di una corretta strategia terapeutica. A tal proposito l'impiego sempre più diffuso della PET (Positron Emission Tomography) ha portato a definire nuovi criteri di stadiazione, di risposta alla terapia e alla stesura di protocolli terapeutici che tengono conto del risultato dell'interim PET.
Il trattamento standard per i pazienti in stadio iniziale a prognosi favorevole o sfavorevole prevede l'utilizzo di uno schema combinato di chemioterapia e radioterapia, mentre per i pazienti con malattia in stadio avanzato è prevista la sola chemioterapia per un numero di cicli più elevato; per questi ultimi l'uso della radioterapia è ancora oggi oggetto di discussione ed eventualmente confinato al solo bulky. L'utilizzo della interim PET, con l'intensificazione precoce del programma terapeutico in caso di positività, ha portato ad un miglioramento del tasso di risposta finale e della sopravvivenza libera di malattia nei pazienti con malattia avanzata; ad oggi i programmi terapeutici non possono trascurare questo parametro.
Infine, i pazienti con malattia ricaduta o refrattaria sono candidati ad un programma di chemioterapia di salvataggio seguita da trapianto autologo di cellule staminali (ASCT). Nuove molecole hanno permesso di migliorare sensibilmente la prognosi di questi pazienti e il loro uso potrebbe a breve entrare a far parte di strategie terapeutiche più precoci.

# Venerdì 19 ottobre 2018

Sala Nico 1 – 12:30 - 17:00

## PATOLOGIA ULTRASTRUTTURALE E NEFROPATOLOGIA

## Sessione II

Moderatori: G. Mazzucco, S. Pizzolitto

### ULTRASTRUCTURAL DIFFERENTIAL DIAGNOSIS OF STORAGE DISEASES

V. Papa, R. Costa, O. Leone[1], G. Cenacchi

*Biomedical and Neuromotor Sciences Department, University of Bologna, Bologna, Italy; [1] U.O. Pathology, S. Orsola-Malpighi Hospital, Bologna, Italy*

Objectives
To describe morphological signs helpful for a diagnostic confirmation or prompting a genetic demonstration of storage diseases.

**Materials**
Ultrastructural studies were carried out on biopsies performed in all consenting patients, clinically suspected of storage diseases between 2001-2018; lysosomal and non-lysosomal deposits were described: 1 GM1 Gangliosidosis (skin), 1 Gaucher disease (bone marrow), 1 Niemann-Pick disease (liver), 25 neuronal ceroidolipofuscinoses, NCL (19 skin, 1 brain, 2 skeletal muscle, 3 peripheral blood), 2 Mucopolysaccharidosis (1 skeletal muscle, 1 liver), 24 CADASIL (skin), 4 Danon disease (3 cardiac and 1 skeletal muscle), 12 Glycogenosis (11 skeletal muscles and 1 liver), 2 Lafora disease (axillary skin), 29 Fabry (20 kidney, 2 urinary bladder, 5 skin, 2 cardiac muscle).

**Methods**
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 CM100 Transmission Electron Microscope, TEM.

**Results**
Type I Gangliosidosis showed large, round lysosomes, with fluffy electron-lucent inclusions and granular content mainly visible in smooth muscle and endothelial cells. Gaucher disease featured histiocytes containing many lysosome filled up of tubular material.
Niemann-Pick was revealed by TEM study of hepatocytes with foamy appearance due to the presence of enlarged lysosomes containing dense irregular and lamellar inclusions, with clear and electron-lucent zones. NCL displayed different ultrastructural patterns as granular osmiophilic deposits, curvilinear and fingerprint profiles. Type I Mucopolisaccaridosis lysosomes showed a rather electron-dense, foamy material indicative of storage. Granular Osmiophilic Material, GOM, are considered the ultrastructural marker of CADASIL in which they are described as contained between plasmalemma and basal lamina, varying in size and shape. Danon disease, was characterized by ultrastrutural presence of large autophagic vacuoles lined with a basal lamina on the inner surface of the sarcolemma and containing multilamellar bodies and electrondense inclusions. Glycogenosis biopsies showed lysosomes storing glycogen particles (type II) or free floating ones especially in subsarcolemmal areas (type V). Lafora disease was ultrastructurally diagnosed by pale oval bodies, with a fibrillary content, without membrane, in sweat gland epithelial cells of axillary skin. Fabry disease was characterized by ultrastructural presence of lysosomal dense lamellar and parallel structures or concentric myelinoid inclusions with lamellar configuration.

**Discussion**
Storage diseases are largely multi-organ ones; the diagnostic marker is to describe morphology of the stored substrate in the cell cytoplasm or within lysosomes, owing to their biochemical failure to verify the respective enzyme deficiency responsible for the substrate accumulation.

**Conclusions**
Biopsies of tissues may serve for a diagnostic confirmation or prompting a genetic demonstration of storage diseases.

## THE ROLE OF MITOCHONDRIA IN HIGH-GRADE OVARY CARCINOMA

R. Rossi[1], A.Signorile[2], F.Fortarezza[1], L. Resta[1], G. Scillitani[3], G. Cormio[4]

*[1] Department of Emergency and Organ Transplantation; [2] Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari, Italy; [3] Department of Biology, University of Bari, Italy; [4] Gynecologic Oncology Unit, National Cancer Institute "Giovanni Paolo II", Bari, Italy.*

Ovarian cancer, one of the most lethal disease in the western coustries, is a very heterogeneous disease characterized by different histological subtypes and different mutations: PIK3CA, PTEN, ARID1A, KRAS, BRAF, RB1, FOXM1, NOTCH 3 pathway. Altered cellular metabolism is considered a hallmarks of cancer and recently mitochondria have been view as an important compartment that fuel metabolic demands of cancer cells.

Deregulation of mitochondrial function, morphology, dynamics, and apoptosis are present during the different phases of carcinogenesis. More mechanisms and proteins are revealed to be involved in the modulation of these processes in different cancer cells, such as cAMP/PKA signalling, SIRT3, OPA1, DRP1, and prohibitins (PHB). Mitochondria are dynamic organelles that undergo fission processes and fusion. These dynamics influence cell migration, differentiation and apoptosis in tumour cells. Fission is associated with the induction of apoptosis in cancer cells. Mitochondrial mitofusins (Mfn), OPA1 and DRP1 are important proteins that govern mitochondrial fusion and fission, respectively. OPA1 is a protein that intervenes in the fusion process, it exists in long (L-OPA1) and short (S-OPA1) forms that are generated by its processing, in response to a pro-apoptotic stimulus. In our studies we explored the number and morphology of mitochondria in ovarian cancers and in control specimens (tubal epithelium), in relation to the expression of AMP signal patway, SIRT3, OPA1 and prohibitin proteins in ovarian cancer tissues.
We consider samples of Ovarian Cancers (OC) in confront of control cases (CT) represented by tubal fimbria in women with absence of neoplastic disease. The samples were collected during surgery and frozen and stored at a temperature of -80°C. These fragments were texted for cAMP assey and mDNA quantification. Samples for electron microscopy were prontly fixed in glutaraldehyde (2.5%) and treated with usual ultrastructure procedures.
Analysis showed an increased cAMP level in OC with respect to CT associated with a decreased complex I activity and an increase of complexes II+III, IV, V and citrate synthase. western blotting showed an increase of SIRT3, OPA1 e PHB2 protein levels.
The electron microscopy showed an augmented mitochondrial number in OC with respect to CT and a significant longer main axis in OC. These morphometrical data are related to a different conformation of mitochondrial in ovarian cancer. The organelle present a relevant major axis and a form often curve. In many case we observed a stricture along the mitochondrial profile very suggestive for a process of fission or fusion. Such pattern was absent in CT.
The present data were correlated with the biochemical data.
In conclusion, our findings show that OC is characterized by deregulation of cAMP level, increased SIRT3, OPA1, PHB2 and mitochondrial mass suggesting an apoptosis resistance acquisition.

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## CRYOFIBRINOGEN-ASSOCIATED GLOMERULONEPHRITIS

A. Barreca

*Anatomia e Istologia Patologica 1U, AOU Città della Salute e della Scienza, Ospedale Molinette, Torino*

Cryofibrinogen is a cryoprotein that was first reported by Korst and Kratochvil in 1955 [1]. It precipitates only when plasma, and not serum, is stored at 4°C for 3 to 8 days. Cryofibrinogen is composed of fibrinogen, fibrin and fibrin-degradation products with or without albumin and/or immunoglobulins [2]. On the contrary cryoglobulins are proteins (immunoglobulins and complement factors) that form a precipitate when both serum and plasma are chilled at 4°C. Cryofibrinogen appears as a precipitate that re-dissolves when warmed to 37°C.

Cryofibrinogenemia is a rare disorder with a prevalence of 2-9% in healthy subjects [3 4 5]. It may be primary (essential) or secondary to other disorders such as infections (bacterial or viral), solid-organ and hematologic neoplasms, autoimmune diseases (Tab. I) [6].

The exact pathogenesis of essential cryofibrinogenemia is not clear.

The mean age at diagnosis is between 50 and 60 years, with a slight predominance in women.

Clinical manifestations are various and diverge from asymptomatic patients to thromboembolic events. Skin manifestations are present in up 80% of cases: purpura, cold sensitivity, livedo reticularis, Raynaud phenomenon, ulcers, gangrene and rarely urticaria. As in cryoglobulinemia, systemic clinical signs can verify, such as myalgia, arthralgia or arthritis.

The kidney represents also a target organ: renal involvement is reported in 4% to 21% of cases, with variable levels of proteinuria and hematuria. Terrier et al [7] described the presence of cryofibrinogen in up to 11% of patients adimitted for management of kidney disorders. Nevertheless reports of kidney pathology related to cryofibrinogenemia are very few and insufficiently detailed.

Table I. Type of disease associated with secondary cryofibrinogenaemia.

| Disease associated with cryofibrinogenaemia | No. |
|---|---|
| *Klebsiella pneumoniae* infection | 3 |
| *Mycoplasma pneumoniae* infection | 2 |
| Herpes zoster virus infection | 1 |
| Epstein–Barr virus infection | 2 |
| Hepatitis C virus infection | 2 |
| Severe sepsis | 1 |
| Erysipela | 3 |
| Thrombosis | 7 |
| Mixed connective tissue disease | 3 |
| Sjögren's syndrome | 2 |
| Dermatomyositis | 1 |
| Follicular lymphoma | 2 |
| Large B-cell lymphoma | 1 |
| T-cell lymphoma | 4 |
| B-cell lymphoma | 3 |
| Chronic myelomonocytic leukaemia | 2 |
| Multiple myeloma | 2 |
| Gastric adenocarcinoma | 1 |
| Hepatocarcinoma | 1 |
| Lung adenocarcinoma | 1 |

Tab. II. Differential diagnosis of cryofibrinogen-associated glomerulonephritis [8].

| | Cryofibrinogen-Associated GN | Cryoglobulinemic GN | Immunotactoid Glomerulopathy | Fibronectin Glomerulopathy |
|---|---|---|---|---|
| Light microscopy Immunofluorescence microscopy | MPGN* Negative for immunoglobulin and complement; segmental fibrinogen | MPGN* Polytypic or monotypic immunoglobulin with complement | MPGN* Often monotypic immunoglobulin with complement | MPGN* Negative for immunoglobulins and complement |
| Electron microscopy | Large microtubular structures, diameters measuring up to 210 nm, with linear fibrils in a matrix | Amorphous or small curved microtubules | Microtubules in stacked piles with intersections, diameters measuring up to 90 nm | Granular deposits with focal fibril formation, no core, 12-16 nm |
| Proteins detected by additional studies | Fibrinogen (proteomics) | Immunoglobulin (proteomics) | Immunoglobulin (proteomics) | Fibronectin (immunofluorescence or immunohistochemistry) |

Abbreviations: GN, glomerulonephritis; MPGN, membranoproliferative glomerulonephritis.
*Most common MPGN pattern of injury.

Recently Sethi et al [8] described two patients with cryofibrinogen-associated glomerulonephritis. Both cases demonstrated a membranoproliferative pattern of glomerular injury with lobular accentuation of tufts and double contours along the capillary walls on light microscopy. Rare fuchsinophilic deposits were reported in a few capillary loops. There is also mild nodular mesangial sclerosis. Immunofluorescence in both cases revealed absent to trace staining for immunoglobulins, including light chains. There was only weak segmental positivity for C3 along capillary walls in one case and weak-moderate segmental staining for fibrinogen in both patients. Immunofluorescence studies with pronase digestion didn't show masked immunoglobulin deposits.

Electron microscopy was crucial for reaching the diagnosis: there were glomerular deposits localized within capillary lumina and in subendothelial sites. These deposits were composed of haphazardly arranged large fibrils with tubular structures whose luminal diameters ranged from 21-211 (mean diameter 158 nm). The distinctive ultrastructural feature for cryofibrinogen was the presence of a prominent central bore with focal double or triple lamellations. The ultrastructural findings of the cryoprecipitate, in one case, were identical to glomerular deposits.

Mass spectrometry demonstrated that the cryoprotein was made up mainly by fibrinogen.

The differential diagnosis involves cryoglobulinemic glomerulonephritis, immunotactoid glomerulopathy and fibronectin glomerulopathy (Tab. II).

The accuracy of cryofibrinogenemia diagnosis is critically dependent upon the method of collection of cryofibrinogen assay: collected blood should be kept at 37°C until plasma centrifugation. It is crucial to respect the stringent conditions of testing to avoid false-negative results.

Cryofibrinogenemia is underdiagnosed as a cause of kidney disease, but it is important to recognize this cryopathology to establish the correct pharmaceutical management and regular follow-ups because of high risk of relapses and secondary occurrence of lymphoma.

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## UN CASO DI GLOMERULONEFRITE CRIOGLOBULINEMICA VS IMMUNOTATTOIDE

F. Fortarezza[1], D. Piscitelli[1], M.G. Fiore[1], R. Rossi[1], I. Carosi[3], M. Rossini[2], L. Gesualdo[2], L. Resta[1]

*[1] Department of Emergency and Organ Transplant, Pathology division, University of Bari; [2] Department of Emergency and Organ Transplant, Nephrology division, University of Bari; [3] Department of Pathology, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo*

Most renal diseases with organized deposits are relatively uncommon conditions. A right diagnosis requires a multidisciplinary approach in which clinical history and pertinent laboratory data play a fundamental role. The renal biopsy is mandatory for a precise nosological classification. Light microscopy and immunofluorescence lead the diagnostic investigation but the ultrastructural examination could be crucial in resolving diagnostic dilemmas. In this relation a case of a 58 old-year male with a history of chronic renal failure and nephrotic-range proteinuria will be presented. The patient underwent three kidney biopsies within three years, each showing a glomerulopathy with organized deposits, morphologically suggestive for cryoglobulinemic nephropathy. No cryoglobulins were found in serum. Also the research of the autoantibodies and the markers of viral hepatitis were negative. The clinical history of the patient was unremarkable for neoplasms, immune disorders or infective pathologies. Considering these clinical data immunotactoid glomerulonephritis was hypothesized. This case represents a diagnostic challenge in definitions of glomerulopathies and highlights the possible overlap that concern these rare disorders.

## A CASE OF LENVATINIB-INDUCED RENAL FAILURE AND REVIEW OF THE LITERATURE

M. Nebuloni[1], A. Tosoni[1], E. Lauri[1], N. Landriani[2], C. Airaghi[2], S. Caruso[2], D. Scorza[2], A. Genderini[2], P. Zerbi[1]

*[1] Pathology Unit, Department of Clinical Sciences; [2] UOC Nefrologia e Dialisi, L. Sacco Hospital, Università degli Studi di Milano, Via Giovanni Battista Grassi 74, Milan, Italy.*

Lenvatinib is an orally bioavailable multi-tyrosine kinase inhibitor (TKI) of VEGFR, FGFR, PDGFR-α, KIT and RET, and is the most effective drug for advanced progressive Iodine-131 refractory (RAI) differentiated thyroid cancer patients. Proteinuria and renal failure (RF) were reported among the most frequent LEN-induced adverse events (AEs), often leading to discontinuations or dose modifications. We described in this paper a case of LEN-induced renal failure with severe proteinuria in a man treated for a metastatic papillary thyroid carcinoma. Kidney biopsy showed a glomerular damage secondary to Lenvatinib therapy. Light microscopy examination revealed a huge number of glomeruli with some degree of mesangial hypercellularity and increase of mesangial matrix. In just one glomerulus, segmental features of mesangiolysis were observed. Capillary basal membranes were thickened for the presence of double contours. Moreover, arteriolar narrowing due to intimal edema, endothelial swelling and focal sub endothelial necrosis was found. No inflammatory reaction was present in the vessels walls. The interstitial showed fibrosis and tubular atrophy. The tubulointerstitial nephropathy was also supposed by clinical evaluation and laboratory tests. Immunofluorescence analysis revealed weak focal and segmental staining for immunoglobulins and complement along the capillary basal membrane.
Ultrastructural examination showed focal sclerosis, focal podocyte foot process effacement and rare electron dense deposits with subendothelial and intramembranous localization. The diagnosis was of tubulointerstitial and vascular necrotic damage, associated with endothelial damage described as thrombotic microangiopathy-like pattern. Moreover, ultrastructural data suggest also the presence of podocyte injury, with foot process effacement.
Effective management was obtained by oral steroids without interrupting LEN.
All these aforementioned features are indicative of drug-derived damage; anti Vegf therapies can induce thrombotic microangiopathy and podocytopaties. In our patient we found a combination of both lesions, associated with an diffuse tubulointerstitial involvement.

## ATOMIC FORCE MICROSCOPY (AFM): AN ADDITIONAL VALUE TO ULTRASTRUCTURAL DIAGNOSTICS

V.G. Vellone[1 2], G. Gaggero[3], L. Peñuela[1 4], R. Raiteri[5]

*[1] Anatomia Patologica, DISC, Università di Genova; [2] U.O. Anatomia Patologica Univesitaria, IRCCS Ospedale Policlinico San Martino, Genova; [3] U.O. Anatomia Patologica, IRCCS Ospedale Policlinico San Martino, Genova; [4] U.O.S.D. Patologia Feto-Placentare, IRCCS Istituto G. Gaslini, Genova; [5] Ingegneria Biomedica, DiBRIS, Università di Genova*

Ultrastructural studies with transmission electronic microscope (TEM) saw their hey-days in the ’70 providing a relevant contribution not limited to pathophysiology but also in daily diagnostics. Often wrongly considered expensive and time-consuming, requiring a differentiated fixation, embedding and cut at the ultramicrotome, the usage of TEM is declining in pathology labs. TEM often remain pivotal to achieve a final correct diagnosis in kidney pathology. With this very preliminary report we would share the experience of our multidisciplinary study groups composed by physicians, biologists and bio-medical engineers proposing a new intriguing tissue imaging technique. With Atomic Force Microscopy we are able to obtain an ultrastuctural imaging of renal tissue using routinely formalin fixed, paraffin embedded kidney biopsies cutting 3-µm slides with a common rotative microtome
The atomic force microscopy (AFM) belongs to a series of scanning probe microscopes (SPM) invented in the 1980s. The functioning principle consists on measuring the interaction forces into a very sharp tip mounted to a cantilever spring in close proximity to the sample by monitoring the deflection of the cantilever. It is possible to obtain a topographic image of the sample by plotting the deflection of the cantilever versus its position on the sample during scanning.
AFM– based imaging evidences amazingly each single component of the renal corpuscle. Small elements as red cells inside of glomerular capillaries can be clearly identified, as well

as mesangium, juxtaglomerular apparatus, and podocytes. Furthermore, this technique allows to observe relationships between different components and plot topography profiles from particular selected zones, especially for measuring distances as thickness, diameters, or spaces.
Details about structure and morphology of small components can be evidenced in AFM imaging by zoom-in scanning. This procedure is possible by selecting the zoom-area for scanning from a previous bigger image, then the zoom-in inversely increases in respect of the scanning- area size. Variables as temperature, humidity, tip shape, scan-speed, etc., become determinants for the quality of the zoom-in image. Small structures' details of the kidney tissue were magnified by applying zoom-in scanning. Intra-glomerular structures as pedicels of podocytes, mesangial cell processes, red cells inside of capillaries; and extra-glomerular structures as Bowman's space, parietal layer of the Bowman's capsule, mesangium, and juxtaglomerular surface roughness, can be remarkably identified. It was also possible to perform real tridimensional (3D) views of the scanned AFM-images from Z-axe data.
The wide resolution range of AFM permits to evaluate samples from the micro-scale to the nano-scale. This characteristic is ideal for studying biological samples and identifying ultrastructural relationships existing between different components of tissues and cells, especially by 2D and 3D structural analyses.
The role of AFM in diagnostics will be elucidates in further studies but the proposed technique appear promising and may represent a new chapter in kidney pathology.

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# Venerdì 19 ottobre 2018

Sala Nico 2 – 08:00 - 10:00

## PATOLOGIA SPERIMENTALE

## Metabolismo ed Immunoregolazione nell'era dei Checkpoint Inhibitors

Moderatori: M. Ponzoni, C. Tripodo

### IMMUNOMETABOLIC CHECKPOINTS OF REGULATORY T CELL DYNAMICS IN CANCER


S. Piconese

*Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma, Rome, Italy silvia.piconese@uniroma1.it*


Though representing a minor proportion of CD4 T lymphocytes in tissues in normal conditions, Tregs undergo dynamic processes of contraction or expansion in response to external signals, thus respectively unleashing or restraining immune effector cells, and critically impacting on protective as well as on immunopathological reactions.
Treg expansion is a key event in tumor immune escape, being Treg infiltration associated with a worse prognosis in most cancers. Much effort is being devoted to the identification of consensus signature of tumor-associated Tregs, in order to identify selective targets for their inactivation. Moreover, in the tumor microenvironment, Tregs are exposed to a variety of signals that may either stabilize or undermine their suppressive activity.
OX40 is a member of the tumor necrosis factor receptor superfamily, whose expression marks a human Treg subpopulation endowed with phenotypical, functional and epigenetic features of suppressive function, proliferation and stability, which is particularly expanded in human cancers. We have recently revealed that Tregs' advantage in the tumor milieu relies on supplemental energetic routes involving lipid metabolism, and that OX40-expressing tumor-infiltrating Tregs displayed a gene signature oriented toward glycolysis and lipid synthesis. Therefore, immunometabolic routes may contribute to shape Treg-mediated immune regulation in cancer, thus representing suitable targets to rescue immune surveillance.

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### METABOLISM AND IMMUNOSUPPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA


S. Deaglio

*University of Turin and Italian Institute for Genomic Medicine, Turin, Italy*


Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world and is characterized by accumulation of mature B cells in the peripheral blood and in the lymphoid organs. Disease outcome is influenced by both a complex pattern of genetic lesions and by a network of stimuli coming from non tumoral neighboring cells in the microenvironment. Tumor-host interactions are particularly

important in CLL as leukemic cells strongly depend on external factors for proliferation and survival. Indeed, whilst the fraction of CD5+ leukemic cells circulating in the PB are arrested in the G0 phase of the cell cycle, those in the BM or residing in the lymphoid tissues actively proliferate at a rate of 0,1-1% of the total leukemic clone per day. This is the result of tumor-favorable local conditions that CLL cells themselves contribute to create, partly through the rieducation of immune response effectors towards tolerance.
Over the past years, increasing number of studies highlighted a role for hypoxia-mediated signals in re-shaping tumor microenvironment towards immune suppression and tumor support. Accordingly, CLL niche has been found to be a highly hypoxic environment. We observed that hypoxia orchestrates local immune tolerance by suppressing T-cell functions and by altering the correct differentiation and homeostasis of T cell populations and macrophages. These effects are, at least in part, mediated by the adenosinergic system. Functionally, under low O2 tension, CLL cells undergo a metabolic adaptation that increases the levels of extracellular ATP and ultimately enhances adenosine production and signaling through adenosine receptors, expressed by leukemic B cells, T lymphocytes and macrophages. Therefore, autocrine and paracrine signalings cooperate to confer pro-survival stimuli to CLL cells and to create a tumor-supportive environment. In line with this, targeting the adenosinergic axis, by acting either on adenosine production or signaling, reverts the effects on cell differentiation and opens the way to specific inhibitors as a new therapeutic strategy in CLL.

# Venerdì 19 ottobre 2018

Sala Nico 2 – 10:30 - 13:45

## UROPATOLOGIA

## Biomarker Tissue data in Kidney, Bladder and Prostate Tumors

Moderatori: M. Colecchia, G. Martignoni

## Slide Seminar

### CASE OF UNUSUAL PRESENTATION OF RENAL NEOPLASIA WITH DOUBLE MORPHOLOGICAL AND IMMUNOPHENOTYPIC CHARACTERIZATION.

S. Massa[1], B. Paolini[1], M. Trombatore[2], M. Ungari[2], M. Colecchia[1]

*[1] Fondazione IRCCS Istituto Nazionale Tumore di Milano; [2] ASST Ospedale di Cremona*

A mesorenal-lower pole 5 cm mass occurred in the right kidney of a 47-years old female and a right nephrectomy has been performed in the hospital of Cremona.
The histological slides have been shared with the department of surgical pathology at the Istituto Tumori di Milano.
Histologically a clear cell renal cell carcinoma, Isup grade II, with immunohistochemical expression of Pax 8, CA IX, RCC, CD 10, claudin, Ck pool, EMA was observed together with a mesenchymal proliferation adjacent to renal carcinoma. It showed positivity to s100, inhibin, GLUT1 and vimentin and negativity for cytokeratin AE1-AE3, claudin and focal expression of CD10. Final diagnosis of mixed tumour of clear cell renal cell carcinoma and hemangioblastoma was signed out.
An analysis to test mutation of the gene VHL was performed and it was negative.
Hemangioblastoma is a rare benign tumor of uncertain origin that can occur sporadically or in association with von Hippel-Lindau (VHL) disease, and arises in the central nervous sys¬tem (CNS) in most cases. However, some cases of hemangioblastoma arise in extraneural sites. In literature 13 cases of sporadic non VHL mutated renal hemangioblastoma have been reported.
Microscopically, the tumor is made up of large polygonal to short spindle cells with eosinophilic cytoplasm with occasional vacuolization and abundant arborizing capillary network. Tumor cells show variable eosinophilic, vacuolated cytoplasm in an arborizing capillary network with nuclear pleomorphism, intranuclear cytoplasmic invaginations, scattered hyaline globules,and psammoma-like calcifications. Some areas show branching hemangiopericytoma-like vessels with tumor cells radiating from the wall.
Immunohistochemically, in published series the tumor cells react strongly and diffusely with antibodies to PAX 8, CD 10, α-inhibin, S100 protein, neuron-specific enolase, and vimentin, and they show focal positivity with antibodies to epithelial membrane antigen and cytokeratin AE1/AE3. Tumor cells are negative for CK7, CK8/18, RCC antigen, synaptophysin, chromogranin, c-kit, D2-40, HMB45, melan-A, cathepsin K, SMA, desmin, CD31, CD34, and estrogen and progesteron receptors. Positive immunoreactivity for PAX8 is unexpected and contrasts to central nervous system (CNS) hemangioblastomas, which are essentially always negative for PAX8.
Hemangioblastoma is the rare benign tumors of uncertain origin that can occur sporadically or in association with von Hippel-Lindau (VHL) disease, VHL disease is a hereditary, autosomal-dominant, neoplastic disease that is associated with various tumour types, including ccRCCs, central nervous system (CNS) and retinal haemangioblastomas, phaeochro¬mocytomas (PCCs) and pancreatic neuroendocrine tumours, in addition to pancreatic and renal cysts. VHL tumour suppressor protein (pVHL) plays a key part in cellular oxygen sensing by targeting hypoxia-inducible factors for ubiquitylation and proteasomal degradation. In renal presentation the hemangioblastomas are reported non VHL related.
In conclusion, it's a very unusual case showing a sinchronous localization of haemangioblastoma and clear cell renal cells carcinoma (CR-RCC) in the kidney. The follow-up is very short to evaluate the outcome of this combined rare renal neoplasia.

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# Venerdì 19 ottobre 2018

Sala Nico 2 – 15:00 - 17:00

## DERMATOPATOLOGIA

## Slide Seminar di giovani dermatopatologi

Moderatori: G. Angeli, C. Clemente

## Patologia Infiammatoria

### PENFIGO VOLGARE ASSOCIATO A VIN

C. Mignona

Una donna di 27 anni con anamnesi familiare negativa per malattie autoimmuni, presenta multiple lesioni lesioni bollose cutanee e del cavo orale, il prelievo bioptico di una delle lesioni mostra un quadro di pemfigo volgare.Nel tempo viene seguita presso la Clinica Odontostomatologica e Dermatologica si alternano periodi di remissione e di riacutizzazione, con comparsa di lesioni estese a tutta la superficie corporea; successivamente compaiono altri sintomi riferibili a Cushing iatrogeno, osteoartrtosi ed osteoporosi, grave scadimento delle condizioni sistemiche, necrosi della testa del femore e dell'omero.Successivamente insorge una lesione vulvare vegetante, asintomatica, caratterizzata da sanguinamento spontaneo. Clinicamente si evidenziano aree di ispessimento associate ad aree di ulcerazione necrotica, con vascolarizzazione aumentata a caratteri atipici. Una biopsia rivela i tipici aspetti da pemfigo volgare, associati ad una lesione displastica di alto grado (VIN di alto grado) anche con aspetti Bowenoidi. Purtroppo le condizioni cliniche della paziente decadono rapidamente, con calo ponderale di 28 KG in due mesi, Hb: 7,50 gr/dl, impossibilità alla deambulazione spontanea, febbre recidivante, infezioni recidivanti opportunistiche, sepsi (immunosoppressori). La lesione vulvare peggiora clinicamente e una nuova biopsia conferma il reperto di lesione bollosa da pemfigo volgare associata a VIN di alto grado con immagini di iniziale invasione stromale. La paziente inizia un percorso di sei cicli chemioterapia, a base di cisplatino, senza miglioramento delle condizioni cliniche. Alla fine della chemioterapia viene effettuato un prelievo citologico che mostra una doppia componente di cellule acantolitiche e di cellule con gravi atipie citologiche. Dopo circa 2 mesi si verifica un grave peggioramento delle condizioni sistemiche fino al decesso. In letteratura è descritto un caso di carcinoma squamoso insorto su pemfigo familiare tipo Haley Haley (Cockayne SE et al nel 2000), in una paziente anziana. Il caso da noi osservato è differente sia dal punto di vista clinico (paziente giovane e quadro clinico devastante), sia dal punto di vista istologico (pemfigo volgare e non familiare).

### SARCOIDOSI SIMIL-LEBBRA

A. Filosa, G. Ferrara

La sarcoidosi rappresenta una malattia sistemica ad eziologia sconosciuta. Ogni organo può essere interessato dalla malattia e le localizzazioni più frequenti sono i polmoni, i linfonodi, gli occhi e la pelle. La diagnosi di sarcoidosi è una diagnosi di esclusione e si basa sulla identificazione istologica di flogosi granulomatosa in assenza di una eziologia infettiva nota o di altre cause. L'aspetto istologico tipico di una lesione sarcoidosica è rappresentato dalla presenza di aggregati di istiociti epitelioidei con poche o senza cellule infiammatorie di accompagnamento; i cosiddetti granulomi nudi non necrotizzanti. Tali granulomi non sono comunque specifici di sarcoidosi dal momento che sono anche descritti in processi infettivi, in reazioni da corpo estraneo, in lesioni secondarie a deficit immunitari, malattie linfoproliferative e reazioni a farmaci. I quadri istopatologici della sarcoidosi cutanea descritti in letteratura sono molteplici, rispetto a quanto si credeva in passato. Esistono forme con flogosi lichenoide, forme che simulano dermatiti di interfaccia associate a spongiosi, forme caratterizzate dalla presenza di granulomi necroibiotici, granulomi da corpo estraneo, granulomi perforanti, granulomi perineurali, di vasculite granulomatosa, di equivalenti morfologici simil-ittiosi. Non si conosce comunque con precisione l'esatta incidenza di ciascuna di queste forme. L'esame istologico della lesione in assenza di una adeguata correlazione clinico-patologica può pertanto portare a diagnosi sbagliate di lebbra, sifilide ed altre forme di granulomi infettivi, oppure alla errata diagnosi di granuloma anulare, necrobiosi lipoidica e reazione da corpo estraneo. La diagnosi di sarcoidosi può essere pertanto considerata in ogni paziente la cui biopsia cutanea mostra una flogosi granulomatosa in assenza di una apparente e dimostrata eziologia.

## Patologia Melanocitaria

### MELANOMA MIXOIDE (MUCINOSO)

N. Nardini

**Clinical features**
This rare histologic variant of malignant melanoma was initially described in metastatic lesions but can also be seen in primary melanomas. It typically presents on the limbs in older individuals (mean age of 60 years), with a male predominance. Although primary lesions can have a similar clinical presentation to conventional melanomas, amelanotic lesions are not uncommon potentially leading to diagnostic delay.
**Histopathological features**
Hitchcock et al defined myxoid malignant melanoma when at least 15% of the tumour showed stromal mucin deposition. In primary tumours there are frequently areas of conventional melanoma. In the myxoid areas, tumour cells may be smaller and can assume a more stellate or spindle-shaped appearance. Tumour cells are diffusely positive for S100, and variably positive for HMB45 and Melan-A. Colloidal iron and alcian blue at pH 2.5 highlight the stromal mucin, but this is negative for the epithelial mucin markers like PAS and mucicarmine.
**Differential Diagnosis**
- Benign mucinous melanocytic neoplasms (myxoid ordinary naevi, myxoid blue naevi, myxoid Spitz naevi)
- Mucinous adenocarcinomas

- Sweat gland tumours
- Cutaneous mixed tumours
- Sarcomas with myxoid features (e.g. myxofibrosarcoma, myxoid liposarcoma, extraskeletal chondromyxoid sarcoma, malignant peripheral nerve sheath tumour)
- Benign soft tissue tumours (schwannoma, nodular fasciitis, myxoid neurothekeoma)
- Mucinoses (papular mucinosis, cutaneous focal mucinosis)

**Discussion**

Mucin deposition in malignant melanoma seems to result from increased production of glycosaminoglycans rich in hyaluronic acid by reactive stromal fibroblasts. Cytokines, such as transforming growth factor (TGF) seem to be important in inducing this phenomenon. Factors derived from mast cells have also been hypothesized to be important in this setting. The prognosis of myxoid melanomas is similar to conventional melanomas when matched for Breslow thickness. Awareness of this rare variant of melanoma is important to avoid misdiagnosis.

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## LESIONI MELANOCITARIE CON ESUBERANTE SINTESI DI PIGMENTO, UN CASO PEDIATRICO

P. Possanzini, A.R. Lombardi, E. Crisanti, F. Scarpellini

*U.O. Anatomia Patologica, Ospedale "G.B. Morgagni L. Pierantoni" Forlì; U.O. Anatomia Patologica, Ospedale "Infermi" Rimini; U.O. Anatomia Patologica, Ospedale "Santa Maria delle Croci" Ravenna; U.O. Anatomia Patologica, Ospedale "M. Bufalini" Cesena*

Tra le lesioni cutanee di difficile interpretazione demoscopica clinica un posto di rilievo lo occupano le lesioni con abbondante produzione di pigmento, che vengono spesso asportate come lesioni melanocitarie atipiche.

Riportiamo un caso di una lesione insorta sullo scalpo di un bambino di 5 anni.

Il quadro clinico è di una papula fortemente pigmentata in accrescimento, di piccola dimensione, con un pattern dermoscopico omogeneo ma di difficile interpretazione.

All'esame morfologico microscopico la lesione è composta da una quota giunzionale e una quota dermica, è ben circoscritta, cupoliforme, con iperplasia dell'epidermide irregolare alternata ad aree di atrofia, e silhouette a "V".

La componente intraepidermica giunzionale è costituita da cellule epitelioidi di piccola e media taglia, nucleolate arrangiate prevalentemente in pattern lentigginoso con citoplasma ricco in pigmento melanico. La componente dermica è predominante, nel derma superficiale e medio si osservano elementi con le stesse caratteristiche morfologiche della componente giunzionale, ed in profondità si osservano elementi fusati bipolari che tendono a seguire il decorso degli annessi, senza evidenza di necrosi, con occasionali mitosi.

In considerazione dell'età e della sede speciale, nonostante le piccole dimensioni della lesione, abbiamo posto diagnosi differenziale tra un nevo blu composto "di Kamino" e un melanocitoma epitelioide pigmentato (PEM).

Per una maggiore accuratezza diagnostica abbiamo quindi deciso di chiedere un secondo parere ad un collega di un centro oncologico di riferimento. Sono state eseguite le indagini FISH per la valutazione di RREB1 (6p25), MYB (6q23), CCND1 (11q13), con esito negativo.

La condivisione dei casi complessi è pratica diffusa tra gli anatomopatologi, le lesioni melanocitarie dei bambini, nonostante siano spesso benigne, si presentano con una clinica atipica e sono di difficile inquadramento diagnostico.

La condivisione dei casi più critici tra colleghi e con centri con maggiore esperienza, utilizzando anche nuove tecniche di condivisione come il "vetrino digitale", porta ad una maggiore accuratezza diagnostica.

# Patologia Neoplastica Linfoide

## LINFOMA T CUTANEO A DIFFERENZIAZIONE T HELPER FOLLICOLARE

F. Alesini

Le cellule T helper follicolari sono un sottogruppo di cellule T helper che facilitano il reclutamento e la maturazione dei linfociti B. Oltre al linfoma T angioimmunoblastico (che rappresenta il paradigma delle lesioni linfoproliferative a differenziazione T helper follicolare) anche alcuni linfomi primitivi cutanei possono mostrare aspetti di differenziazione T helper follicolare. Questi aspetti si manifestano attraverso l'espressione di alcuni markers immunofenotipici (Bcl-6, ICOS; CXCL13, PD-1 e CD10) e la presenza di un microambiente neoplastico ricco in linfociti B. Lo studio presente è quello di confrontare gli aspetti clinici e patologici di due entità linfoproliferative primitive cutanee:il disordine linfoproliferativo T CD4+ a piccole e medie cellule (prima chiamato linfoma T cutaneo a piccole e medie cellule pleomorfe CD4+, inserito come entità provvisoria nella penultima classificazione WHO/EORTC ed oggi rivisitato nella sua nomenclatura per riflettere il suo basso potenziale di malignità) e la micosi fungoide, variante intertriginosa, che si manifesta d'emblée sottoforma di lesioni tumorali nelle regioni in cui sono presenti pieghe cutanee in assenza di precedenti/concomitanti stadi di chiazza o placca ed ha una spiccata tendenza alla progressione nonché una brutta prognosi nella maggior parte dei casi. Lo studio mette in evidenza le differenze cliniche tra le due entità sopra descritte, ne confronta le caratteristiche patologiche ed immunofenotipiche, con particolare riguardo al microambiente neoplastico e studia l'espressione dei markers tipici delle cellule a differenziazione T helper follicolare per individuare alcuni possibili target terapeutici per l'utilizzo di terapie anticorpali mirate di nuova generazione.

## NEOPLASIA A CELLULE DENDRITICHE BLASTICHE PLASMOCITOIDI

E. Mattioli

Paziente con una vistosa lesione della spalla, rilevata ma centralmente depressa, che a prima occhiata mi ha subito dato un'impressione di natura linfoide, ma è risultata negativa alla classica batteria CD3/CD20/CD4/CD8, così come a molti altri marcatori linfoidi; la presenza di una focale positività per S100, unitamente alla necessità di considerare altre ipotesi, mi ha portato ad esplorare (fuori strada) la possibilità di una neoplasia epiteliale, neuroendocrina, melanocitaria ed anche

secondaria (da polmone), sempre senza riscontri. Dopo una conferma della natura linfoide tramite CD45(LCA), siamo arrivati ad inquadrarla come neoplasia a cellule dendritiche blastiche plasmocitoidi, una recente entità caratterizzata dalla positività a CD56 e CD123 e prima considerata appartenente al gruppo dei linfomi a cellule NK. Il successivo colloquio con la figlia del paziente ha confermato la natura di localizzazione di patologia sistemica con la notizia della comparsa, nel frattempo, di numerose altre lesioni, alcune delle quali poi involute spontaneamente, e del vistoso decadimento delle condizioni generali del paziente (si tratta di una neoplasia aggressiva).

# Venerdì 19 ottobre 2018

Sala Cigno – 08:10 - 10:00

## PATOLOGIA DEI TRAPIANTI

## Alterazioni Morfologiche degli Organi da Donatori in Morte Circolatoria (CDC): Esperienze a Confronto

### HYPOTHERMIC OXYGENATED PERFUSION OF KIDNEY AND LIVER GRAFTS BEFORE TRANSPLANT: THE BOLOGNA EXPERIENCE.

F. Vasuri

**Background**
The use of extended criteria donors (ECD), including donors after cardiac death (DCD), increases the risk of delayed graft function (DGF) in kidney transplant and early allograft dysfunction (EAD) in liver transplant. The hypothermic oxygenated perfusion (HOPE), together with other perfusion strategies under trial, was studied in experimental and clinical settings in our Institution to maintain/improve the graft function. Preliminary phase (experimental study). Twenty kidneys (not suitable for transplantation) treated with 5 different perfusion conditions for at least 20 hours, demonstrated that perfusion in hyperbaric oxygenation and HOPE correlated with a significant increase in ATP level on graft tissue, with a downregulation of eNOS and HIF-1α expression. Light microscopy (with immunohistochemical staining for CD31 and CD34) and transmission electron microscopy were applied for the evaluation of endothelial preservation.

**HOPE clinical trial (safety study)**
HOPE was applied as preservation technique in 10 liver grafts and 10 kidney grafts from ECD donors and therefore transplanted. As controls, 30 liver grafts and 30 kidney grafts were selected, matched for clinical data donor/recipient age and surgical conditions. Histopathological analysis showed no significant differences among HOPE and control livers (9 histological variables analyzed), and among HOPE and control kidneys (13 histological variables analyzed). At follow-up, no cases of EAD were recorded in the HOPE liver group, versus 26.7% of control livers (p=0.076, chi-squared test). AST and GGT levels at 1 month after transplant were significantly higher in the control group than in HOPE livers (p=0.026 and p=0.007 respectively, Mann-Whitney test). In kidney grafts no significant differences are recorded until now in follow-up, albeit the incidence of DGF was 20% and 40% in the HOPE and control kidney groups respectively.

**Conclusions**
After preliminary studies aimed to set up the technique, the hypothermic oxygenated perfusion (HOPE) is likely to preserve graft tissue integrity and metabolic activity for long times, with good impact on EAD and DGF.

# Venerdì 19 ottobre 2018

Sala Cigno – 10:30 - 13:30

## PATOLOGIA TESTA-COLLO

## Sessione I

## Memorabilia

Moderatori: S. La Rosa, P. Morbini

### VASCULAR LESIONS: NEW INSIGHTS IN VASCULAR MALFORMATIONS

L. Moneghini

Classifications and diagnosis of diseases are a challenge because of finding of genes mutations.
Identification of specific mutations in vascular malformations with overgrowth of soft tissue replaces different clinical and pathological patterns into a new classification in a variety of districts as head and neck.
PROS (PIK3CA related overgrowth spectrum) is a recent example where different vascular malformations, mostly lymphatic, with or without soft tissue overgrowth, are united by a single genic alteration, the mutation of PIK3CA gene.
Another example is GNAQ mutation related vascular malformation with or without soft tissue overgrowth.
Clinical and imaging evaluation should be mandatory to perform adequate sampling for histological examination and genetic analysis of gene mutation even if only in 1% of extracted DNA.
ISSVA (International Society for the Study of Vascular Anomalies) and SISAV (Società Italiana per lo Studio delle Anomalie Vascolari) are committed to publish new Classification and Guidelines in order to account for each clinical, imaging, histological and genetic feature.

# Sessione II

# Focus su...

Moderatori: F. Ionna, L. Moneghini

## FOCUS ON MASC (SECRETORY CARCINOMA OF SALIVARY GLANDS)

A. Skálová

The secretory carcinoma (SC) of salivary glands, originally described as mammary analogue secretory carcinoma (MASC) by Skálová et al. in 2010 [1], is a low-grade malignancy recently included in the 4th edition of WHO Classification of Tumours of Head and Neck in 2017 [2]. As the original name implies, there is histomorphological, immunophenotypical and genetical resemblance to the secretory carcinoma of the breast, which also harbor the same genetic translocation t(12; 15)(p13;q25) resulting in ETV6-NTRK3 gene fusion [1 3]. This specific fusion is confirmatory for SC and has not been reported in any other salivary gland tumors. Recently, new rearrangements ETV6-RET [4] and ETV6-MET [5] have been found in small subset of SCs, possibly related to more aggressive behavior.

Histologically, SC consists of uniform eosinophilic cells with vacuolated cytoplasm arranged in cystic, tubular, solid or papillary manners. The cystic spaces are characteristically filled by periodic acid-Schiff (PAS)-positive secretory material.

More than 300 articles on SC have been published so far, but only few are dedicated to the description of the cytomorphological features [6]. For decades the reporting of salivary gland cytology has been full of diversity with the use of descriptive reports with no categories or with surgical pathology terminology. The development of a brand new uniform system for reporting salivary gland fine needle aspirations (FNAs) has been aimed. The Milan System for Reporting Salivary Gland Cytology follows the trend of thyroid and cervical Bethesda and urinary Paris reporting systems where each category has a defined risk of malignancy (ROM) and suggested therapeutic approaches [7].

The treatment of SC has been variable, ranging from simple excision to radical resections, neck dissections, adjuvant radiotherapy and/or adjuvant systemic chemotherapy. Locoregional radiation therapy can be considered for larger tumors or those with positive margins or perineural invasion. Systemic chemotherapy may be implemented for distant metastases, however, little is known about clinical outcomes and optimal treatments. Therefore, recognizing SC and testing for ETV6, NTRK3 and RET rearrangement may be of potential value in patient treatment, though use of and response to tyrosine kinase inhibitors in SC is limited so far [8].

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## LA CHIRURGIA ROBOTICA NEL DISTRETTO TESTA COLLO, PRIME CONSIDERAZIONI SU 67 CASI RAZIONALE

F. Ionna

La chirurgia robotica, una tecnica emergente per il distretto testa collo, trova indicazioni nei tumori benigni e maligni dell'orofaringe, dello spazio sopraglottico, dello spazio parafaringeo e per il trattamento delle apnee notturne ostruttive.

Descriviamo la Nostra esperienza con l'intento di valutare: i benefici chirurgici, le indicazioni, gli svantaggi.

**Materiali e metodi**

Dal 2013 ad oggi ,presso la Nostra Struttura son state eseguite 67 procedure chirurgiche TORS per patologie benigne e maligne dell'oro-ipofaringe, spazio sopraglottico e spazio parafaringeo. Abbiamo valutato i seguenti outcomes: durata dell'intervento, presenza/assenza di sanguinamento intra operatorio, presenza/assenza di complicanze post operatorie, dolore post operatorio.

Le localizzazioni trattate: 48 orofaringe, 5 spazio parafaringeo,14 ipofaringe/laringe sovraglottica.

**Risultati**

L'intervento è durato in media 90 minuti, in 12 casi è stato associato al vuotamento linfonodale laterocervicale. Nel 67 % si trattava di patologia maligna ( carcinoma squamoso +linfoma).

Complicanze:1 sanguinamento intra operatorio , 1 sanguinamento post operatorio e a 7 giorni (paziente radiotrattato), 2 casi in cui si è resa necessaria la tracheotomia.

Degenza post operatoria era tra i 3 e i 40 gg ( in media 10 gg) a seconda se associata o meno anche chirurgia del collo. Nel 15% dei casi il dolore nell'immediato post operatorio non ha richiesto l'uso di analgesici, nel 60% il dolore è stato controllato solo con FANS o paracetamolo e nel 25 %è stata utilizzata pompa antalgica ( fans+ tramadolo)nelle 24 h successive all'intervento. I pazienti nel 60% dei casi hanno riferito dolore ancora a 7 giorni dall'intervento da richiedere utilizzo di antidolorifici(fans /paracetamolo).

L'edema post operatorio è stato controllato con betametasone per vie ev. nei primi 4 giorni.

1 paziente deceduta a 20 giorni dall'intervento per emorragia.

**Conclusione**

Vantaggi di tale procedura: visione "3D full Hd",possibilità di operare in spazi angusti con una gestualità fine e precisa .riduzione delle complicanze intra operatorie, buon controllo del campo chirurgico per le neoformazioni parafaringee( lesioni ben circoscritte).

Offre nuove e sicure opzioni di trattamento per tumori in stadio precoce dell'ipofaringe, base lingua, regione tonsillare, assume un nuovo ruolo per stadiare, citoridurre stadi T>2 (facilitazione terapeutica), "modulare" le terapie adiuvanti e le conseguenti tossicità acute e tardive delle stesse.
È fondamentale la selezione dei pazienti candidabili a tale chirurgia.
Fattore limitante: dimensioni degli strumenti non ancora sufficientemente miniaturizzati e versatili per l'utilizzo ottimale nel nostro distretto.

# Venerdì 19 ottobre 2018

Sala Orione – 08:00 - 17:00

## CITOPATOLOGIA

### CITOLOGIA DEL CAVO ORALE: NUOVE PROSPETTIVE E POSSIBILITÀ

R. Navone

*Former Director of Department of Biomedical Sciences and Human Oncology of the University of Turin, E-mail: roberto.navone@unito.it*

**Introduction**
Oral mucosal squamous cell carcinoma (OSCC) is a frequent malignant neoplasia (in sixth place for cancer-related mortality worldwide). The most important risk factors identified so far are tobacco and alcohol. Moreover, a potential role of oral HPV (human papilloma virus) infection in the onset of OSCC [1 2], could represent an adjunctive predictive factor. There has been no significant improvement in survival rate over the last 30 years (< 50% of patients with oral or pharyngeal cancer survive more than 5 years), as this type of tumour is more often diagnosed at an advanced stage. An Indian study [3] has demonstrated that screening programmes, based on periodic objective oral cavity visual examination, reduce OSCC mortality rate in high risk individuals. However, there is insufficient data on programmes based on other parameters like exfoliative cytology [4 5].
As definitive diagnosis of the OSCC and its precursors (dysplasias) are still based mainly on scalpel biopsy, this may be a contributing factor for the poor prognosis of oral neoplasia. Indeed, scalpel biopsy is not usually done in all oral lesions observed, but rather reserved for only those with high clinical suspicion (class I). Evidently it would be useful, particularly for the apparently innocent but potentially malignant (class II) oral lesions (OPML), to establish a first level test6, to identify only lesions that should be further investigated with a second level test, as is the case with the Pap test and colposcopy with biopsy, for carcinoma of the uterine cervix. Despite the fact that diagnostic oral cytology has been well known for many years, it has not yet been as widely adopted as has cervicovaginal cytology. Paradoxically, nowadays seemingly pelvic examination and Pap smears are more acceptable than oral cavity examination [6]. Exfoliative oral diagnostic cytology was quite popular in the 1960s and 70s [7]. More recently oral conventional cytology has lost ground [7]. Several factors, such as diagnostic (interpretative) difficulties, or the sampling technique itself may be responsible for this. Many oral lesions have resistant keratotic surfaces, so samples are often inadequate because they are often too superficial and/or bloody. Recent reports indicate that both efficacy and efficiency of oral cytology may be enhanced by the addition of new ancillary techniques that enhance sensibility and specificity. The new approaches include: liquid-based cytology, flow and image cytometry, and microhistology. The various options will be examined.

**Liquid-Based Cytology**
Liquid-based cytology, which to date has been used mainly for the Pap test, has given promising results both in terms of sample quality (no cellular artefacts). Navone et al [8] studied 473 patients referred to the Oral Medicine Section of the University of Turin for scalpel biopsy due to the presence of OSCC or OPML. All patients, after sampling for cytology, had surgical biopsy and histological examination. Eighty-nine of the 473 samples were processed using conventional cytology and 384 by liquid-based cytology (Thin Prep). There were 12.4% of inadequate cases in conventional oral cytology versus 8.8% in liquid-based cytology; the sensitivity, specificity, predictive positive value and predictive negative value were 85.7%, 95.9%, 95.4% and 87.0% respectively for conventional samples, versus 95.1%, 99.0%, 96.3% and 98.7% for liquid-based cytology. More recent data [9 10] on 927 patients, all with histological control, gave similar results (sensibility 94.7%, specificity 98.9%, predictive positive value 95.9%).

**DNA Analysis with Flow and Image Cytometry**
As DNA cellular content (ploidy) has been reported to be a reliable marker in oral oncology for both malignant and premalignant lesions, DNA ploidy has been studied both by flow and image cytometry. Our group [11 12] examined 211 OPML with liquid based cytology and flow cytometry and 45 with image cytometry. A conventional histological diagnosis was done in each case by scalpel biopsy. Flow cytometry demonstrated aneuploidy (an abnormal number of chromosomes), in 60.0% of OSCC, in 22.8% of the OPML without dysplasia and in 56.7% of the OPML with dysplasia. Image cytometry demonstrated aneuploidy in 77.7% of OSCC, in 25.0% of OPML without dysplasia and in 65.4% of OPML with dysplasia. In fresh samples, also with flow cytometry, Pentenero et al11 demonstrated that DNA aneuploidy and dysplasia in 60 OPML were strictly associated with cigarette smoking and site of the lesion and Donadini et al12, studying 109 OPML and 82 "oral distant fields" (ODF), characterized by clinically normal appearing mucosa situated at a distance from co-existing OPML, observed the prevalence of single near-diploid sublines in ODF and non-dysplastic OPML. Whereas multiple highly aneuploid sublines were widespread in dysplastic OPML and OSCC. Then, near-diploid aneuploidization in ODF and OPML appeared as early events of oral carcinogenesis, in agreement with the concept of "field cancerization"12.

**Oral HPV-DNA Studies**
Navone et al [13 14] investigated one hundred and sixty-two subjects, 77 males and 85 females: 160/162 had normal cytology results, 1 had a low-grade oral lesion (OIN 1) and 5 had HPV-DNA test positive (the HR-HPV types were 16, 31, 53; 16, 31; 73 in three females; two males had low risk HPV, 62 and CP6108 respectively: in these five cases the cytology was normal or with keratosis. Combining liquid based diagnostic oral cytology and tests for HPV infection seems able to select a subgroup of patients with potential predictive factors for OSCC development and thus requiring proper follow-up schedule even in absence of visible oral lesions. The prospective evaluation of healthy subjects with oral HPV infection could give important information about its role in the develop-

ment of OSCC. A combination of oral cytology and tests for HPV infection could represent a significant diagnostic step forward early diagnosis of OSCC and dysplasia, as yet demonstrated with the experience in uterine cervical carcinoma screening with PAP test and DNA-HPV.

**Microhistology ("The Curette Technique")**

So as to increase the quantity of cells available for cytological examination and the other complementary techniques, Navone et al [15 16] set up and applied a new sampling technique that was no longer based on the use of a "Cytobrush", but on "scraping" using a dermatological curette. It was immediately evident that, not only did this technique provide more abundant material for cytology and ploidy10, but that the samples were richer in "accidentally" acquired small fragments that could be used as micro-biopsies, included in paraffin and examined histologically. So, OSCC and OPML (even those with the lowest suspicion index) may be investigated by cytology and microhistology, for an early identification of cancer and/or precancerous oral lesions so as to prevent transformation into carcinoma. Lastly, the "microbiopsies" (over 400 cases), recovered together with the material used for liquid based cytology, allowed for a definitive histological diagnosis in more than 90% of the cases [16].

**Conclusions**

Conventional, and especially liquid-based exfoliative cytology, is able to provide satisfying diagnostic information. Its sensibility is higher than that for the Pap test, whilst its specificity is the same. "Ancillary" techniques, as flow and/or image cytometry for DNA, allow for the detection of aneuploid OPML, i.e. the identification of lesions that are at risk of evolution. By combining "liquid based" diagnostic oral cytology and HPV DNA testing it seems that a subgroup of patients with potential predictive factors for PMLs and OSCC development may be selected. Therefore, these subjects are to have regular follow-ups even in absence of visible oral lesions. HPV becomes the main risk factor in those subjects where alcohol and tobacco are less representative. By combining "liquid based" diagnostic oral cytology and HPV infection testing it seems that a significant diagnostic step towards an early diagnosis of OSCC, as has already been demonstrated in uterine cervical carcinoma screening with the PAP test and DNA-HPV.

Lastly, the sampling with the "curette technique", which covered ample surface areas and/or multiple lesions and provided "microbiopsies", made for a reduction in the number of patients that had to return and the number of diagnostic (scalpel) biopsies carried out, leading to a positive cost/benefit ratio and less discomfort for the patients. Moreover, this "second level" but relatively simple and "patient friendly" technique, make it a good candidate for use in the field of general dentistry, as it is there that most of the preneoplastic and neoplastic lesions are observed for the first time. The adoption of this strategy could make a contribution towards the reduction of the present percentage of late diagnosis in OSCC.

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# Sessione I

Moderatori: V. Ascoli, F. Zanconati

## CYTOPATHOLOGY AND HPV: THE GYNECOLOGIST'S POINT OF VIEW

C. Carriero

*Dipartimento Interdisciplinare di Medicina - Sezione Ginecologia, Università degli Studi di Bari "Aldo Moro"*

Obstetrician–gynecologists have the opportunity to provide holistic care for their patients. This includes taking a comprehensive history, diagnosing and treating conditions that are identified with a comprehensive history and focused examinations, and providing evidence-based and evidence informed clinical preventive services. Gynecologists can play a critical role in engaging patients in shared decision making, encouraging and facilitating healthy behaviors, and counseling about a wide array of effective preventive health practices. There is great variability in how these services are offered among health care delivery systems, and gynecologists should work within their local systems to promote their role in providing primary and preventive care services for women. Updated guidelines from the American College of Obstetricians and Gynecologists [1] stress the role of obstetrician/gynecologists in providing preventive care for women throughout their lifetime. The recommendations coincide with the release of a new Well-Woman Chart from the ACOG-led Women's Preventive Services Initiative (WPSI) [2].

The gynecologist's most important and critical role in prevention of cervical cancer is colposcopy, encompassing both diagnosis and management of premalignant disease [3].

Improvements in the performance of cervical screening may be limited by the diagnostic performance of colposcopy. Nonetheless, colposcopy remains the best available tool to assess women considered at high risk for having or developing cervical cancer.. Introduction of vaccination against HPV as well as the switch to primary HPV test-based screening is likely to change the profiles of women presenting to colposcopy services and provide management difficulties for the colposcopist.. HPV-based

cervical cancer screening is more effective than screening with cytology. The effects of HPV-based screening depend on the organization of the program and on adherence to algorithms for screening triage. Otherwise, it is likely that HPV-based screening will increase the referral rate to colposcopy including more women with no detectable cervical lesion. HPV vaccination will require many years to evaluate any beneficial effects on cervical cancer incidence and mortality [4]. The prevalence of cervical intraepithelial neoplasia grade 3 may decrease for women having had HPV vaccination. The incidence of cervical intraepithelial neoplasia grade 3 and cervical cancer in second and subsequent rounds of HPV-based screening are likely to decrease compared to cytology-based screening. In HPV-based screening, the numbers of women with no detectable or minor abnormalities at colposcopy and with screen-detected glandular disease are likely to increase..The development of quality assurance across Europe accompanying these program becomes essential and it is a central objective of the European Federation of Colposcopy [5]. Finally, excisional treatments of precancerous lesions of the cervix are associated with a significantly increased risk of preterm birth and obstetric morbidity. Considering the natural history of CIN, it is advisable, particularly for women under 40-45, to abstain from treatment in case of Low-grade lesion (LSIL). The long-term expectant follow-up of cervical LSIL (up to 48 months) has showed to be effective: prompt detection and treatment of progressed lesions and regression or persistence of the remainder, and safe:no risk of invasive cancer during follow-up intervals [6].

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## Sessione II

Moderatori: M. Bonzanini, A. Crescenzi

### COMPARISON BETWEEN THE 2014 ITALIAN AND THE 2017 BETHESDA SYSTEMS FOR REPORTING THYROID CYTOLOGY


G. Fadda

*Associate Professor of Pathology - Head, Cytopathology Section - Università Cattolica del Sacro Cuore - Fondazione Policlinico Universitario "Agostino Gemelli"– Largo Francesco Vito, 1, 00168 Roma (Italy) email: guido.fadda@unicatt.it*


**Introduction**

The accurate preoperative diagnosis of thyroid nodular lesions represents a real problem for physicians, endocrinologists and surgeons. The pivotal role of the pathologist is to characterize as best as possible these lesions to enable the patient to receive a timely and appropriate treatment. FNAC (fine-needle aspiration cytology) is the only test that can provide a definitive preoperative diagnosis especially for benign and malignant nodules. Their sensitivity and specificity are reported to be 68–98% and 56–100%, respectively in different studies [1 2].
FNAC also represents the most accurate and cost-effective method for identifying patients suffering from thyroid nodules candidate to surgery from those who can be clinically managed. The Italian reporting system for thyroid cytology committee was established in the years 2011-2014 with the aim of updating of the previous SIAPEC-IAP system devised in 2007 [3]. The committee who accomplished this task, sponsored by the SIAPEC-IAP (Italian Society for Anatomic Pathology and Cytology, joint with the Italian Division of the International Academy of Pathology) in agreement with the endocrinological societies SIE (Italian Society of Endocrinology), AME (Association of Medical Endocrinologists) and the Italian Association of Thyroid (AIT), was composed by 10 experts in thyroid diseases (5 pathologists and 5 endocrinologists) The previous SIAPEC-IAP reporting system was a five-tiered scheme which included the following categories: TIR 1-non diagnostic; TIR2 - negative for neoplasia; TIR3 - indeterminate/follicular proliferation; TIR4- suspicious for malignant neoplasm; TIR5- positive for malignancy. The newly devised Italian reporting system [4] introduces the additional subgroup of TIR1C (cystic) in the non-diagnostic group and the subdivision of the indeterminate category (TIR 3 of the previous SIAPEC-IAP system) in TIR 3A (low-risk indeterminate lesion - Figure 1) and TIR 3B (high-risk indeterminate lesion- Figure 2). The American Bethesda Reporting System for Thyroid Cytology (TBRSTC) published in 2008 (5, 6) and updated in 2017 [7] included six instead of five categories: the nondiagnostic, benign and malignant groups were similar to the Italian and British classifications whereas the indeterminate lesions (IL) where furtherly subclassified in three diagnostic groups: 1) Atypia of undetermined significance and Follicular lesions of undetermined significance (AUS/FLUS); 2) Follicular neoplasm or suspicious for follicular neoplasm (FN/SFN) and 3) suspicious for Malignancy (SM) [6].

**The Italian Reporting System 2014**

Based on the previous published experiences of the British [8] and American national reporting systems the Italian committee developed a project with the purpose to i) revise the morphologic criteria of inclusion in each category; ii) to update the clinical actions with more innovative diagnostic techniques; iii) to validate the new system with a multicenter study Thus, the project of the 2014 Italian system is different from the previous and features some structural differences in comparison to the above mentioned systems . The first point is the different interpretation of the nuclear atypia of the follicular cells (excluding oncocytic cells). In fact the architectural atypia remains the basis for distinguishing low-risk and high-risk lesions (TIR 3A from TIR 3B) but a significant degree of nuclear atypia warrants the inclusion of a lesion in a suspicious category (either TIR 3B or TIR 4) which involve the surgical consultation [9 10]. Based on these parameters, the Italian committee expects that the low-risk category (TIR 3A), which does not include cases with severe atypia of thyrocyte, may decrease to a 5-10% ROM compared to the 5-15% of the omologous categories of the British and American systems. The new Italian reporting system has also included in

the suggested actions for the non-diagnostic category (TIR 1) the possibility to use, in cases of repeated non-diagnostic results, the core-needle biopsy (CNB) technique. CNB allows the sampling of the lesion with a 20 to 22 spring-activated needle useful to obtain a thin biopsy which can be processed as histological specimen. This technique has been extensively studied by Korean and Italian groups and it can be also used to get material for performing immunohistochemical procedures such as galectin-3, HBME-1 and cytokeratin 19 in indeterminate lesions [11 12]. Immunocytochemical stainings may be also applied to the material processed by liquid-based cytology (LBC), which is specifically mentioned in the reporting system though it is recommended to be used only in institutions with specific experience [13 14].

**Indeterminate lesions**

Indeterminate lesions still remains a controversial subject in the classification of FNA specimens. Numerous studies have demonstrated that this category may represent up to 20% of thyroid diagnoses and it represents the so-called "grey zone" in which both benign and malignant entities are included [15 16]. This category may also result as follicular variant of papillary thyroid carcinoma (FVPC) at the histological examination as the lack of the distinctive nuclear features of PTC may fall short of a definitive diagnosis of malignancy. All these evidences result in a high number of unnecessary surgeries which may cause additional morbidity and increase of health care costs. They represent the major limit of the morphology and the main cause of the reduced diagnostic accuracy of the technique in case of follicular-patterned neoplasms in which only the capsular or vascular invasions are the cornerstones for the diagnosis of carcinoma.

A recent paper published by an international group [17 18] has introduced the pathological entity of non-invasive follicular tumor with papillary-like structures (NIFTP) which has also been included in the 2017 edition of the WHO "Blue Book" [19]. This entity represents the low-malignant potential counterpart of the follicular variant of PTC and is the most important responsible of the false-negative diagnoses in thyroid cytology). The introduction of the NIFTP in the histological practice will likely decrease the ROM for the diagnostic categories of the thyroid reporting systems [20 21].

If the surgical removal of a nodule diagnosed as IL is the preferred option as many as 70% of patients will undergo an unnecessary operation. Based on TBSRTC, the Atypia of undetermined significance/Follicular lesion of undetermined significance (AUS/FLUS) category has a 5-15% risk of malignancy a value which increases to 15-30% in the groups of Follicular Neoplasm/Suspicious for Follicular neoplasm (FN/SFN) and Hurthle cell neoplasm (HCN - 21). The 2014 Italian reporting system for thyroid classification SIAPEC-AIT 2014 has introduced the new subclasses of TIR3A and TIR3B, identified on the basis of both architectural and cytological atypia, with the purpose of decreasing the amount of unnecessary surgeries. The recent study by Straccia and colleagues [22] on 4,043 cytological cases in the period 2014-2016 analyzed the frequency of the diagnostic categories of the Italian system. The following results: TIR1: 9.8%; TIR1C: 1.3%; TIR2: 71%; TIR3A: 5.5%; TIR3B: 4.6%; TIR4: 2.5%; TIR5: 5.2% were perfectly in keeping with the expected frequencies. Four-hundred seven (56%) of the 721 cases classified as indeterminate (TIR 3A, TIR 3B, TIR 4) and malignant neoplasm (TIR 5) underwent surgical resection. This study examined also the change in ROM for the different diagnostic categories considering NIFTP as malignant neoplasm then excluding it (14 vs 16% for TIR 3A, 24 vs 28% for TIR 3B, 85 vs 83% for TIR 4, no changes for TIR 5) The results of this study confirm the reduction of ROM in the indeterminate categories reported by Faquin and coll and Strickland and coll. for the Bethesda system [23 24].

The TIR3A subgroup, (low-risk indeterminate lesion-LRIL), is comparable to the AUS/FLUS category of BSRTC and the Thy3a class of the British Thyroid Association (BTA). It is characterized by increased cellularity with numerous microfollicular structures (which represent less than 60% of the overall cellular component) in a background of poor colloid amount. A mild degree of atypia can be sometimes observed in the nuclei of the follicular cells. The overall proportion of microfollicles, however, is not sufficient for the diagnosis of follicular neoplasm. Degenerative changes may be present, as sometimes observed in non-neoplastic lesions. At times sparsely cellular samples containing predominantly microfollicular groups, also with oncocytic features ("Hurthle cells"), in a background of scant colloid, can fulfil the criteria for inclusion in this category.

The TIR 3A category also includes partially compromised specimens (because of preparation artifacts or blood contamination), with cytologic or architectural alterations that neither can be confidently classified as benign nor otherwise categorized. This subcategory is expected to have an estimated maximum ROM of 10%.

The TIR3B subgroup is similar to the FN/SFN category of BSRTC and the Thy3f category of BTA identifies nodules with an expected risk of malignancy of 15-30%. It is characterized by high cellularity with a predominant microfollicular/trabecular arrangement (>60% of the cell component), with focal cytologic atypia (mostly moderate, rarely severe) suggestive for a "follicular neoplasm" (FN). Samples composed almost exclusively of oncocytes (Hurthle cells - oncocytic neoplasm - Figures 3 and 4) are included in the TIR 3B subcategory without taking in consideration either the architectural or the cytological atypia. The above mentioned study of the Italian Reporting system [22] subclassified the TIR3B category in non-oncocytic high risk lesions and Hürthle cell lesions. Among the 109 cases analyzed, the overall ROM for the category was 28% but the specific ROM for oncocytic lesions was as low as 9% whereas it rose to 50% for non-oncocytic lesions.

This subcategory also includes samples characterized by nuclear alterations suggestive of papillary carcinoma, that do not permit to reliably exclude malignancy, but are too mild or focal to be included in the TIR 4 (suspicious for papillary carcinoma) category.

For all these reasons the clinical actions referred to the TIR 3 subgroups are different. A clinical and sonographic follow-up lesions and the repeat of the FNA within six months are the clinical suggestions for TIR3A, while surgery is the most appropriate indication for TIR3B nodules. However, the surgical or conservative management of these novel subcategories depends upon both the cytological and clinical data and requires the consultation between cytopathologists and clinicians.

**The 2017 Bethesda System**

In 2017 TBSRTC has been updated by the same authors (Cibas and Ali) with the contribution of an international panel. The work has been based upon the review of the recent literature data and some differences in respect to the previous system are present [7].

The most important points of difference between the 2008 Bethesda reporting system and the current one are:

i) the recalculation of the ROM for the different categories, particularly for AUS/FLUS (which becomes 10-30% instead of 5-15% of the previous edition) and for FN/SFN (25-40% instead of 15-30%);
ii) the possible effect of the introduction of the new pathological entity of NIFTP which is expected to decrease the ROM for the previous categories by at least 30%;
iii) the pivotal role of the molecular analysis in identifying the most appropriate clinical management for indeterminate lesions.

**Conclusions**

The 2014 update of the Italian reporting system and the 2017 Bethesda system are meant to provide the cytopathologists a manageable tool for the daily practice in diagnosing the thyroid nodules. Meanwhile, the assessment of the morphologic criteria for including each case in the proper reporting category and the identification of the molecular tests which may help in diagnosing the most difficult (more often indeterminate) cases are one of the purposes for a future improvement of the cytological technique [25 26]. The most important point a cytopathologist should keep in mind when involved in the cytological characterization of a thyroid nodule is that the morphologic picture is the most important driver of the clinical management (whether surgical or clinical) and it is also the basis for validating all molecular techniques. Finally, in the absence of marked atypical features of the follicular cells, the future impact of the NIFTP to the thyroid cytological practice suggests a careful use of the diagnoses of suspicion to avoid the increase of unnecessary surgical procedures.

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## CYTOLOGY OF SALIVARY GLAND LESIONS


E.D. Rossi

*Fondazione Policlinico Unversitario Agostino Gemelli-IRCCS, Roma, Italy*


Tumors of the salivary gland include a wide range of benign and malignant neoplasms that often pose diagnostic challenges for both the cytopathologist and surgical pathologist. Salivary gland fine needle aspiration (SG-FNA) is well established as an effective tool in the preoperative diagnosis of salivary gland lesions: it is safe, rapid, and causes few complications. SG-FNA has gained its popularity because it can be easily applied in an outpatient setting to sample and diagnose most non-neoplastic and neoplastic salivary gland

lesions. Moreover, in most cases, SG-FNA can effectively identify many common benign tumors, and it is effective at discriminating between low- and high-grade malignant tumors. Despite the advantages of SG-FNA, there are certain factors which can influence its overall utility. These include the FNA technique, the use of ultrasound guidance, rapid-on-site evaluation (ROSE), sample preparation techniques, experience of the cytopathologist, and the general inherent heterogeneity of SG tumors as a group.
The accuracy of SG-FNA is high for the diagnosis of most common salivary gland tumors such as pleomorphic adenoma and Warthin tumor. The accuracy is also high for distinguishing benign and low-grade neoplasms from high-grade carcinomas; however, the specificity of SG-FNA for sub-typing a particular neoplasm shows a range (48-94%) of diagnostic accuracy. This is due to cytologic overlap of several of the less common salivary gland tumors. Availability of ancillary studies has helped, in part, to address this diagnostic issue. Nonetheless, there is still a subset of salivary gland lesions where a specific cytologic diagnosis cannot be made based upon cytomorphology alone, leading to challenges for the treating clinician to devise a specific management plan. Previously, there was no standard cytologic evidence-based reporting system which adequately addresses the cytomorphologic spectrum of salivary gland lesions. Ideally, a uniform reporting system would provide a more effective structure to convey useful diagnostic information to the treating clinician. This is addressed by the new Milan System for Reporting Salivary Gland Cytopathology.

## IL PUNTO DI VISTA DEL CLINICO

V. Triggiani

*Dipartimento Interdisciplinare di Medicina, Sezione di Medicina Interna, Geriatria, Endocrinologia e Malattie Rare, Università degli Studi di Bari "A. Moro", Bari.*

La citologia da agoaspirato rappresenta ancora oggi il gold standard per la diagnosi di natura della patologia nodulare tiroidea. La diffusione dell'impiego dell'ecografia e della pratica dell'agoaspirato, in particolare sotto guida ecografica, tuttavia, è ritenuta attualmente essere tra le principali cause dell'aumento di incidenza del carcinoma tiroideo registrato negli ultimi decenni, a fronte di una mortalità che è rimasta bassa e costante nel tempo. L'andamento delle curve di incidenza e di mortalità del carcinoma tiroideo suggerisce infatti una "overdiagnosis", condizione che si determina quando in soggetti asintomatici viene diagnosticata una malattia che non sarà mai sintomatica né causa di mortalità precoce. Infatti, in molti casi si tratta di piccoli carcinomi papillari in buona parte scarsamente evolutivi, come evidenziato da studi condotti in Giappone su pazienti con microcarcinomi tiroidei diagnosticati alla citologia, non sottoposti a intervento chirurgico e seguiti semplicemente con controlli clinico-ecografici periodici, che non mostrano nella gran parte dei casi, anche con follow up superiori ai 10 anni, alcuna crescita significativa o insorgenza di metastasi linfonodali.
L'aumento di diagnosi di carcinoma tiroideo differenziato ha portato negli ultimi 10-15 anni ad un incremento di circa il 60% degli interventi di tiroidectomia totale, seguiti spesso da terapia con radioiodio e con ormone tiroideo in molti casi a posologie TSH-soppressive, venendosi a configurare frequentemente una condizione di "overtreatment" con l'aumento delle possibili complicanze e conseguente impatto negativo sulla qualità di vita.
Un problema che è rimasto in buona parte non risolto è poi quello delle citologie indeterminate, anche se la classificazione della SIAPEC del 2014, introducendo la sottoclassificazione delle citologie indeterminate in Tir3A e Tir3B, ha dato la possibilità al clinico di prevedere in molti casi un follow up clinico-ecografico e la possibile ripetizione dell'agoaspirato, laddove in precedenza vi era una più diffusa indicazione alla chirurgia cosiddetta "diagnostica". Con la contestualizzazione del reperto citologico con l'aspetto ecografico e con lo sviluppo rapido delle metodiche di indagine molecolare, dovrebbe pertanto essere nel tempo sempre più limitato il ricorso alla chirurgia diagnostica.
Di fronte a tali problematiche, anche alla luce delle linee guida, il clinico sta modificando progressivamente il proprio atteggiamento. Una indicazione importante è quella di evitare gli screening ecografici, in particolar modo in soggetti non a rischio per tumore della tiroide e di evitare le ecografie tiroidee non appropriate. L'ecografia deve mirare sempre più ad una stratificazione del rischio, al fine di selezionare sempre meglio i noduli da sottoporre ad agoaspirato. In caso di citologia indeterminata, va valutata la risposta del citologico alla luce del quadro ecografico, facendo ricorso eventualmente alla diagnostica molecolare, conoscendone limiti e costi, laddove questa sia disponibile. In ogni caso, occorre cercare di limitare il più possibile gli interventi chirurgici a scopo diagnostico, e limitare, se possibile, l'estensione dell'intervento, al fine di ridurre anche la possibile insorgenza di complicanze e danni permanenti. Di fronte all'esame istologico, occorre tenere conto del significato di entità di recente introduzione come il NIFTP con il conseguente effetto "delabeling" sul paziente. In caso di diagnosi di carcinoma tiroideo, tenere conto nella valutazione del paziente della classificazione TNM per quanto concerne la mortalità, con le modifiche introdotte dall'8^ edizione, ma, soprattutto, della valutazione del rischio di recidiva, basandosi essenzialmente sulle indicazioni delle linee guida dell'American Thyroid Association 2015. Per il trattamento e il follow up del paziente occorre tener conto di tali aspetti, limitando ai casi che effettivamente lo richiedano il trattamento con radioiodio e la terapia TSH soppressiva.
Fondamentale è, pertanto, limitare l'overdiagnosis e quindi l'overtreatment di forme clinicamente poco significative di pazienti a basso rischio, ma nello stesso tempo evitare l'underdiagnosis e l'undertreatment di pazienti ad alto rischio, non sottovalutando, pertanto, il possibile comportamento aggressivo di alcuni tumori tiroidei che vanno adeguatamente identificati e trattati.

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## LIQUID BIOPSY IN TUMORS

U. Malapelle

*Department of Public Health, University of Naples Federico II*

The advent of predictive molecular pathology has led to multiple advances in the molecular characterization of many tumor

types. When cancers are diagnosed in the advantage stage, collection of tumor tissue sample is not always feasible, and circulating free DNA (cfDNA) extracted from liquid biopsy can be a real alternative. Gene mutations detection in cfDNA is currently used in clinical practice and provides important information for patient management. Among the most important clinical applications on liquid biopsy, monitoring baseline and resistance alterations and can be used to non-invasively track disease evolution. An important improvement in this setting can be obtained by using Next Generation Tecnologies, such as Next Generation Sequencing and Multiplex Code Barcodes Technology.

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# Sabato 20 ottobre 2018

Sala Nico 1 – 08:30 - 11:45

## PATOLOGIA TESSUTI MOLLI

## Novità in Patologia dei Tumori Mesenchimali

Moderatori: A.P. Dei Tois, A. Franchi

### GIST: OLTRE KIT E PDGFRA


R. Ricci

*Istituto di Anatomia Patologica, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "A Gemelli", Largo F.Vito 1, 00168, Roma*


I tumori stromali gastrointestinali (GIST) hanno rappresentato un paradigma della terapia molecolare mirata nei tumori solidi sin dalla scoperta, nel 1998, del ruolo patogenetico fondamentale giocato in queste neoplasie dalle mutazioni attivanti del KIT. Tale evento ha infatti permesso di ottenere successi terapeutici eclatanti utilizzando l'imatinib, molecola in grado di interferire con numerose isoforme mutanti del KIT. Pochi anni dopo è stato evidenziato il ruolo giocato dalle mutazioni attivanti di un'altra tirosin-kinasi, il recettore per il fattore di crescita derivato dalle piastrine di tipo alfa (PDGFRA), in buona parte dei GIST che non mostravano mutazioni del KIT, anche queste in parte sensibili all'imatinib. Era il 2003, e queste scoperte avevano globalmente evidenziato il motore molecolare alla base dell'oncogenesi di circa l'85% dei GIST, in buona parte dei casi con sensibilità alla terapia con imatinib. Scotomizzando il sesto dei GIST a patogenesi ancora ignota, i casi con mutazione attivante nota ma primariamente resistenti, ed ancora senza chiari dati sullo sviluppo della resistenza secondaria, il clima generale sull'approccio ai GIST appariva relativamente trionfalistico sulla base di una patogenesi molecolare apparentemente lineare e di risultati terapeutici relativamente sensazionali. I progressi della conoscenza hanno successivamente evidenziato varie altre cause molecolari nei GIST, costituite da difetti della neurofibromina, del BRAF, del complesso della succinatodeidrogenasi (SDH) e del RAS, in possibili contesti sindromici1. In parallelo, il progressivo accumulo dei dati di follow-up ha mostrato la resistenza secondaria che di norma insorge in casi inizialmente sensibili al glivec. Contestualmente, nella terapia dei GIST sono state studiate varie molecole con àmbiti di efficacia alternativi a quelli del glivec, alcune delle quali approvate per l'uso clinico. Infine, il diffuso utilizzo di tecniche di analisi molecolare ad ampio spettro ha recentemente mostrato il possibile riscontro nei GIST, con una prevalenza molto bassa, di svariati altri difetti molecolari, non sempre mutuamente esclusivi rispetto a quelli "canonici"2. In sintesi, una sempre più esigua frazione di GIST va apparentemente mostrando la possibile presenza di una sempre più ampia varietà di difetti molecolari possibili3. La complessa situazione che ne risulta richiede un'attenta valutazione, con la necessità di distinguere le alterazioni che rivestono un ruolo patogenetico e, in particolar modo, quelle attaccabili farmacologicamente in modo efficace, allo scopo di tradurre in progressi clinicamente significativi la sempre più abbondante messe di dati offerta dalla letteratura scientifica.

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### NUOVE ALTERAZIONI MOLECOLARI IN PATOLOGIA MESENCHIMALE


A. Righi


I tumori dei tessuti molli sono relativamente rari, ma sono diagnosticamente difficili in quanto comprendono un ampio spettro di entità diagnostiche. I progressi sostanziali che sono stati fatti negli ultimi anni con le nuove tecniche di biologia molecolare hanno consentito di identificare ulteriori alterazioni cromosomiche e genomiche ricorrenti in un sottogruppo significativo di tumori dei tessuti molli, consentendo altresì di iniziare a comprendere maggiormente i meccanismi biologici dello sviluppo e della progressione tumorale. L'integrazione di questi risultati negli algoritmi diagnostici routinari rappresenta una nuova sfida quotidiana per i patologi che si occupano di tumori dei tessuti molli, in quanto queste nuove conoscenze ottenute con le analisi molecolari hanno portato e presumibilmente porteranno alla ri-o sotto-classificazione di alcune entità diagnostiche con auspicabili miglioramenti nel trattamento dei singoli pazienti. Negli ultimi anni, le nuove conoscenze molecolari applicabili in ambito diagnostico hanno riguardato i tumori vascolari e adipocitari, i sarcomi indifferenziati a cellule rotonde e quelli miogenici e i tumori della guaine nervose periferiche. Alcuni dei meccanismi molecolari scoperti hanno consentito di mettere a punto anticorpi specifici per tali meccanismi migliorando notevolmente l'accuratezza e la riproducibilità diagnostica nei tumori dei tessuti molli. Un' analisi approfondita e critica dei risultati ottenuti con le analisi di biologia molecolare mediante una continua correlazione clinica, morfologica e immunoistochimica, è necessaria per riconoscere quali alterazioni geniche siano

specifiche di una determinata neoplasia e di conseguenza utili nella pratica diagnostica quotidiana.

# Sabato 20 ottobre 2018

Sala Nico 1 – 12:00 - 13:00

## DERMATOPATOLOGIA

## I Simulatori nella Patologia Cutanea

Moderatori: C. Clemente, G. De Rosa

### I SIMULATORI NELLA PATOLOGIA CUTANEA INFIAMMATORIA

A. Cassisa

*ASST-Mantova Ospedale Carlo Poma, Servizio di Anatomia Patologica*

L'approccio alla patologia delle lesioni infiammatorie cutanee attraverso la cosiddetta pattern analisi ha come limite il fatto che patologie del tutto differenti possono provocare modalità di infiammazione simile.
Lo stesso limite si pone se privilegiamo l'interpretazione di singoli dettagli morfologici fuori dal contesto generale.
La strategia per evitare il più possibile i pitfalls è di tenere sempre in considerazione la correlazione clinico-patologica.
A questo riguardo non è scontato che il patologo riceva tutte le informazioni necessarie per la risoluzione del caso ma se ha sufficiente esperienza nel campo della dermatopatologia infiammatoria, può diventare soggetto attivo nel suggerire al clinico gli approfondimenti clinici e laboratoristici necessari a confermare un'ipotesi diagnostica basata sulla sola interpretazione dei preparati istologici.
In questa breve trattazione vengono considerati alcuni esempi pratici riconducibili a quattro tipi di situazioni in cui entrano in gioco i "simulatori".
1 Sopravalutazione di un dettaglio istologico al di fuori del contesto generale.
È il caso in cui si osserva la presenza di elementi linfoidi morfologicamente atipici. L'interpretazione diagnostica può virare plausibilmente verso una patologia linfoproliferativa. Gli esempi di questo gruppo sono: lichen scleroatrofico con aspetti simil micosi fungoide, aspetti tipo micosi fungoide associati a regressione di lesioni neoplastiche, Ipercheratosi Nevoide del capezzolo con aspetti tipo micosi fungoide.
2 Pattern infiammatorio a basso ingrandimento fuorviante perché associato ad una patologia poco frequente. In questo gruppo consideriamo: la presentazione nodulare pseudolinfomatosa della sifilide, una forma PLEVA-like di sifilide, il granuloma annulare da Borrelia.
3 Mancata correlazione di un pattern infiammatorio ben definito alla patologia di base quando questa è conosciuta.
Esempi: exagerated insect bite-like reaction in corso di linfoma sistemico, istiocitosi cutanea associata a CLL, inflammatory oncotaxis.
4 Mancato riconoscimento di modificazioni cosiddette "invisibil".
Esempi: mid-dermal elastolysis, anetodermia.
Questi pochi esempi servono a capire che la pratica della dermatopatologia nel campo delle lesioni infiammatorie è particolarmente complessa e non permette di limitarsi al semplice riconoscimento di un pattern ma richiede di integrare informazioni complesse. Il primo step è sicuramente l'interpretazione corretta dei dettagli isto-morfologici. A questo devono seguire, almeno nei casi selezionati: lo studio dell'immunofenotipo, che serve a comprendere quali sono i players implicati nel processo infiammatorio, le informazioni cliniche, che non devono mai mancare, la comprensione dei meccanismi fisiopatologici che collegano il tutto.

# Sabato 20 ottobre 2018

Sala Nico 2 – 11:00 - 13:00

## PATOLOGIA MOLECOLARE E MEDICINA PREDITTIVA

## Applicazioni Cliniche delle Tecnologie Molecolari ad alta Processività

Moderatori: A. Marchetti, G. Stanta

### IL PATOLOGO NEL MONDO BRCA

A. Scarpa

*Anatomia Patologica, Università di Verona*

I notevoli progressi nella comprensione delle funzioni dei geni BRCA1 e BRCA2 hanno portato allo sviluppo di nuovi approcci terapeutici mirati ai tumori con perdita di funzione di questi geni. BRCA1/2 sono fondamentali per la riparazione del DNA mediante ricombinazione omologa (homologous recombination repair, HRR), che è coinvolta nella riparazione delle lesioni che coinvolgono ambedue i filamenti del DNA (double strand breaks, DSB).
BRCA2 lega direttamente la DNA ricombinasi RAD51 e la localizza sul DNA danneggiato; in assenza di BRCA2 l'HRR è compromessa. La localizzazione di RAD51 è compromessa anche nelle cellule mutanti BRCA1, ma questo accade indirettamente in quanto BRCA1 controlla i processi di trasduzione del segnale coinvolti nella HRR.
HRR è una forma conservativa di riparazione del DNA, in quanto ripristina la sequenza del DNA nella sua forma originale. In assenza di HRR le DSB vengono riparate utilizzando forme non conservative di riparazione del DNA (non-homologous end joining repair, NHEJ). NHEJ ripara le DSB con un processo semplice in cui le due estremità del DNA spezzate vengono unite senza utilizzare una sequenza di DNA omologa per guidare la riparazione e possono comportare l'introduzione di mutazioni, in particolare delezioni, del DNA. L'utilizzo del NHEJ in assenza di BRCA1 o BRCA2 provoca una caratteristica firma mutazionale (mutational signature) che consiste in un'elevata frequenza di delezioni del DNA fiancheggiate da brevi sequenze di DNA ripetute in tandem del nei punti di rottura della delezione.
Le cellule carenti di HHR, e in particolare di BRCA1 o BRCA2 sono sensibili a farmaci che bloccano la normale progressione delle forchette di replicazione e ne provocano il collasso, spesso causando DSB. I sali di platino (cisplatino o carboplatino) e la mitomicina C (MMC) causano legami crociati

covalenti all'interno della doppia elica del DNA che bloccano la progressione della forchetta di replicazione. Linibitore della topoisomerasi camptotecina e gli inibitori di PARP come olaparib bloccano anche la forcella di replicazione ma tramite un meccanismo diverso: ovvero, prevenendo il normale rilascio delle proteine di riparazione del DNA (topoisomerasi e PARP1, rispettivamente). Queste proteine di riparazione del DNA "intrappolate" probabilmente prevengono la progressione delle forchette di replicazione. In assenza della funzione BRCA, le cellule non riescono a riparare il danno al DNA causato da questi agenti e progrediscono verso una qualche forma di morte cellulare programmata o tentano di riparare le risultanti lesioni del DNA usando processi come NHEJ, che possono causare una grossolana instabilità genomica a livello che è incoerente con la vitalità cellulare. La risposta a farmaci dei tumori mutanti BRCA quali i sali di platino e olaparib possono essere bloccate da eventi che inducono resistenza secondaria con ripristino delle funzioni HRR, quali mutazioni secondarie in BRCA1/2, l'inattivazione di 53BP1 o di MAD2L2. Lo spettro dei tumori che presentano inattivazione di BRCA e quindi sensibilità a farmaci peculiari comprendono carcinomi della mammella, particolarmente i basal-like e tripli negativi, dell'ovaio, del pancreas, e della prostata resistenti alla castrazione. I tumori con inattivazione di BRCA hanno alcune caratteristiche molecolari in comune, sono caratterizzati da estrema instabilità genomica e presentano frequentemente mutazioni di TP53 e amplificazione di MYC. Essi hanno anche alcune caratteristiche morfologiche relativamente comuni quali la presenza di infiltrato linfocitario. Il patologo è e sarà chiamato sempre più spesso a rispondere alle esigenze di caratterizzazione morfologica di queste neoplasie oltre che a fornire il materiale più adeguato ai fini della caratterizzazione molecolare.

## PHARMACOLOGICAL RESISTANCE EVALUATION BY USING NEXT GENERATION SEQUENCING

U. Malapelle

*Department of Public Health, University of Naples Federico II*

Next Generation Sequencing (NGS) represent a pivotal technology that can allows simultaneously the evaluation of multiple gentic alterations by using a small amount of DNA. Whole clinical exome assessment and target re – sequencing of clinical relevant cancer genes represents the approach best suited to monitor developing of resistence to target treatment in clinical practice. The traslation of NGS in clinical setting expected to increase the diagnostic performance of resistance mutations to better define the therapeutic option for cancer patients in the lendscape of personalized medicine.

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# I Marcatori Molecolari dell'Immunoterapia

Moderatori: G. Tallini, G. Troncone

## THE MICROSATELLITES

T. Venesio

*Anatomia Patologica, Laboratorio Patologia Molecolare, FPO-IRCCS, Candiolo*

In human genome there are more than 100,000 short tandem repeat (STR) sequences named microsatellites (mono-, di-, tri, or tetranucleotide repeats) that, being prone to "slippage" during DNA replication, require a highly proficient DNA repair system for their integrity. The DNA repair system in charge of this task is the Mismatch Repair pathway (MMR). Alterations affecting four key proteins of this pathway—MLH1, MSH2, MSH6, and PMS2—were identified as the causative germline mutations responsible for Lynch syndrome in 1993-1996. In normal DNA replication with proficient MMR, small DNA errors are initially detected and bound by MSH2/MSH6 and MSH2/MSH3 heterodimers. MLH1/PMS2 heterodimers are subsequently recruited for excision and synthesis of the corrected new strand. Deficiency in MMR proteins causes the accumulation of mutations at the microsatellites, leading to the onset of the so-called microsatellite instability (MSI).
This process results in the further accumulation of DNA alterations inactivating several proteins necessary for cell homeostasis and thus facilitating carcinogenesis. Patients affected by germline mutations in MMR genes develop specific tumor types, mainly nonpolyposis colon and endometrial cancers. However, sporadic somatic mutations in the MMR pathway can also be found in tumors unrelated to the hereditary Lynch syndrome and often due to the MLH1 inactivation by methylation.
Two forms of testing are commonly used in screening tumors for a deficiency in MMR or MSI: immunohistochemical staining (IHC) for altered proteins and polymerase chain reaction (PCR) testing for MSI. IHC, which evaluate the presence of the MMR proteins, is inexpensive and readily available at most institutions, but it may miss abnormalities caused by some mutations leading to qualitative changes in the tested proteins (i.e.missense mutations). MSI testing by PCR is considered the gold standard as it provides a good reproducibility measure of functional MMR activity, allow the identification of abnormalities due to different mutation types in the MMR
proteins, and the MSI caused by other defects. In 1998, the National Cancer Institute (NCI) proposed a standardized microsatellite panel for MSI testing including five microsatellite markers: two mononucleotide repeats (BAT25 and BAT26) and three dinucleotide repeats (D2S123, D5S346, and D17S250). Tumors with MSI at more than 30%–40% of sites are considered MSI-H, those with less than 30%–40% mutations are considered MSI-L, and those without instability are micro satellite stable (MSS). More recently, the updated NCI guidelines has suggested the adoption of a new panel of five mononucleotide markers (BAT 25, BAT26, NR21, and NR24) on the base of its improved sensitivity and specificity in respect to the old one. Multiple next-generation sequencing
platforms are actually being explored to improve the detection of MSI across the whole genome, with some platforms

showing concordance with traditional PCR testing. Overall, reports on MSI-H frequency have varied greatly due to the lack of standardized test, but so far the higher percentages have been reported in endometrial, gastric, and colon cancer. MSI tumors are known to trigger a strong immune response in the host because of their higher mutational load (TMB high), as shown by next-generation sequencing studies reporting that these cancers harbor more than 1,000 coding somatic mutations per tumor cell genome compared with the 50 to 100 somatic mutations found in MSS tumors. The impaired MMR results in frame shifting mutations giving rise to inactive proteins that can be presented through the tumor's MHC I to cytotoxic T-lymphocytes (CTLs) as neoantigens, with an immunoediting process regulated through a series of checkpoint receptors, including programmed death 1 pathway (including PD-1 and its ligand PD-L1). Accordingly, MSI tumours have shown a durable response to PD-1 blockade. These evidences support MSI status as a helpful pan-cancer biomarker predictive for response to immunotherapy.

# Sabato 20 ottobre 2018

Sala Cigno – 08:30 - 12:00

## PATOLOGIA OLTRE FRONTIERA

## Il Patologo Reale

Moderatori: D. Fenocchio, L. Viberti

### COSTRUIRE IL FUTURO: L'ASSISTENTE PATOLOGO

E. Stigliano

Il laboratorio di Anatomia, Istologia e Citologia Patologica (Sala Autoptica compresa) è ormai da considerarsi un laboratorio di alta specializzazione, infatti al Professionista Sanitario sia Medico che Biologo/Biotecnologo sono richieste le relative specializzazioni in Anatomia Patologica o Patologia Clinica.
Al Tecnico Sanitario di Laboratorio Biomedico (TSLB) operante in Anatomia Patologica è richiesta la sola laurea triennale o titoli equipollenti.
Alla luce dei nuovi modelli sanitari regionali "Hub e Spoke", con l'applicazione di avanzate tecnologie informatiche e telematiche in ambito sanitario (Telapatologia) e soprattutto per uniformarci ai modelli europei/internazionali, sia in termini di professionalità, competenze, formazione e responsabilità, sarebbe auspicabile che anche il TSLB possa diventare un " Tecnico di laboratorio Specialista in Anatomia Patologica", attraverso un percorso formativo universitario post-lauream (Master, Laurea Specialistica, altro), designando quindi un nuovo profilo professionale sanitario, l' Assistente Patologo.
Nel 2001 presso l'Università Cattolica del Sacro Cuore di Roma, per iniziativa del sottoscritto, è stato istituito il primo Master Universitario di primo livello in " Tecnica e diagnostica delle autopsie e procedure istopatologiche" con l'obiettivo di divulgare questa disciplina attraverso la conoscenza, garantire quindi una formazione specifica e soprattutto elevare le competenze del Tecnico Sanitario di Laboratorio Biomedico nell'ambito dell'Anatomia Patologica Autoptica e Istopatologica, rapportandola alla figura dell' Assistente Patologo, figura già nei paesi anglosassoni.
Da diversi anni anche APOF-ONG propone nell'ambito dei Progetti di Cooperazione Internazionale la figura dell'Assistente Patologo sia nell'organizzazione del laboratorio che nel fornire supporto ai medici anatomopatologi attraverso formazione specifica del personale tecnico locale sull'intero processo lavorativo circa le procedure, dalla riduzione/campionamento macroscopico (Gross Pathology) alla digitalizzazione dei preparati e relativo invio via web (Digital Pathology).

### CYTOLOGY-BASED CERVICAL CANCER SCREENING IN DEMOCRATIC REPUBLIC OF THE CONGO: REPORT AND ANALYSIS OF FOUR YEARS OF ACTIVITY

M.E. Laurenti[1 6], S. Guzzetti [2 6], D. Fenocchio [3 6], L. Viberti[4 6], A. Kakule Musafiri [5 6], G.M. Corbetta[5 6], S. Arnaud[6]

*[1] Dipartimento di Medicina Sperimentale - Sezione di Anatomia e Istologia Patologica - Università degli Studi di Perugia; [2] S.C. di Anatomia Patologica, ASL Città di Torino, Torino, Italy; [3] Citologia e Istologia Diagnostica Ospedale Santa Maria della Misericordia di Perugia; [4] Anatomia Patologica IRCSS Candiolo, Torino; [5] Anoalite Hospital, Mungbere, RDC; [6] Associazione Patologi Oltre Frontiera (APOF, Pathologists Beyond Borders), NGO*

**Background**
Cervical cancer (CC) is a major health problem in low resource settings and Sub-Sahara Africa is the region with the highest incidence of cervical cancer worldwide. There were an estimated 266,000 deaths from CC worldwide in 2012, of which almost nine out of ten (87%) occurred in less developed regions [1].
In the late 20th century, developed countries achieved considerable reduction in CC incidence and mortality through systematic use of cytology-based screening programs However the paucity of human and economic resources and the poor reliability of public health systems have often prevented the development of such model of preventive medicine in low and middle-income countries (LMICs) [2].
CC is a potentially preventable disease and can be entitled to primary prevention with vaccination, to secondary prevention with different screening methods and to tertiary prevention by early diagnosis and treatment of invasive forms [3].
In low resources settings, WHO recommends a "screen and treat" approach with VIA (visual inspection with acetic acid), VILI (visual inspection with Lugol iodine) or HPV-DNA test followed by an ablative technique (like cryotherapy), without colposcopic or histopathologic confirmation; this strategy minimize the number of visits and allow more women to complete the treatment. In fact, many women cannot afford to travel to health facilities multiple times and it is very difficult to track them and recall those who need further diagnostic procedures [2].
Effective cytology-based screening programs are difficult to set up in resource constrained areas, mostly because the lack of human resources, proper education and training and organized services; thus, many studies focused that could be more cost-effective to provide a screen and treat approach with a maximum of two to three visits lifetime starting at the age of 35 years [4].
Even though this policy seems to have streamlined the layout of cervical cancer screening in LMICs, they do not completely overcome the need for structured health services and health information systems, continually training and updates for

health workers at every level and sustainability of cryotherapy in primary care settings.

**Materials and nethod**

The Italian NGO "Associazione Patologi Oltre Frontiera" APOF launched a project for the establishment of a pathology lab at the Anoalite Hospital in Mungbere, a town located in the North-East region of Democratic Republic of the Congo, and provided the whole equipment, a comprehensive specific training for technicians (including cytopathology diagnostic) and introduced a telepathology service to assist cytological diagnosis and to provide for the histological ones through a whole slide scanner. One aim of this project was to set up a cytology-based cervical cancer screening program, where cytotechnicians were called to send out negative cases by themselves and to identify any suspect smear (a role defined as cytoscreener) to be further diagnosed through telepathology. Being them trained cytotechnicians, a simple and low-cost telepathology system was applied: they took photomicrographs of remarkable areas of the specimen at different magnification levels and uploaded image files in the management information system. Then, a group of Italian pathologists examined the images and posed the definitive diagnosis. Finally, each year whole slides of all positive cases and at least 5% of randomly negative cases are reassessed to perform an internal quality assurance control.

We analysed data regarding the last four years of activity, from 2014 to 2017, in order to assess the correlations between the first diagnosis proposed by cytoscreeners (≥ASCUS), the final diagnosis delivered by static images-based telepathology and the whole slide review diagnosis. We used two different statistics, namely Cohen's kappa (κ) and Gwet's AC1.

Results. The total number of Pap-smear was 10.039; 13 cases missed complete information and were excluded. Negative diagnosis accounted for 95,3% while five hundred forty-one Pap smears were considered abnormal by cytoscreeners and thus sent for definitive diagnosis thorough telepathology; 85% of these (460 cases) were diagnosed ad ≥ASCUS, with 1,9% of high-grade lesions (189 cases). The concordance between cytoscreeners diagnosis and final diagnosis made through telepathology was 0,61 with Cohen's κ and 0,73 with Gwet's AC1, while the telepathology diagnosis vs. whole slide review showed a κ value of 0,88 and an AC1 of 0,91. Histologic confirmation of 119 cases out of 189 high grade lesions revealed a very high sensibility 94,6% but poor specificity (34,6%), with high positive predictive value (CIN1+) of 83,8%.

**Conclusion**

These results show that cytoscreeners can select useful fields of the slide to demonstrate cellular alterations, even if they are often unable to assign the proper grade of dysplasia; the remote-working pathologist can pose the right diagnosis in almost all cases, as demonstrated by the high concordance with whole slide review. The use of telepathology is effective in this context but essential improvements are needed to face other issues, especially regarding colposcopic confirmation of high grade cases; further treatment of patients should be arranged with a minimum gap between first diagnosis and secondary procedures, and as conveniently as possible for the women, to reduce loss to treatment. Since continuous training, quality control and supportive supervision would be necessary in any case (even in primary screening visual approach like VIA or VILI) we find more useful and ethical to improve cytology-related skills in this context. Because a pathology lab already exists and a reliable diagnostic is available, is thus possible to avoid overtreatment of women and, possibly, to keep Pap smear as triage whenever an affordable, easy HPV-DNA test will be available [5].

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## SLIDE SEMINAR OF AFRICAN CASES: EXTRA-VAGINAL CYTOLOGY


S. De Crescenzo

*Resident pathologist, Department of Experimental Medicine, Section of Pathologic Anatomy and Histology - "S. Maria della Misericordia" Hospital, Perugia (Italy)*


**Materials and methods**

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

In this hospital, a telepathology service to assist cytological diagnosis and to provide histological ones has been introduced.

The director, Dr. Gian Maria Corbetta, in collaboration with a NGO called "Pathologists Beyond Borders Association, (APOF)", planned a comprehensive specific training course for cytotechnicians (including cytopathology diagnostic) to prepare cytological specimens, identify more significant regions of interest (ROI) for pathological diagnosis and get static digital images of these areas of slide, which are stored in a web platform, called Sinapto, in addition to further clinical data of every patient. Then, an Italian volunteer pathologist who have access to the network can view the images and pose the final diagnosis in the report.

However cytological examination is not always sufficient for a proper diagnosis, and an histopathological study can be necessary. Similarly to what is done through Sinapto, histological samples are scanned and then stored in a cloud storage (Dropbox®), with the advantage of a dinamic image, therefore not as static as the cytological one.

In this way, a final shared diagnosis between pathologists can be formulated.

**Results**

The casuistry provided in a period of 48 months (2014 – 2017) consists of 291 cases of extravaginal cytology, allocated as follows: 40 cases of imprinting cytology, 77 cases of exfoliative cytology and 174 cases of fine needle aspiration cytology.

**Discussion**

The aim of our slide seminar is to show static images of some

interesting African cases and demonstrate the potential of telepathology in cytological and histological diagnosis, providing rapidly a diagnostic possibility to those centers with few resources, in order to improve patient care in Developing Countries.

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# Sabato 20 ottobre 2018

Sala Orione – 08:30 - 13:00

## AITIC

## Citologia Ginecologica e Screening

Moderatori: M. Iacobellis, M. Russo

### CITODIAGNOSTICA DA SCREENING HPV CORRELATA SU CAMPIONI LBC CERVICO VAGINALI

F. Pagano

*ARCHIMED srl. Busto Arsizio (VA)*

Lo striscio cervico vaginale o anche Pap test ha ormai oltre 50 anni utilizzo, questo metodo semplice e poco costoso ha portato l'incidenza del cancro alla cervice uterina, nelle donne che si sottopongono con cadenza triennale a questo esame, ad una drastica riduzione.

Le donne tra i 25 e i 64 anni secondo le Linee Guida devono effettuare un Pap test ogni 3 anni, che o viene ridotto se si riscontrano condizioni di rischio o nel caso di lesioni preneoplastiche.

L'esame "tradizionale" consiste nel prelevare cellule con spatolina del collo uterino cercando di raggiungere la giunzione squamo colonnare maggior sede delle alterazioni, strisciare il materiale ottenuto su vetro e fissarlo con spray, successivamente colorate e valutate a microscopio nei laboratori.

Questo tipo di esame è definito come "esame citologico" e serve ad evidenziare la presenza di alterazioni neoplastiche o pre-neoplastiche, che permettono al clinico di adottare misure terapeutiche sia per prevenire che la malattia si verifichi, ma anche a curarle una volta diagnosticate.

Questa tecnica di prelievo può presentare delle criticità, circa il 50% delle cellule prelevate tendono a rimanere adese alla spatola e non si trasferiscono sul vetro di lettura, ma anche ammassi di quantità di materiale che tende ad addensarsi in parti del vetri rende non possibile la lettura.

Per ottenere vetri sempre più leggibili, si è sviluppato da qualche anno un nuovo metodo di conservazione, allestimento e lettura dei vetri denominato "citologia in fase liquida" e più specificamente Pap in fase liquida.

Questa metodica permette una maggiore rappresentatività cellulare, una migliore conservazione del materiale e la possiblità di inserire il materiale prelevato in un triage.

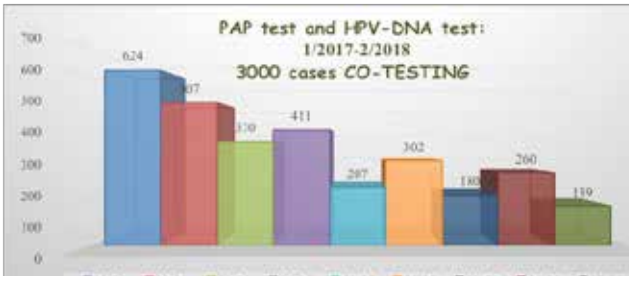
Liquide Based Citology o LBC (citologia in fase liquida) utilizza nuove tecnologie, rispetto alla citologia convenzionale, perché trasferisce all'interno di un contenitore apposito, che contiene liquidi fissativi, le cellule prelevate nella loro quasi totalità, migliorando la loro morfologia in lettura e permettendo il triage del materiale.

I metodi meccanici per allestire campione dal LBC sono molteplici, ma tutti tendono ad eliminare tutto ciò che può interferire con la diagnosi come muco, detriti cellulari e sangue, ed alcune di queste tecniche LBC, hanno ricevuto l'approvazione FDA (Food and Drug Administration).

Le differenze che si possono notare tra materiale cervico vaginale allestito in maniera tradizionale e campioni ottenuti da LBC mostrano:

- una riduzione del numero di campioni citologici cervico vaginali non valutabili e quindi inadeguati
- aumentata sensibilità diagnostica riducendo i casi sospetti che possono allertare immotivatamente la paziente
- una maggiore rappresentatività cellulare del materiale cervico vaginale prelevato
- una migliore visualizzazione delle cellule endocevicali ed endometriali
- automazione del processo preanilitico che permette una riproducibilità del risultato in merito ai vetri allestiti
- un incremento significativo nell'individuazione delle lesioni squamose intraepiteliali a basso e alto grado
- utilizzare il materiale cellulare, contenuto nella fiala per possibili test molecolari aggiuntivi come la ricerca di HPV DNA o RNA, permettendo un inutile secondo prelievo alla paziente.

Casi co-testing




| | | | |
|---|---|---|---|
| HPV DNA neg | PAP test neg | 2240 | 74,67 |
| HPV DNA pos | PAP test pos | 560 | 18,67 |
| HPV DNA pos | PAP test neg | 162 | 5,40 |
| HPV DNA neg | Pap test pos | 38 | 1,26 |

| CYTOLOGICAL DIAGNOSES hr-HPV neg/ PAP test pos (Total = 38 CASES (1,26%, 95% CI:0.92-1.74) | | |
|---|---|---|
| HSIL | 1 | 2,64 |
| LSIL | 10 | 26,31 |
| ASCUS | 27 | 71,05 |

## Immunoistochimica

Moderatori: M. Carlucci, D. Loisi

### INSTABILITÀ DEI MICRO SATELLITI (MSI-HIGH O LOW) E TUMOR MUTATIONAL BURDEN NEL CARCINOMA DEL COLON-RETTO: NON SOLO LYNCH SYNDROME I

S. Trubini, A. Ubiali

*Laboratorio di Biologia Molecolare della U.O. Anatomia Patologica – AUSL Piacenza*

L'instabilità dei microsatelliti (MSI) è un'alterazione molecolare che comporta mutazioni a livello di porzioni di microsatelliti. I microsatelliti sono tratti di DNA composti da motivi ripetuti che si presentano come alleli di lunghezze variabili. L'MSI può derivare da mutazioni ereditarie o originarsi somaticamente. Nello specifico, la sindrome di Lynch deriva da mutazioni ereditarie di geni del sistema di riparazione del DNA (MisMatch Repair genes, MMR). I tumori sono classificati come "MMR defective" (dMMR) se presentano mutazioni somatiche o germinali in questo sistema. L'MSI si trova più comunemente nei tumori del colon (15% del totale) e dell'endometrio; sono queste le neoplasie maggiormente associate alla sindrome di Lynch. Tuttavia, recenti analisi hanno rilevato MSI in ben 24 tipi di cancro, suggerendo che è un fenotipo tumorale ricorrente.

L'MSI è associato ad una migliore prognosi di malattia, ma fino al recente avvento di inibitori del checkpoint immunitario (immunoterapici), l'uso predittivo dei test MSI era limitato. Uno studio proof-of-concept comprendente 87 pazienti con 12 diversi tipi di cancro ha dimostrato il valore predittivo dello stato di MSI per la risposta dei tumori solidi ad un agente anti-PD-1. La predittività di MSI ha portato alla prima approvazione di questa categoria farmacologica da parte della FDA a maggio 2017. Ulteriori test hanno mostrato una risposta migliorata per i pazienti con elevata instabilità dei microsatelliti (MSI-high, MSI-H) a vari agenti anti-PD-1. Questi risultati elevano lo status di MSI come terzo biomarcatore predittivo, realmente indipendente, per inibitori del checkpoint immunitario, insieme a PD-L1 ed al carico mutazionale del tumore (tumor mutational burden, TMB) [1]. La stima del TMB dal profilo genomico completo è stata dimostrata come legata alla risposta ai farmaci immunoterapici nel melanoma metastatico, nel carcinoma della vescica uroteliale e nel carcinoma polmonare non a piccole cellule. Dato che le risposte dei pazienti a questi farmaci possono essere estremamente durature, è fondamentale identificare il maggior numero possibile di potenziali responder, utilizzando sia l'immunoistochimica per PD-L1, sia il TMB, sia infine la stima dell'instabilità microsatellitare. Il numero totale di casi di CRC che potrebbero trarre beneficio dall'immunoterapia, infatti, potrebbe potenzialmente essere significativamente esteso se TMB fosse usato per prendere decisioni relative al trattamento immunoterapico, piuttosto che lo stato MSI da solo.

Attualmente, a livello biomolecolare l'MSI è più comunemente rilevato attraverso la reazione a catena della polimerasi (PCR) mediante analisi del frammento (FA) di cinque regioni satelliti conservate. Questo è considerato il gold standard per il rilevamento genico della MSI. La FA tuttavia presenta alcune criticità. Innanzitutto, non è sempre possibile per i casi con quantità limitate di tessuto, inclusa l'analisi delle metastasi del cancro, che vengono comunemente presentate come biopsie e possono contenere poche cellule normali. Inoltre, richiede campioni sia di tumore che di tessuto normale. Infine, la determinazione dell'MSI mediante analisi FA e MMR da immunoistochimica (IHC) viene eseguita come test standalone ed è dispendioso in termini di tempo e denaro eseguirla su tutti i pazienti con cancro, dato che l'incidenza di MSI è solo del 5% circa tra le diverse neoplasie. Il sequenziamento di nuova generazione (Next Generation Sequencing, NGS) in futuro potrà rappresentare un'alternativa valida per il superamento di queste problematiche [2].

Al momento, visti i costi economici e tempistici delle tecniche biomolecolari, nella maggior parte dei centri viene precedentemente eseguita una determinazione upfront con test immunoistochimici (IHC) per la valutazione dell'espressione delle principali proteine coinvolte nella riparazione del DNA. Utilizzando IHC per MLH1, PMS2, MSH2 e MSH6, è possibile determinare lo stato MMR del tumore. La rilevazione di tutte e quattro le proteine nel tumore indica uno stato di mismatch repair normale. La perdita dell'espressione di MLH1 è quasi sempre accompagnata dalla perdita del suo partner eterodimerico, PMS2. L'uso di IHC per la rilevazione delle proteine PMS2, MSH2 e MSH6 è l'indicatore più diretto dello stato di mutazione della linea germinale. La perdita di PMS2, in presenza dell'espressione MLH1, o della perdita dell'espressione MSH2 o MSH6 designa il tumore come dMMR e può indicare sindrome di Lynch. Tutti gli individui con sospetta sindrome di Lynch devono essere rimandati a una consulenza genetica. In generale, comunque, tutti i casi con negatività per qualsiasi proteina nel nostro centro sono rinviati alla biologia molecolare.

**References**

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# Sabato 20 ottobre 2018

Glass House – 08:30 - 10:20

## PALEOPATOLOGIA

## Patologia Cardiovascolare nell'Antichità

Moderatori: G. Fornaciari, L. Ventura

### ATHEROSCLEROSIS IN THE ITALIAN MUMMIES (15TH-20TH CENTURY)

R. Gaeta[1], L. Ventura[2], G. Fornaciari[1]

*[1] Division of Paleopathology, Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Italy; [2] San Salvatore Hospital, Division of Pathology, L'Aquila, Italy.*

**Background**

Atherosclerosis, disease in which the lumen of an artery narrows up to occlusion caused by buildup of plaque composed

of fatty material on the inner walls, is one of the most common pathology among the developed countries and every year thousands of people die by the complications of the atherosclerotic disease (in Central Europe: 201 man and 117 women per 100,000 inhabitants per year [1]). Due to its connection with the dietary habits, the disease had been considered peculiar of the modern sedentary and well-nourished society, with diet characterized by a rich intake of meat, sugar and fat. However, the presence of atherosclerosis in mummies of different temporal horizons and different geographic contexts [2]]suggests the potential that other risk factors or causes could result in atherosclerosis.

**Materials and methods**

We report the results of the macroscopic and histologic studies performed on six Italian mummies from different regions and belonging to a wide range of time. The oldest subject is Oetzi, the so-called Iceman, a natural mummy of the Copper Age (3300-3100 BC) whose atherosclerotic disease has also been investigated from a genetic point of view [3]. Ferrante I of Aragon, king of Naples (1423-1494 CE) suffered the consequences of a excessive and unregulated diet [4]. Girolamo Macchi (1649-1734 CE), Major Writer of Santa Maria della Scala Hospital of Siena, died at an advanced age and evident traces of atherosclerotic calcifications are present along the abdominal aorta. Two mummies with atherosclerosis have been found in Comiso, Sicily, and belonged to two subjects who lived in the 18th and 19th centuries and died respectively at 50 and 30 years. Finally, the most recent mummy (20th century) comes from Abruzzo and belonged to an unidentified individual of the poor class.

**Discussion**

The problem of atherosclerosis in Antiquity is a topic that has aroused much debate in recent times. Besides being a fascinating field of research, it has an important impact on modern medicine since atherosclerosis is among the most widespread pathologies in Western populations.

The six investigated mummies have unequivocal findings attributable to atherosclerosis in varying degrees of severity. In some cases, the diagnosis was macroscopic, while for some mummies it was possible to perform histologic analyses of the arterial vessels.

**Conclusions**

These cases confirm that atherosclerosis is also a disease of ancient times. The presence of atherosclerosis in pre-contemporary individuals could suggest that the disease may not only be uniquely characteristic of a specific diet or lifestyle, but it could be also an inherent component of human ageing. Additional future surveys will help to clarify the history of this disease.

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## CARDIOVASCULAR DISEASES IN PATHOLOGY MUSEUM.


L. Ferrari

*SC Anatomia Patologica Ospedale Cardinal Massaia Asti*


**Introduction**

Since antiquity the heart was a major organ, due to cultural and religious reasons. The ancient Egyptians believed that the heart was the seat of though and the witness of the moral fitness during the judgment of Osiris and therefore it would still needed in the afterlife. The heart was the only organ left behind during mummification. The Holy Bible mentions the heart almost 1000 times as the core of the being, but the preservation of the heart after the death was forbidden. To Christians the heart was a relevant relic and it was often preserved after the death of a saint, so as St.Gregorio Barbarigo. A very particular relic of heart is the “Miracle of Lanciano”. The hearts of the Kings or of relevant persons were preserved too, often as dry specimens so as the heart of Prince Eugene of Savoy and sometimes as wet specimens, so as the heart of Napoleon Bonaparte or that of Fryderyk Chopin. In Europe there are more than 700 famous preserved hearts, but none of them was collected for scientific purpose. Thanks to Giovanni Battista Morgagni, the father of modern anatomical pathology, the knowledge of anatomical conditions became essential to understand the basis of diseases. This new importance of the pathological organs allowed the born of Pathology Museums.

**Material**

Historically the primary function of Pathology Museums was to show specimens for didactic purpose. The oldest specimens were wax-works, which were artificial models, but later the preservation of real part of the body was allowed thanks to the use of chemical substances so as sublimate which avoid the post-mortem processes. The results of this technique were dry specimens. Many dry specimens of cardiovascular pathology belong to Pathology Museum in Italy, they are especially dry specimens of aortic aneurisms, so as for example the specimens of Pathology Collection of Turin (Fig. 1). But this technique was very dangerous for the health of the anatomic preparers therefore the wet specimens were preferred, especially after the discovery of formol. The old wet specimens stored in Pathology Museums show often the natural history of many diseases (Fig. 2) since most of the Pathology Collections in Italy are historical collections dating back to the XIX and the XX century. They are some modern pathology collections so as these of University of Roma and University of Padua: to the last collection belong more than 7000 modern specimens.

**Methods**

The dry specimens are suitable for the study with modern technique also without rehydration. The wet specimens are suitable for the study with modern techniques so as immunohistochemistry, if the fluid allowed the preservation of antigens.

**Results**

The dry and wet specimens of Pathology Museums are an irreplaceable biological storage of old disease often disappeared thanks to the modern therapies since their preservation is not only morphological for didactic purpose but often biological for modern research.

**Conclusions**

These collections are relevant for history of medicine and


are a field of modern research for pathology as well. Therefore their preservation is mandatory.

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## PELVIC PHLEBOLITHS. IMPORTANCE OF AN UNDER-REPORTED FINDING IN PALEOPATHOLOGY

L. Ventura

*U. O. C. di Anatomia ed Istologia Patologica, Ospedale San Salvatore, L'Aquila, Italy*

### Introduction

Phleboliths are venous calcifications representing the end product of thrombosis. They are frequently seen within the pelvis, in the veins around bladder, prostate, uterus and rectum, but also in supra-pelvic organs and vascular tumors. Sometimes they may be difficult to differentiate from distal ureteral stones on radiographs, but pelvic phleboliths are generally considered of no clinical significance. For this reason they are actually neglected in modern radiology and histopathology reporting. They are common in people from economically developed countries, representing a marker of western pattern of diseases. Their presence in mummies has been described only incidentally in literature.

### Methods

Aim of this study is to report phleboliths in 3 italian natural mummies, and evaluate their significance in understanding biopathologic features of the subjects. The small series included a well-preserved female mummy from Goriano Valli (inner Abruzzo region) belonged to a XX century woman (Fig. 1), a well-preserved natural female mummy from Scicli (south-eastern Sicily) dating back to XX century (Fig. 2), a well-preserved natural male mummy from Modica (south-eastern Sicily) dating back to the end of XVIII century (Fig. 3). All of them underwent radiology and computed tomography (CT) scanning and in the first subject histology was performed using autopsy samples.

### Results

The age at death of the individuals was in the range 50-80

Figure 1.

Figure 2.

Figure 3.

Figure 4.

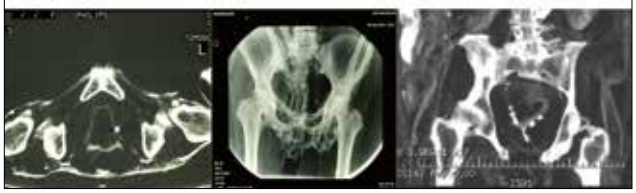
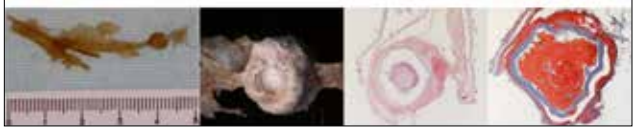


Figure 5.



years. Two of them showed evidence of high social class/good nutritional status, whereas the mummy from Goriano had a pelvic mass of possible ovarian origin. Phleboliths were observed near the walls of pelvic organs (Fig. 4). In the first case autopsy allowed close inspection of the nodules, showing their classic concentric calcification pattern at microscopy (Fig. 5).

**Conclusions**

These findings suggest that phleboliths are not an uncommon finding in mummies, as they may be easily identified during radiology or CT scanning. They share pathogenesis and location with their modern counterparts, and can be easily distinguished from ureteral calculi or calcified lymph nodes by radiology, CT, and histology. Their search should be improved in skeletal material as they represent a useful marker of age at death, social status, and a clue to the respective diseases.

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# Giovedì, 18 ottobre 2018

Sala Roof Garden – 17:00 - 19:00

## CITOPATOLOGIA

### MALIGNANCY RISK AND HISTOLOGICAL CORRELATION OF BREAST FINE- NEEDLE ASPIRATION CLASSIFIED AS ATYPICAL (C3). A RETROSPECTIVE STUDY ON 499 CASES

F. De Rosa, A. Gencarelli, E. Clery, I. Migliatico, E. Vigliar, G. Troncone, C. Bellevicine

*Dipartimento di Sanità Pubblica, Università degli Studi di Napoli "Federico II"*

**Background.** Fine needle aspiration (FNA) of clinically and/or radiologicallysuspicious breast masseshasrepresentedthemostimportanttooltoreachadiagnosisandguidethefurthermanagement of these patients. However, in the last years, the use of core needle biopsy has become increasingly frequent due to different reasons. 1) A trained on-site cytopathologist, who ensure the correctness of both the aspirative and smearing technique lowering the rates of inadequate samples, is not always available. 2) Unlike the Bethesda System for thyroid or the recent Milan System for the reporting of salivaryglandcytology,aninternationalstandardized reportingsystemforbreastFNAisstilllacking. This result in a poor reproducibility among different cytopathologist cause incomprehension between cytopathologist and clinician, and may lead to an inappropriate treatment of the patients.To overcome these issues, the International Academy of Cytology (IAC) have recently proposed during the International Yokohama Congress of 2016, the implementation of a five-tiered reporting system, 1 based on the previous system from the Australian National Mammographic Screening Pathology Q Group (1993) and NCI (1996). Unfortunately, as in other cytopathology classification systems, the "atypical" (C3) diagnostic class represent a "gray zone" with variable malignancy risk. In our Institution we have adopted since 2010 a classification system similar to those proposed by the NCI in1996,whichinturnhasrepresentedtogetherwiththeAustralianscheme,thebasisofthereporting system now recommended by the IAC. In order to clarify the histological basis and the malignancy risk of the "atypical" (C3) diagnoses, we have retrieved from our files the cytopathology reports of breast FNA signed-out from 2010 to 2017, closely investigating those with a matched histopathological diagnosisavailable.

**Materials and Methods.** The laboratory information database of the study institution was searched to obtain the diagnostic reports recorded in the system from January 2010 to December 2017 related to breast FNA classified as "C3". During the study period, an overall of 4625 breast FNA were performed, including 499 (10.7%) C3. Of these latter, 289/499 (57.9%) had a matched histology available. Basing on the histopathology follow-up, the over all malignancy risk of the C3 diagnosis was determined with a careful analysis of the histological outcomes.

**Results.** Considering the 289 C3 FNA with an available histology, 213/289 (73.70%) cases showed a benign histological diagnosis, whereas 76/289 (26.30%) resulted in a malignant outcome (Tab. I). Of these latter, the majority (46/76, 60.5%) were either usual ductal (36/46, 78.2%)orlobular(10/46,21.7%)

Table I. Histological correlation of the breast FNA classified as C3, from 2010 to 2017. In the last column, thefrequencyofthehistologicaldiagnosisamongallC3FNAsandamongeitherbenignormalignant diagnoses(parenthesis).

| | Histology | N. of cases | % |
|---|---|---|---|
| C3 FNAs with matched histology | | 289 | 57,92 of total C3 FNAs |
| Benign lesions | | 213 | 73,70 |
| | Fibroadenomas | 86 | 29,76 (40,38 of benign) |
| | Fibrocystic breast disease | 60 | 20,76 (28,17) |
| | Fibrocystic breast disease with atypia | 3 | 1,04 (1,41) |
| | PASH | 3 | 1,04 (1,41) |
| | Intraductal papillomatosis | 16 | 5,54 (7,51) |
| | Intraductal papillomatosis with atypia | 1 | 0,35 (0,47) |
| | Benign Phyllodes Tumours | 20 | 6,92 (9,39) |
| | Myofibroblastoma | 1 | 0,35 (0,47) |
| | Usual ductal hyperplasia | 3 | 0,35 (1,41) |
| | Atypical ductal hyperplasia | 5 | 1,04 (2,35) |
| | Reactive changes | 3 | 1,03 (1,41) |
| | Steatonecrosis | 4 | 1,38 (1,88) |
| | Normal breast parenchyma | 8 | 2,77 (3,76) |
| Malignant tumors | | 76 | 26,30 |
| | Carcinoma in situ | 6 | 2,08 (7,89 of malignant) |
| | Microinvasive Breast Cancer | 9 | 3,11 (11,84) |
| | Ductal carcinoma | 35 | 12,11 (46,05) |
| | Lobular carcinoma | 10 | 3,46 (13,16) |
| | Mixed ductal/lobular carcinoma | 1 | 0,35 (1,32) |
| | Intracystic papillary carcinoma | 3 | 1,04 (3,95) |
| | Papillary and ductal carcinoma | 1 | 0,35 (1,32) |
| | Cribriform invasive carcinoma | 1 | 0,35 (1,32) |
| | Tubular carcinoma | 8 | 2,77 (10,53) |
| | Squamous cell carcinoma | 1 | 0,35 (1,32) |
| | Periductal stromal sarcoma | 1 | 0,35 (1,32) |

invasivecarcinoma. As showed in Table I, 30/76 (39.4%) C3FNA with a malignant outcome showed either unusual or challenging histological features. In particular, 15/76 (19.7%) C3 FNA cases showed a ductal carcinoma in situ with or without neoplastic micro- invasive foci. Fifteen C3 FNAs (15/76, 19.7%) included unusual histotypes such as papillary neoplasm, tubular carcinoma, squamous cell carcinoma and periductal stromal sarcoma (Tab. I). Among the 213 benign cases,the vast majority werediagnosed as fibroadenoma (86/273,40,38%) or fibrocystic breast disease on histology. However, 44/213 cases (20.6%) showed a range of diagnosis usually difficult to recognize on cytology specimens, including low-grade phyllodes tumor and intraductal papillomatosis (Tab. I).

**Conclusions.** In our experience, a diagnosis of C3 rendered on breast FNA showed a malignancy risk of 26.3%, justifying its "atypical, probably benign" definition. The microscopic complexity or unusual features observed in a part of both malignantand benign histological follow-up may explain the difficulties of assign these cases to a straight forward benign or malignant class basing only on the cytological features of these lesions.Infact, these entities not only may show challenging cytological features, but are also difficult to recognize even on core biopsy.

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## MORPHOLOGICAL AND MOLECULAR ANALYSIS OF A SERIES OF HYALINIZING TRABECULAR TUMOR. CYTO-HISTOLOGICAL CORRELATION

M. Dell'Aquila[1 2], S. Capodimonti[1 2], C. Gravina[3], I. Sapuppo[1 2], T. Musarra[1 2], V. Fiorentino[1 2], S. Sfregola[1 2], A. Cocomazzi[1 2], M. Martini[1 2], L. Revelli[2 3], G. Fadda[1 2], L.M. Larocca[1 2], E.D. Rossi[1 2]

*[1] Division of Anatomic Pathology and Histology, Fondazione Policlinico "A. Gemelli", IRCCS; [2] Università Cattolica del Sacro Cuore, Rome Italy; [3] Department of Endocrine-Surgery, Rome Italy*

**Objectives.** Hyalinizingtrabeculartumor(HTT)isarareneoplasmthataffectsthethyroidgland. This pathological entity is essentially benign, however it poses a serious challenge in diagnostic cytopathology.Asamatteroffact,onfineneedleaspirationcytology(FNA),cellularsmearsofHTT are often misdiagnosed as papillary thyroid cancer (PTC) or as medullary thyroid cancer (MTC). These false positive diagnoses often cause overtreatment as complete total thyroidectomies representing an unnecessary surgical approach for a benignlesion.

Cytological smears usually show cells that can be either isolated or arranged in groups with little to no cohesion. Cells are often poligonal or spindle shaped. Pseudo-inclusions and nuclear grooves are not infrequent. Hyaline material secreted by the tumor represents one of the pathognomonic features of this entity. However, it is often misinterpreted either as amyloid substance, wrongly suggesting a MTC diagnosis, or as colloid substance. According to the literature, the cytological interpretation of the majority of HTT cases is diagnosed as either suggestive or outright positive for PTC.

Ancillary studies including also the membrane and cytoplasmic positivity for the ki67 clone mib-1 and the negativity for HBME-1, are useful when HTT is suspected.

Molecular diagnostic techniques have the potential to be extremely useful in the diagnostic discrimination between PTC and HTT. Since *BRAF* mutations are only found in PTC, the presence of a *BRAFV600E* mutated case would rule out a HTT diagnosis, while wild type results might not exclude a HTT diagnosis. In the current study, we analyzed and evaluated a series of 25 HTT with cyto-histological correlation. To the best of our knowledge, this is one of the largest casuistry for a single center reported in the literature.

**Materials and methods.** We presented 25 HTT cases collected from September 2001 to July 2018 in the Department of Anatomic Pathology and Histology of the Fondazione Policlinico Universitario Agostino Gemelli of Rome - IRCCS. Cytological cases were processed with liquid based cytology (LBC). Immunocytochemistry for HBME-1 and Galectine-3 antibodies were performed on both LBC and histological specimens. The search for the *BRAFV600E* mutation was performed on the histological specimens.

**Results.** Ourgroupincluded25resectedthyroidlesionsdiagnosedasHTT, with size ranging from 1.5 mm to 3 cm. Our series included two males out of 25 patients (8%), while 23 were off emale sex. Cytological diagnoses were available in 16 cases out of 25 (64%). The cytological cases were classified according to the Italian classification system for reporting Thyroid cytopathology. They included: Benign lesions favoring Goiter-TIR2 (one case, 6.25%), Follicular neoplasm of low risk of malignancy-TIR3A (three cases, 18.75%); Follicular Neoplasm with high risk of malignancy-TIR3B (five cases, 31.25%), Suspicious for Malignancy favoring-PTC-TIR4 (five cases, 31.25%); Malignant favoring-PTC-TIR5 (two cases, 12.5%). The evaluation of HBME-1 carried out on either cytological or histological samples was performed on 13 cases with negative yields. On the other hand, Galectine-3 was negative in 10 out of 13 cases (77%), while it was positive in three cases. *BRAFV600E* was wildt ype in all of our samples.Concerning the surgical management of our 25 HTT cases, 23 had a total thyroidectomy, one had a partial thyroidectomy and one was a totalization of a previous thyroidectomy. All the cases in this study were incidental findings.

**Conclusions.** The morphological evaluation of our series emphasized that the majority of HTT are cytologically diagnosed in the indeterminate proliferations, including also the category of Suspicious for malignancy. This data underline the issues in the morphological evaluation alone due to the presence of some cells with the features of malignancy such as mild nuclear pleomorphisms and nuclear pseudoinclusions. According to our results, the combined evaluation of ultrasound findings, morphology and ancillary techniques (including ICC and molecular testing) might lead to consider HTT among the differential diagnoses. In HTT it is not rare to finding congruities between an ultrasound imaging report suggestive for a benign lesion and cytological features of malignancy, especially when immunocy to chemistry isnegative.

Since *BRAF* mutations were absent in all of our samples, our study confirms the literature data, pointing out the absence of *BRAF* mutations in HTT. Moreover, we found that this molecular diagnostic test has an important value in order to differentiate PTC from HTT on cytological smears for which the morphological features are equivocal. A wild type yield cannot completely rule out a PTC diagnosis, however, we believe that its use in cytology can help the cytopathologist to get on the right track for HTTdiagnoses.

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## EVALUATION OF ANAL LESIONS WITH CONVENTIONAL AND LIQUID BASED CYTOLOGY. COMPARISON OF RESULTS IN A LARGE INSTITUTIONAL SERIES

I.M. Sapuppo[1 2], C. De Marco[1 2], T. Musarra[1 2], V. Fiorentino[1 2], M. Dell'Aquila[1 2], S. Sfregola[1 2], G. Fadda[1 2], C. Ratto[2 3], G.F. Zannoni[1 2], E.D. Rossi[1 2]

*[1] Division of Anatomic Pathology and Histology; [2] Università Cattolica del Sacro Cuore; [3] Department of Surgery, Fondazione Policlinico "A. Gemelli", IRCCS, Rome, Italy*

**Objectives.** Anal cytology is an important preventative screening method for patients at risk for anal carcinoma. According to the literature, high risk patients include men who have sex with men (MSM), HIV-positive men and women, women with a history of lower genital tract neoplasms, and transplant recipients. Anal cancer is not a common cancer representing only the 26th most common cancer in the United States with only 0.4% of all new cancer cases. Several different papers highlighted that anal cytology may be useful for evaluating anal lesions especially those associated with human papillomavirus (HPV) in individuals at increased risk for anal cancer. While conventional exfoliative cytology (CC) is the most commonly adopted method due to some advantages including a low-cost and the fact that it is a nonaggressive method, liquid-based cytology (LBC) emerged as an alternative and valid approach also for anal cytology. The diagnosis of anal intraepithelial neoplasia (AIN) and the identification of malignancy represent the most important diagnostic questions for anal cytological samples. According to the literature, AIN is frequently associated with HPV infection and can be detected by cytological screening. This increasing and relevant role of anal cytology is a central part of the anal cancer screening in patients at high risk for anal neoplasia. Although studies of the efficacy of anal cancer screening methods would be of great importance for groups at high risk for AIN, few such studies have been conducted. The aim of the present study was to assess the concordance of CC and LBC in diagnosing anal pre-neoplastic lesions, and to compare cytological results with the two different cytological preparations.

**Material and methods.** We recorded all the anal samples in the period between January 1999 and December 2017. We analyzed and compared the cytological features between the two preparations. Concordance between the two cytology methods was calculated, as were the associations between cytology results and histological findings.

**Results.** A total of 589 anal Pap smears were performed during the study period including 74 female and 515 male patients. The series included 281patients with negative cytology, and 308 patients who had abnormal Pap tests. Among these cohort of 308 patients, 42 patients including 36 males and 6 females, had a cyto-histological correlation. They included: three ASC-US, 37 Low Grade SIL, two HSIL (high grade squamous intraepithelial lesion). Concordance between the two methods was statistically significant ($P<0.05$) and the positive cytology was identified with both methods independently. Concerning the three ASC, our histological diagnoses included all cases with AIN-I. For the LSIL, 62% of the cases resulted in a histological diagnosis of AIN-I and/or condiloma whilst 19% resulted in a histological diagnosis of AIN-III and/or squamous carcinoma. The two HSIL resulted in two moderately and poorly differentiated squamous carcinoma. The morphological features were identified with both conventional and LBC with adequate material. Molecular biology results showed that patients with LSIL tested positive for the highest number of HPV subtypes. The associations between positive biopsy and high grade HPV, HPV 16, and multiple HPV infections were not statistically significant.

**Conclusions.** Conventional and liquid-based cytology are equally effective in screening for anal preneoplastic and neoplastic lesions. Anal cytology represents a valid cost-effective screening tool for evaluating human papillomavirus-related disease of the anal canal, especially in at-risk populations, principally MSM and those with HIV disease. In fact, high-risk male patients are at significant risk of epithelial cell abnormality and histopathologically verifiable anal intraepithelial lesions. Education related to better follow-up and collection methods of anal Pap smears is required to reduce the number of unsatisfactory rate and false negative results.

## NODULAR FASCIITIS OF THE PAROTID GLAND: THE "SIMULATOR" IN THE CYTO- HISTOLOGICAL DIAGNOSIS OF SALIVARY GLAND LESIONS

S. Sfregola, M. Dell'Aquila, C. De Marco, V. Fiorentino, T. Musarra, M. Palumbo, I. Sapuppo, G. Fadda, E.D. Rossi

*Division of Anatomic Pathology and Histology, Fondazione Policlinico Universitario "Agostino Gemelli", IRCCS, Rome, Italy*

**Background.** Nodular fasciitis (NF) is a clonal, self-limited proliferation. Although NF most commonly occurs in the extremities of adults, it has been described in many other anatomic sites including the neck, forehead, cheek, orbit, scalp, oral cavity, gnathic bones, ear canal, and sinonasal tract. NF involving the parotid gland is exceedingly rare in any patient, with only a few dozen reports in the English literature.

Clinically, NF is most common in the 3rd or 4th decade of life but it can occur over a wide age range without a clear gender predilection. Symptomatic onset generally arises over the course of a few weeks but with unspecific signs and symptoms, such as swallowing and pain or discomfort. On imaging modality, NF can show infiltrative borders as it extends along fascial planes and permeates into adjacent structures, mimicking a malignant, invasive tumor. The radiologic differential diagnosis for nodules showing these characteristics includes both solid and cystic benign and malignant salivary gland neoplasms. Among the possible entities, we need to exclude Pleomorphic adenoma (PA), Acinic cell carcinoma (ACC), Secretory carcinoma (SC), and Mucoepidermoid carcinoma (MEC), neurogenic tumors such as neurofibroma and schwannoma, dermal based tumors such as dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP), and various other lesions including, melanoma, fibromatosis, hemangioma, and sarcoidosis. The lesional sampling by FNA is commonly performed, even though NF can be easily mistaken for an aggressive neoplasm due to its high cellularity and the overlapping morphological features. As a result, NF is often managed with unnecessary parotidectomy, which is associated with a high-risk for significant surgical complications including facial nerve injury. According to the literature, one of the largest series of NF is a recent multi-institutional study including 15 cases with cyto-histological correlation. The results obtained from this study suggested that combination of cytomorphological findings of FNA specimens (bland, single, spindled cell proliferation with elongated cytoplasmic processes and bland nuclei) and ancillary studies (including immunocytochemistry-ICC and molecular testing) can lead to detecting NF and to consider a conservative management.

The purpose of this study is to present the clinical and cytomorphologic findings of NF of the parotid gland by fine needle aspiration (FNA).

**Methods.** The anatomic pathology archive of the University of the Sacred Heart was searched for salivary gland FNA cytology specimens with a confirmed histological diagnosis of nodular fasciitis in the period between 1998 and 2017. The clinical history, pathologic diagnosis, cytomorphologic findings, and im-

munocytochemical results were recorded. The cytological cases were processed with both conventional smears and liquid based cytology (LBC).

**Results.** A total of two cases were identified; the average age was 29,5 y/o with one female and one male patient. Clinically, both lesions have been described as ipoechoic masses and the average time from symptom onset to clinical presentation was seven weeks. The average lesion size was 3 cm by radiographic measurement. On both conventional and LBC smears, the two cases (100.0%) were classified as spindle cell neoplasm, not otherwise specified (NOS). On average, smears were composed of predominantly single (50.0%), spindled (100.0%) cells with short unipolar (100.0%), elongated (100.0%) nuclei, and absent nucleoli(100.0%).Concerning the architectural pattern, 100.0% showed a tissue culture appearance and 50% contained abundant myxoid stroma with pleat- like folding. No one showed significant cytologic atypia and/or inflammatory components. Due to the spindle bland features of the two lesions, immunocytochemistry was not carried out as long as it would not have changed the finale cytological diagnosis of "spindle cell neoplasm".

**Conclusions.** NF is an extremely rare entity in the salivary gland but a few dozen cases have been described. This entity can show some morphological overlap with a wide range of benign and malignant lesions. The morphological evaluation of cytological samples composed of any bland, single, spindled cell proliferation with elongated cytoplasmic processes and bland nuclei may suggest including NF into the possible differential diagnoses. As a consequence, the cytological diagnosis should inform the clinician to consider more conservative management in the correct clinical context.

Furthermore, the limited application of ancillary techniques, including ICC and FISH for the *MYH9-USP6* fusion, makes it especially important to recognize the morphologic features.

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## URINE CYTOMORPHOLOGY OF MICROPAPILLARY AND PLASMACYTOID VARIANTS OF UROTHELIAL CARCINOMA

P. Straccia[1], M. Martini[1], G. Fadda[1], E. Sacco[2], F. Pierconti[1]

*[1] Division of Anatomic Pathology and Histology, Università Cattolica del Sacro Cuore, Foundation "A. Gemelli" University Hospital, Rome, Italy; [2] Urologic Clinic, Department of Surgical Sciences; Università Cattolica del Sacro Cuore, Foundation "A. Gemelli" University Hospital, Rome, Italy*

**Background.** Among various histologic variants of urothelial carcinoma (UC), some variants such as the micropapillary and plasmacytoid variants exhibit very aggressive clinical behavior. Therefore, it is important to identify the morphologic variants of UC early because these variants often present as high grade and with advanced-stage disease at the time of diagnosis. To date, only a small number of cytology cases have been reported on either of these variants. Herein, we report 10 cases of UC with combined micropapillary and plasmacytoid features based on urine cytology.

**Methods.** We performed a retrospective chart review of all patients with carcinomas of bladder with predominant plasmacytoid and micropapillary histology who had been seen from January 1, 2005, through December 31, 2017. A total of ten cases (5 cases of plasmocitoid variant and 5 cases of micropapillary variant of bladder cancer) with urine specimen, processed using ThinPrep method, were evaluated. Clinical follow-up was obtained by chart review whitin a year after diagnosis. The cytomorphologic features were compared between two histological variants.

**Results.** We investigated 10 patients with the diagnosis of micropapillary and plasmocitoid variant of bladder carcinoma subjected to urine cytology in a period from 2005 to 2017. The ratio man to women was 5:1 with a median age of 60 years (range: 45-77 years).

The cytologic features, single-cell pattern, true papillae, flat sheets/nests, three dimensional clusters, micropapillae (inside-out, acini-like, or cauliflower with nuclei located peripherally), nuclear grade, cytoplasm quantity, cytoplasmic vacuoles, and necrosis, were evaluated in micropapillary variant. The cytologic features of plasmacytoid are characterized by large, discohesive, isolated tumor cells that have abundant, thick cytoplasm and eccentrically located, hyperchromatic nuclei with coarse chromatin and inconspicuous nucleoli.

**Conclusions.** In summary, these cases of urothelial carcinoma(UC) with two rare morphology variants is presented with urine cytologic findings corroborated by histology. Because these variants of UC show a very poor prognosis, pathologists and urologists should be alerted to these rare, but clinically very aggressive variants of UC.

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## FINE-NEEDLE CYTOLOGY OF INTRAGLANDULAR PAROTID LYMPH NODE. AN USEFUL PROCEDURE IN THE MANAGEMENT OF SALIVARY GLANDS NODULES

G. Vitagliano[1], A.L. Peluso[2], C. Baldi[2], P. Zeppa[2]

*[1] Dipartimenti di Anatomia Patologica, Università di Napoli "Federico II"; [2] AOU San Giovanni di Dio e Ruggi d'Aragona, Università di Salerno*

**Introduction.** There are 15-20 parotid and periparotid lymph nodes; 10-11 are located along the retromandibular vein, 4-7 are superficial and 1- 2 are localized in the parotid (pLNs). Reactive pLNs require clinical surveillance and only lymphoma or the rare metastases may need histological diagnosis and classification. therefore preoperative pLNs diagnosis is advisable. pLNs identification may be missed by ultrasound (US) because of the similarities of their US pattern to other parotid diseases and because of their low incidence. Fine-needle cytology (FNC) is used in the pre-operative diagnosis of parotid nodules, therefore pLNs may undergo to FNC to assess their nature and corresponding pathological processes. In this study a series of pLNs diagnosed by FNC

is presented to assess their cytology, the ancillary techniques and clinical implications.

**Materials and Methods.** A series of 18 consecutive FNC from pLNs collected in 5 years were retrieved from the files of the UOC of Pathology, AOU, University of Salerno. All the cases were identified by clinical and US and submitted to US-assisted FNC for preoperative diagnosis. n all the cases FNC and rapid on-site evaluation (ROSE) were performed as previously described. According to ROSE, flow cytometry (FC) was performed in all the cases. Immunoglobulin Heavy Chain (IGH) Gene Rearrangement PCR in 2 cases and immunohistochemistry (IHC) on cell- block in 3 cases using CD3,CD20,CD10 and T-Bet, were performed as previously described2-3. Obtained data were checked by follow-up and histological controls when available.

**Results.** Nine of the 18 cases were US suspected or identified as pLNs. The remaining 9 cases were considered adenoma (5), cyst (2) or "undefined nodule" (2). US-guided FNC and rapid on-site evaluation (ROSE) identified all the pLNs. According to the cytology features, combined to FC and ICC pLNs were then diagnosed reactive (16), MALT (1) and B-cell, low-grade non-Hodgkin lymphoma (1). Clinical follow- up (16) histology (2) confirmed the FNC diagnoses of reactive processes, MALT and grade1 follicular lymphoma (Tab. I).

**Conclusions.** FNC is a sensitive procedure in the identification of pLNs. FNC is useful in the management of pLns indicating follow-up for reactive processes and surgical excision for neoplasia only, sparing useless excisions for reactive processes.

Tab. I. Clinical ultrasound and fine-needle cytology data of 16 intra-parotid lymph nodes.

| Case n. | Age/ sex | Site-size (mm) | Clinical-us | Shape, hilus, us | Finc | Flow-cytometry or immunocytochemistry | Fnc diagnosis | Pcr | Histology or follow-up |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 45-F | left-8 | lymph-node | oval | polymorphous, lymphoid cells | CD5:36%, CD19:63%, CD10:1%, CD2-3-7:57% | reactive | no | reactive |
| 2 | 52-F | left-15 | undefined nodule | round | polymorphous, lymphoid cells | CD5:34%,CD19:66%,CD10:1%, CD2-3-7:60%, lambda:32%,kappa:67%, CD20:75% | reactive | no | reactive |
| 3 | 63-M | right-10 | lymph-node | oval | polymorphous, lymphoid cells | CD5:55%,CD19:37%,CD10:1%, CD2-3-7:54% | reactive | no | reactive |
| 4 | 36-F | left-12 | lymph-node | oval | polymorphous, lymphoid cells | FC not contributive. ICC: CD3+, CD20+ | reactive | no | reactive |
| 5 | 51-M | left-16 | adenoma | oval | polymorphous, lymphoid cells | CD5:50%,CD19:45%,CD10:1%, CD2-3-7:53% | reactive | no | reactive |
| 6 | 24-M | right-15 | adenoma | round, no hilum | polymorphous, lymphoid cells | CD5:34%,CD19:65%,CD10:1%, CD2-3-7:58%,lambda:30%, kappa:64%,CD20:70% | reactive | no | reactive |
| 7 | 27-F | left-14 | lymph-node | round | isolated and solid groups | Not contributive. ICC: CD3+, CD20+ | reactive | no | reactive |
| 8 | 57-F | left-10 | lymph-node | oval | polymorphous, lymphoid cells | CD5:33%,CD19:64%,CD10:1%, CD2-3-7:55% | reactive | no | reactive |
| 9 | 52-F | right-16 | lymph-node | oval | polymorphous, lymphoid cells | CD5:36%,CD19:66%,CD10:1%, lambda:34%,kappa:62% | reactive | no | reactive |
| 10 | 56-F | left-9 | adenoma | round, no hilum hypo-echoic | monomorphic, small lymphocytes | CD5:4%,CD10:1%,CD38:70%, CD20:99%, lambda:98%,kappa:2%, ICC:T-BET+ | NHL MALT | IGH rearrangement | MALT lymphoma |
| 11 | 53-F | right-11 | cyst | oval | polymorphous, lymphoid cells | Not contributive. ICC: CD3+, CD20+ | reactive | no | reactive |
| 12 | 60-M | right-13 | lymph-node | oval | lymphoid and acinar cells | Not contributive. ICC: CD3+, CD20+ | reactive | no | reactive |
| 13 | 57-F | left-10 | adenoma | round, no hilum hypo-echoic | monomorphic, cleaved lymphocytes | CD5:1%,CD10:1%,CD38:78%, CD23:85%, CD20:99%, lambda:99%, kappa:67% | NHL B-cell low-grade | IGH rearrangement | follicular lymphoma |
| 14 | 55-M | right-10 | lymph-node | round | polymorphous, lymphoid cells | CD5:37%,CD19:64%,CD10:3%, CD2-3-7:59%,lambda:33%, kappa:65%,CD20:73% | reactive | no | reactive |
| 15 | 47-M | left-9 | lymph-node | round | polymorphous, lymphoid cells | CD5:33%,CD19:65%,CD10:1%, CD2-3-7:60%,lambda:34%, kappa:66%,CD20:75% | reactive | no | reactive |
| 16 | 58-F | right-8 | adenoma | oval, no hilum | polymorphous, lymphoid cells | Not contributive. ICC: CD3+, CD20+ | reactive | no | reactive |
| 17 | 62-F | left-15 | cyst | oval, no hilum | polymorphous, lymphoid cells | CD5:35%,CD19:58%,CD10:1%, CD2-3-7:62% | reactive | no | reactive |
| 18 | 51-F | right-17 | undefined nodule | oval | lymphoid cells | CD5:36%,CD19:60%,CD10:1%, CD2-3-7:63%,lambda:32%, kappa:67%,CD20:75% | reactive | no | reactive |

## CYTODIAGNOSTIC ACCURACY OF SALIVARY GLANDS TUMORS IN YOUNG PATIENTS

I. Cozzolino[1], M. Montella[1], A. Ronchi[1], R.L. Sabetta[1], G. Ferrara[1], G. Colella[2], R. Franco[1]

*[1] Pathology Unit, Department of Mental and Physical Health and Preventive Medicine; [2] Maxilofacial Unit, Università della Campania 'Luigi Vanvitelli'*

**Objective.** Salivary gland tumours are very uncommon neoplasias in young patients and its histopathology is highly varied due to the heterogeneous cellular composition. The role of the preoperative fine-needle cytology (FNC) is controversial. The purpose of this study is to investigate the accuracy of cytological diagnosis and potential clinical utility of FNC paediatric salivary gland lesions.

**Materials.** At the time of our survey, the files of the Vanvitelli university contained 22 cases of salivary gland tumors in young patients, submitted to US-guided FNC. We consider patients up to 19 yrs in this group. In this study the features of inclusion were: 1) patients younger than 19 yrs; 2) lesions of both major and minor salivary glands; 2) all pathologic lesions (inflammatory, hamartomatous, neoplastic, and those reflecting more widespread disease). In 20 of 22 cases ROSE was performed for cellularity adequacy, while the patient was still in the outpatient; in the cases where scantily or non-cellular and/or blood contaminated samples were obtained, another pass was performed. ROSE also oriented the need of ancillary techniques; in fact, when possible, other passes were performed for immunocytochemistry (ICC) and/or for flow cytometry (FC).

**Results.** The series include 8 (36%) males and 14 (64%) females, with a median age of 14 yrs (ranging from 1-19). The targeted anatomical sites were: 9 left parotids; 9 right parotids; 2 left submandibulars; 1 palate; 1 cheek. The FNC samples were adequate and representative in 22/22 cases for morphological diagnosis. The cytological diagnosis was pleomorphic adenoma in 7 cases, acinic carcinoma in 1 case, adenoid cystic carcinoma in 1 case, mucoepidermoid carcinoma in 2 cases, Warthin tumour in 1 case, schwannoma in 1 case, lipoma in 1 case, hamartoma in 1 case, pilomatrixoma in 1 case, intraglandular cyst in 1 case, inflammatory processes in 3 cases, intraparotid lymph node in 1 case, myoepithelial neoplasm in 1 case. In 5 cases surgical resection was performed and histological examination confirmed the cytological diagnosis in 4 cases (2 mucoepidermoid carcinoma, 1 pleomorphic adenoma, 1 acinic carcinoma); myoepithelial neoplasia was a pleomorphic adenoma.

**Conclusions.** Preoperative FNC of salivary gland lesions is a simple, useful and accurate tool in diagnosing benign from malignant lesions and in planning appropriate approach for treatment.

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## DISSEMINATED CRIPTOCOCCOSIS FIRST DIAGNOSED BY FINE NEEDLE ASPIRATION OF LYMPH NODE: REPORT OF TWO CASES

E. Kuhn[1], F. Poli[1], S. Lega[1], L. Caprara[1], S. Cortecchia[1], M. De Lillo[1], A. Cancellieri[2], F. Morgagni[3], M. Puccetti[1]

*[1] Pathology Unit, Azienda Unità Sanitaria Locale (AUSL) di Imola, Italy; [2] Pathology Unit, Ospedale Maggiore, Bologna, Italy; [3] Pneumology Unit, AUSL Romagna, Lugo (RA), Italy*

**Objectives.** Cryptococcal infection is a relevant opportunistic disease that usually starts after inhalation of the yeast with a lung involvement. Clinical manifestations vary from asymptomatic infection to pneumonia with fever and, rarely, acute respiratory distress syndrome. Later, a cryptococcal infection can disseminate to many sites, including lymph nodes, and present primarily as multiple lymphadenitis. We report two cases of cryptococcal infections first diagnosed on cytology specimens obtained from lymphadenitis.

**Material and Methods.** Fine needle aspiration was performed on enlarged lymph nodes from two patients. In both cases, the aspirated material was smeared on glass slides and stained with Papanicolaou and May-Grunwald-Giemsa stains and processed by cell block technique. Histochemical staining was performed for periodic acid-Schiff (PAS).

**Results.** Both patients were immunocompromised men. One was HIV-infected 40-year old, with a recent onset of bilateral hilar and mediastinal lymphadenopathies and a 2 cm lung nodule. The other was 68-year old, treated with steroids, with intermittent fever, multidistrectual (laterocervical, mediastinal and retroclavear) lymphadenopathies, a 1.5 cm lung nodule, interstitial lung disease and a history of treated skin melanoma. The cytological material was obtained from a lower paratracheal lymph node and a laterocervical lymph node, respectively. On cytologic examination, both specimens presented numerous ovoid and spherical structures, 5-15 µm in diameter, with internal empty appearance and a PAS- positive thick wall, morphologically compatible with Cryptococcus. In the first case, the background material was completely necrotic and filamentous acellulated, while in the second case, the microorganisms were extracellular as well as intracellular, interspersed with a mixed inflammatory cell population, mainly composed of lymphocytes and histiocytes, In the second case, a fungal culture from lymph node material identified Cryptococcus Neoformans.

**Conclusions.** Lymphadenopathy is a rare manifestation of cryptococcal dissemination, however, a prompt diagnosis is essential to start an effective treatment and prevent life-threatening events. Lymph node fine needle aspiration demonstrates great utility for a rapid and accurate diagnosis of cryptococcal lymphadenitis.

## THE ROLE OF 7-GENE TEST IN THE PRE-SURGICAL RISK STRATIFICATION OF INDETERMINATE THYROID FINE NEEDLE ASPIRATIONS: PRELIMINARY DATA FROM TWO YEARS OF DIAGNOSTIC PRACTICE

I. Migliatico, R. Sgariglia, M. Nacchio, F. Scognamiglio, E. Vigliar, U. Malapelle, G. Troncone, C. Bellevicine

*Dipartimento di Sanità Pubblica, Università degli Studi di Napoli "Federico II"*

**Background.** The aim of this study was to investigate the role of the "7 gene test" in the pre-surgical risk stratification of indeterminate thyroid fine-needle aspirates (FNA) routinely performed at our Institution.

**Methods.** Starting from April 2016, n=991 indeterminate thyroid FNAs were prospectively tested by the "7-gene test", a real-time PCR based assay which asses BRAF, N-H-KRAS, RET/PTC and PAX8/PPARG genomic alterations. In order to calculate the pre- and post-test risk of malignancy (ROM), only FNA with available histological follow-up (207/991 FNA, 20.9%) were included in the study. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was adopted for the microscopic diagnosis.
**Results.** FNA classified as atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) showed a 25.9% pre-test ROM whereas post-test ROM was 42.6% in mutation-positives (MT-pos) and 14.5% in mutation- negatives (MT-neg) cases, respectively. Considering the MT-positive cases, the cases harbouring BRAF-like mutations (BRAFV600E, RET/PTC1, RET/PTC3) showed a statistically significant higher ROM (80%) than those with RAS-like mutations (N-H-KRAS, PAX8/PPARg) (32.4%, p=0.010). Follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) FNA showed a 44.1% pre-test ROM. Conversely, FN/SFN post-test ROM was 80% in MT-pos and 29.1% in MT-neg cases. Although BRAF- and RAS-like mutations were associated to different ROM even in FN/SFN cases (100% vs 71.4%), this difference did not reach a statistical significance (p=1,0). Suspicious for malignancy (SFM) FNA showed a 93% pre-test ROM; this latter figure almost overlapped to post- test ROM of both MT-pos (100%) and MT-neg (84.6%) FNAs. In particular, BRAFV600E-mutated FNAs were consistently associated with a papillary carcinoma on histology, irrespective of TBSRTC categories.
**Conclusions.** Our preliminary data show that the "7-gene test" may contribute to the risk stratification in AUS/FLUS and FN/SFN categories, thanks to the significant difference in post-test ROM between MT-pos and MT-neg FNAs, confirming the high positive predictive value of BRAFV600E and BRAF-like mutations over the RAS-like genomic alterations. Conversely, considering the high pre-test ROM, the "7-gene test" does not add any additional information in FNAs classified as SFM.

## DNA HPV TESTING AND DIAGNOSTIC CYTOPATHOLOGY FOR ORAL CANCER SCREENING

F. Botto Micca[1], E. Amodeo[1], C. Lapucci[2], F. Crò[2], M. Pentenero[3], S. Gandolfo[3], R. Navone[1]

*[1] Centro Diagnostico CDC, Anatomia Patologica e Citopatologia, Torino; [2] Synlab Italia, Castenedolo (BS); [3] Dipartimento di Oncologia dell'Università di Torino (Unità di Medicina e Oncologia Orale)*

**Objectives.** The survival rate for squamous cell carcinoma of the oral cavity (OSCC) remains low, as it is often diagnosed late due to no reliable diagnostic method of selecting people with high risk of transformation. In presence of precancerous lesions, liquid based oral cytology alone can provide useful information (sensitivity, specificity and PPV have been reported to be as high as 94.7%, 98.9% and 95.9%) and gives good first level screening results [1 2]. Conversely, there are no reliable test in order identify subjects with high risk of malignant transformation in absence of precancerous lesions. Although the most important risk factors are tobacco and alcohol, a potential role of oral HPV infection in the onset of OSCC, could represent an adjunctive predictive factor [3].
**Methods.** We are currently screening apparently normal subjects with a first level method, i.e. liquid-based cytology combined with investigation with DNA-HPV test. Samples were obtained by the cytobrush on the most commonly involved sites for oral carcinoma (floor of the mouth, tongue, gums and cheek lining).
**Results.** One hundred and thirty one subjects were enrolled, 63 males and 68 females: 130/131 had normal cytology results, 1 had a low-grade oral lesion (OIN 1) and 4 had HPV-DNA test positive (the HR-HPV types were 16, 31, 53 and 16, 31 in two females; two males had low risk HPV, 62 and CP6108 respectively: in these four cases the cytology was normal or with keratosis).
**Conclusions.** Combining liquid based diagnostic oral cytology and tests for HPV infection seems able to select a subgroup of patients with potential predictive factors for OSCC development and thus requiring proper follow-up schedule even in absence of visible oral lesions. Relevance. The prospective evaluation of healthy subjects with oral HPV infection could give important information about its role in the development of OSCC. It is also important to choose the appropriate HPV test in as much as many HPV commercial kits were originally developed for cervical carcinoma screening. To date, the classification of high and low risk HPV genotypes has been based on cervical cancer evidence, but this nomenclature could be misleading in the presence of OSCC. However, in case of a proven causal role of HPV, a combination of oral cytology and tests for HPV infection could represent a significant diagnostic step forward early diagnosis of OSCC, as yet demonstrated with the experience in uterine cervical carcinoma screening with PAP test and DNA-HPV.

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## INDETERMINATE THYROID NODULES AT LOW-RISK (TIR3A) AND HIGH RISK (TIR3B) OF MALIGNANCY: A CYTOMORPHOLOGICAL STUDY ON THE NEW ITALIAN REPORTING SYESTEM FOR THYROID CYTOLOGY

E. Rullo[1], G. Minelli[2], D. Bosco[1], F. Nardi[1], G. Grani[3], C. Durante[3], V. Ascoli[1]

*[1] Department of Radiological, Oncological and Pathological Sciences, Sapienza University of Rome, Italy; [2] Unit of Statistics, National Institute of Public Health (Istituto Superiore di Sanità), Rome, Italy; [3] Department of Internal Medicine and Medical Specialties, Sapienza University of Rome, Italy*

**Objective.** The Italian reporting system for thyroid cytology1 subdivides indeterminate lesions into TIR3A (low-risk) and TIR3B (high-risk) subcategories providing a practical guide rather than considering morphological issues in detail. We aimed to assess which cytological features are valuable and whether these are effective in TIR3A/TIR3B subclassification and in predicting histological outcomes.
**Methods.** Thyroid fine-needle aspirates from 111 indeterminate nodules were reviewed blinded to clinical information, TIR3A/TIR3B subclassification and histology to assess which cytological features pooled into artifacts, smear background, architectural and nuclear atypia, and oncocytes differentiate TIR3A from TIR3B, and benign from malignant histological outcomes.
**Results.** Of the cytological features examined, some were specific for TIR3B (high cellularity, nuclear atypia, oncocyte predominance, transgressing vessels) or TIR3A (artifacts,

low cellularity, oncocyte sparseness) while others (including microfollicules/trabeculae) were non-specific. By different distributions of these features, three subgroups were identifiable in TIR3B (follicular lesions with oncocytic changes, follicular lesions without oncocytic changes, and follicular lesions with nuclear atypia) but none in TIR3A. Nuclear atypia was a significant indicator of malignancy whereas high cellularity and microfollicules/trabeculae were not discriminants of any histological outcome; oncocytic features were misleading to intercept malignancy.

**Conclusions.** Most of the assessed features are good predictors of histological outcomes. TIR3A accounts for undefined nodules due to the absence of characterizing features in contrast to TIR3B that is associated with a wider number of distinguishing features.

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# DIGITAL PATHOLOGY

## A DIGITAL PATHOLOGY APPROACH TO UNCOVER NEW IMMUNOGENIC PATHWAYS IN ORAL SQUAMOUS CELL CARCINOMA

F. Merolla[*1], D. Russo[*2], G. Ilardi[2], S. Varricchio[2], A. Borzillo[2], R.M. Di Crescenzo[2], F. Martino[2], V. Napolitano[2], M. Cervasio[2], L. Stasio[2], M. Mascolo[2], L. Postiglione[3], G. Di Spigna[3], A. Celetti[4], S. Staibano[2]

*[1] Department of Medicine and Health Sciences "V. Tiberio", University of Molise, Campobasso, Italy; [2] Department of Advanced Biomedical Sciences, University of Naples "Federico II", Italy; [3] Department of Translational Medical Sciences, University of Naples "Federico II", Italy; [4] Institute for the Experimental Endocrinology and Oncology "Gaetano Salvatore", Italian National Council of Research, Italy; [*] Equally contributed to this work*

**Aim.** Malignant tumors of head and neck region include a great variety of lesions, the great majority of whom being squamous cell carcinomas of oral cavity [1]. Oral squamous cell carcinoma (OSCC) accounts for 2-4% of all malignant lesions and is the most common neoplasia of head and neck district. Its frequency is increasing in last decades, but its prognosis is still unfavourable. Nowadays, there is not a universal consensus about prognostic and predictive markers for OSCC. Chromatin Assembly Factor 1 (CAF-1) is an heterotrimeric complex made of three subunits: p150, p60 and p48 protein [2-4]. The complex pays a role as histone chaperone and it is involved in the deposition of histones H3 and H4 in newly synthetized DNA [5]. CAF-1 is involved in DNA replication process, ensuring the correct progression through S-phase [6-8]. Moreover Chromatin assembly factor is also involved in DNA repair mechanisms, mainly involved in repair associated chromatin formation [8 9]. While CAF-1 p150 subunit appears to be more active in interphase DNA damage repair processes, interacting with PCNA on the damaged DNA, specifically during nucleotide excision repair (NER) [10 11] and also during double strand breaks repair [12], CAF-1 p60 has been described to be more specifically connected to cell replication. Loss of p60 leads cells to incorrectly replicate DNA, consequently accumulating DNA damage [13], this resulting in potential increase in neoantigen exposure and consequently to increased tumor immunogenicity, a strong predictive feature for response to immune checkpoint inhibitors drugs. Prognostic value of CAF-1 p60 in Oral squamous cell carcinomas has been investigated and proved in several works [14 15]. The aim of this study is to investigate the correlation between CAF-1 p60 and tumor-elicited immune response, focusing on p60 role as immunotherapy responsiveness predictive factor and their value as prognostic factors.

**Materials and methods.** We performed an automated immunohistochemical analysis of CAF-1 p60 and immune markers using QuPath, an Opensource software for image analysis, comparing their expression in 105 cases of oral squamous cell carcinoma. Tumor samples were arranged in tissue micro arrays (TMAs) in order to take advantage of the procedure's beneficial cost- benefit ratio.

**Results.** From the results analysis, we confirmed on a larger tumor series that increased expression of CAF-1 p60 associates to poor prognosis in OSCC; moreover, our observations let us envisage a role for loss of CAF-1 p60 expression as promising immunomodulating treatment response predictive marker.

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# PATOLOGIA SPERIMENTALE

## BIOINFORMATICS ANALYSIS OF THE CLINICOPATHOLOGICAL SIGNIFICANCE AND THE PROGNOSTIC ROLE OF SURFACTANT PROTEIN D IN LUNG, GASTRIC, BREAST AND OVARIAN CANCERS

B. Belmonte[1], A. Mangogna[2 3], D. Bonazza[2], C. Agostinis[4], A. Gulino[1], I. Ferrara[1], V. Cancila[1], G. Ricci[2 4], A. Romano[2], R. Bulla[3], F. Zanconati[2]

*[1] Tumor Immunology Unit, Department of Health Sciences, Human Pathology Section, University of Palermo, Palermo, Sicily, Italy; [2] Department of Medical, Surgical and Health Science, University of Trieste, Trieste, Italy; [3] Department of Life Sciences, University of Trieste, Trieste, Italy; [4] Institute for Maternal and Child Health, IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico) Burlo Garofolo, Trieste, Italy*

**Background.** Surfactant protein D, also known as SP-D, is a collagenous glycoprotein encoded by SFTPD gene belonging to the collectins family (collagen-containing C-type lectin). SP-D is a pattern recognition molecule that has pulmonary as well as extra-pulmonary localization [1]. In addition to its canonic role in the maintenance of surfactant homeostasis in the lung, SP-D plays a critical role as regulator of immunity and inflammation [2]. We have performed a bioinformatics analysis to investigate whether SP-D can be considered as a potential prognostic marker for human epithelial malignancies by focusing on carcinomas of the lung, stomach, breast and ovary. Subsequently, by immunohistochemistry, we have investigated the expression of SP-D in the same healthy human tissues and in their malignant counterparts.

**Methods.** For the bioinformatics analysis we used Oncomine, a cancer microarray database (www.oncomine.org), which allowed evaluating the expression level of SFTPD gene in different types of cancer and to compare the differences in mRNA level between normal and neoplastic tissues [3]. The prognostic significance of SP-D expression and survival in lung, gastric, breast and ovary cancer were analyzed by Kaplan–Meier plotter (www.kmplot.com) [4]. Immunohistochemistry (IHC) on formalin-fixed and paraffin-embedded (FFPE) samples was performed using a polymer detection method.

**Results.** In lung cancer we detected a significantly lower SP-D mRNA expression both in adenocarcinoma and squamous cell carcinoma than in the normal pulmonary parenchyma ($p < 0.05$). Moreover SP-D mRNA expression was positively related with the overall survival rate of lung cancer patients, even lung adenocarcinomas and squamous cell carcinomas were analyzed separately (Fig. 1A). IHC staining for SP-D confirmed a different expression in the healthy pulmonary parenchyma and in both histotypes of lung cancer. Consistently, the same bioinformatic analysis applied to gastric and breast cancer revealed a lower SP-D mRNA expression in gastric adenocarcinoma, even stratified into diffuse-, intestinal-, and mixed-type adenocarcinomas by Lauren's classification ($p < 0.05$) and in invasive ductal breast carcinomas compared to gastric mucosa and normal ductal mammary epithelium, respectively ($p < 0.05$). SP-D mRNA expression was negatively related to an overall survival rate of the patients with gastric cancer ($p = 0.00011$) (Fig. 1B). If stratified by Lauren's classification, SP-D mRNA expression was negatively related to the overall survival rate of patients with intestinal-type adenocarcinoma ($p = 0.00091$), without distant metastasis and Her2-negative ($p = 0.0023$) (Fig. 1B). In invasive ductal breast carcinomas, we observed a negative association between SP-D mRNA expression and a favorable prognosis in patients with Luminal-A grade-1 and -2 cancers (respectively $p = 0.01$ and $p = 0.0059$) (Fig. 2A). IHC staining for SP-D confirmed its lower expression in gastric adenocarcinomas and invasive ductal breast carcinomas than in the normal gastric mucosa and ductal mammary epithelium, respectively. As far as ovarian cancer was concerned, a higher SP-D mRNA expression was detectable in d than in normal ovary ($p < 0.05$). This data was also confirmed by real-time PCR in primary cells isolated from four samples each of human ovarian serous cystadenocarcinoma and normal ovarian tissues. SP-D mRNA expression showed a negative relationship with both overall or progression-free survival (respectively $p = 0.016$ and $p = 0.0035$) rates of patients with serous cystadenocarcinoma, if stratified by stage -1 and -2 (Fig. 2B). Furthermore, IHC staining for SP-D confirmed a higher expression in serous cystoadenocarcinoma compared to the normal ovarian parenchyma.

**Conclusions.** We conclude that, while in lung cancer it might be considered as a favorable prognostic factor, in gastric, breast, and ovarian cancers SP-D might have a different clinicopathological significance, representing an unfavorable prognostic factor. The apparently conflicting role of this pattern recognition receptor might find explanation on the heterogeneous immune contextures of the investigated tumors, which arise in the setting of dramatically different inflammatory milieus. Correlation between the levels of SP-D and patients' outcome requires further investigation. Our in silico analyses, candidate SP-D as potential novel marker with prognostic significance in epithelial malignancies, which actual impact deserves to be investigated in forthcoming ad-hoc-designed studies.

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## TRANSCRIPTOME SEQUENCING OF THE TRANSITION FROM NORMAL EPITHELIUM TO INVASIVE CANCER REVEALS INSIGHTS INTO THE CARCINOGENESIS OF HPV+ AND HPV- VULVAR NEOPLASIA

A. Garuti[1], L. Ferrando[1], M. Paudice[2], C.M. Biatta[2], G. Anselmi[3], M. Pelle[1], R. Fiocca[2 3], E. Fulcheri[2 4], S. Ferrero[5 6], A. Ballestrero[1 7], V.G. Vellone[2 3], G. Zoppoli[1 7]

*[1] Dipartimento di Medicina Interna (DiMI), Università degli Studi di Genova, Italy; [2] Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate (DISC), Università degli Studi di Genova, Genoa, Italy; [3] UOC Anatomia Patologica Universitaria, Ospedale Policlinico San Martino, Genova, Italy; [4] UOSD Patologia Feto-Placentare, Istituto G. Gaslini, Genova, Italy; [5] Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia e Scienze Materno-Infantili (DiNOGMI), Università degli Studi di Genova, Genoa, Italy; [6] Clinica di Ostetricia e Ginecologia, Ospedale Policlinico San Martino, Genova, Italy; [7] UOC Medicina Interna Ind. Oncologico, Ospedale Policlinico San Martino, Genova, Italy*

**Background.** Vulvar Squamous Cell Carcinoma represents a rare neoplasm with bimodal distribution by age and complex pathogenesis. This neoplasm often affects fragile patients with

Figure 1. Pathological significance of SP-D expression in lung cancer **(A)** and gastric cancer **(B)**.

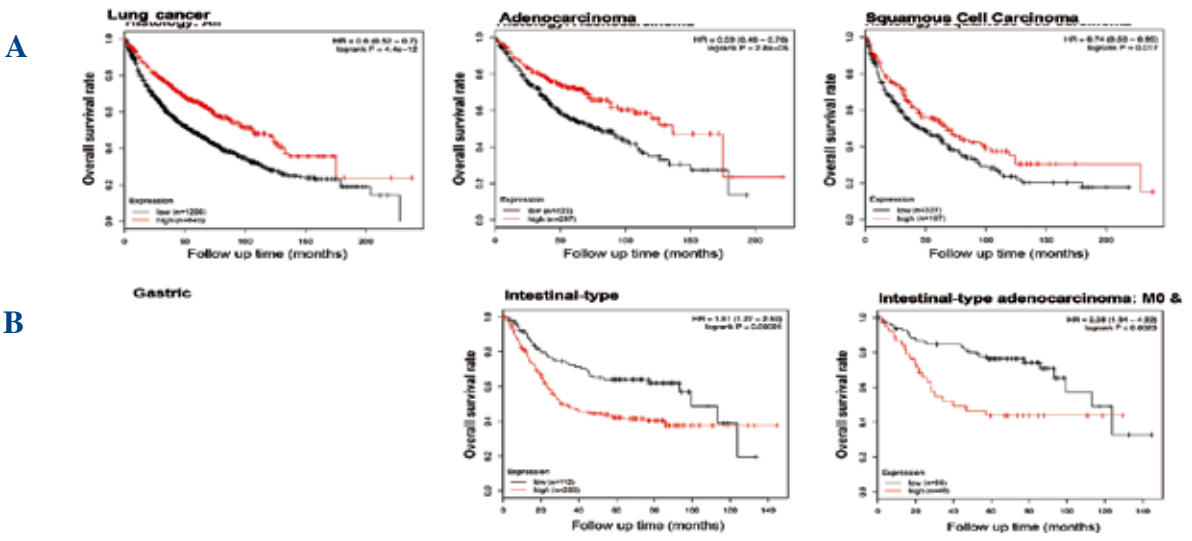
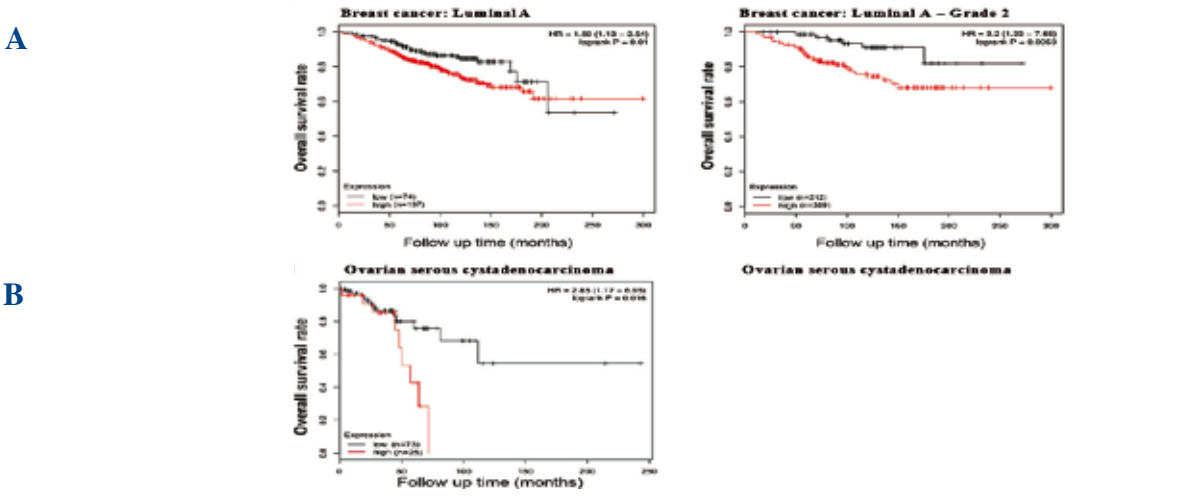



Figure 2. Pathological significance of SP-D expression in breast cancer **(A)** and ovarian cancer **(B)**.




a high frequency of therapeutic failures. The primary aim of the study was to assess gene expression profiles in patients affected by HPV- and HPV+ Vulvar Squamous Cell Cancer (VSCC). Furthermore, we sought to analyze similarities and differences in gene expression levels related to spatially contiguous tissues from the same patients (normal, preneoplastic, and cancerous specimens), in order to shed light on the carcinogenesis process in VSCC.

**Materials and Methods.** From 2014 to 2016, HPV- and HPV+ VSCC samples along with their paired normal and preneoplastic tissues were obtained from patients diagnosed at the Academic Unit of Obstetrics and Gynecology of the Policlino San Martino University Hospital. Patients undergoing neoadjuvant treatment or hormone therapy were excluded from the study.

**RNA** extraction was accomplished using Promega kit (Maxwell® RSC RNA FFPE Kit), and libraries preparation was carried out with Ion AmpliSeq™ Transcriptome Human Gene Expression Kit protocol. Transcriptome sequencing was carried out by Ion S5 system (Thermo Fisher). RNA quantity was assessed with Tape Station 2200 (Agilent Technologies). Raw counts were trasformed onto logarithmic scale using R package edgeR. Poorly expressed or unexpressed genes throughout all samples were removed from analysis. We performed differential gene expression analysis using the BioConductorLimmapackage within the R Environment for Statistical Computing.To identify differential gene expression between HPV+ and HPV- patients we conducted a preliminary study considering only tumor and normal HPV- tissue samples. Prior data indicated that the minimum average read counts among prognostic genes in the control group was 20 and the maximum dispersion was 0.5. We supposed that the total number of interesting genes was 10,000 and the top 100 genes were prognostic. Setting the desired minimum fold change to 3, we needed 16 subjects in each group to be able to reject hypothesis with

a power of 0.8 using exact test and with an associated false discovery rate of 0.1.

**Results.** To identify differential genes expression levels, we collected and analyzed 48 biospecimes (16 normal tissues, 16 preneoplastic tissues, 16 cancer tissues) from 16 patients (8 HPV+ VSCC, 8 HPV- VSCC) with a mean age of 71 (±11) for HPV+ group, and 73 (±10) for HPV-. We normalized raw counts and removed from analysis samples with less than 1,000,000 reads (7 samples). Transcripts with less than 5 reads per million in 30% of samples were filtered from the dataset, and remaining genes were log2-transformed. We evaluated differential gene expression levels in tumor samples HPV+ vs HPV-. Of 10,430 genes, 29 genes showed statistically significant differences ($|logFC| > 1$, $fdr < 0.1$) between the two groups (Fig. 1A). We performed a principal component analysis to assess the distribution of samples on a bidimensional space (Fig. 1B). Similarly, we investigated differential gene expression profiles in HPV-tumor samples vs HPV- normal samples. 1847transcripts were identified with a $|logFC| > 1$ and $fdr < 0.1$ (Fig. 1C). Contiguous spatial expression analysis of the 10th percentile of genes in HPV- tissues were carried out. 87 transcripts were overexpressed through the 3 stages, whilst 13 genes showed underexpression profiles.

**Conclusions.** Trascriptome sequencing represent a powerful tool in modern pathology, investiganting the alterations of gene expression in the transition from normal epithelium to invasive neoplasia can shed light in the pathogenesis and identify new molecular targets for an individualized therapy.

## QUANTITATIVE IMAGE ANALYSIS OF PROLIFERATION AND MICROVESSELS DENSITY IN A MOUSE XENOGRAFT MODEL OF BREAST CANCER

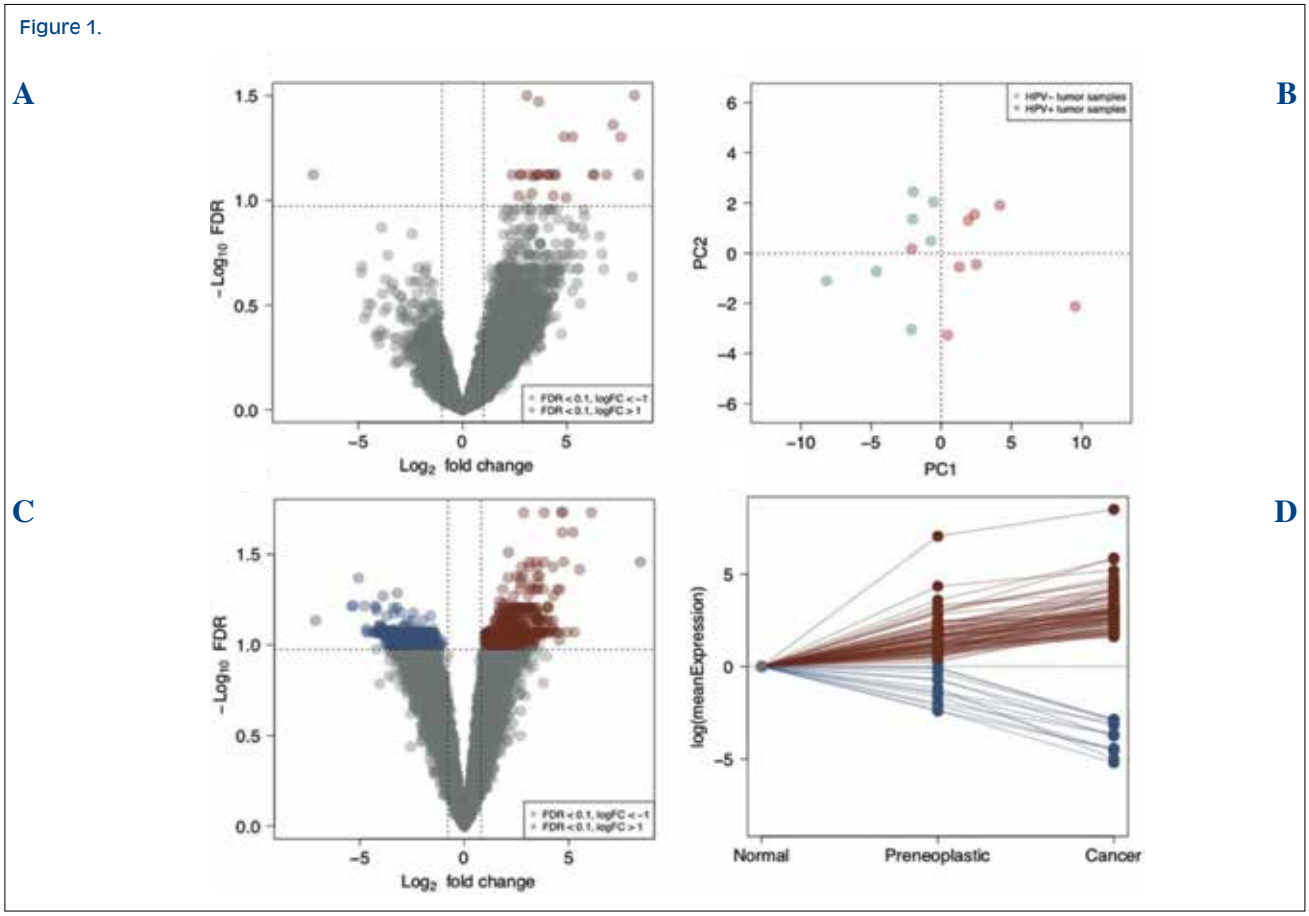
M. Paudice[1], J. Taramasco[1], L. Penuela[2], F. Buffelli[2], E. Fulcheri[2], I. Caffa[3], A. Nencioni[3], V.G. Vellone[1 4]

*[1] Anatomia Patologica, DISC, Università di Genova; [2] UOSD Patologia Feto-perinatale, IRCCS Istituto Giannina Gaslini, Genova; [3] Geriatria, DIMI, Università di Genova; [4] UO Anatomia Patologica Universitaria, IRCCS Ospedale Policlinico San Martino, Genova*

**Objective.** Proliferation index and tumor angiogenesis are considered prognostic factors in many malignancies. However, their quantification by immunohistochemistry (IHC) is not completely objective, being in part operator-dependent. We evaluated the proliferation index and microvascular density (MVD) by IHC in a mouse xenograft model of breast cancer and we quantified them using an image analysis software. Moreover, we focused on IHC changes after different treatment protocols.

**Methods.** The mice were allocated to six cohorts with different treatment protocols (controls, A, B, C, A+C, B+C). Twenty samples of breast cancer were histologically analyzed. We considered histological features as grading, necrosis, fibrosis, Tumor Infiltrating Lymphocytes (TIL), mitotic index (n° mitoses/10HPF), apoptotic cells (n° apoptotic cells/10HPF), and immunohistochemical markers for proliferation index (Ki67) and microvascular density (CD31 and CD105). IHC staining has been quantified by image analysis software Fiji-ImageJ®. After the selection of regions of interest (ROI), digital photographs of 4080x3072 Pixels^2 x 600 were taken and saved in Tiff format. The proliferation index with Ki67 was evaluated by manual counting, percentage of area (%A) and

Figure 1.



percentage of cells with semi-automatic counting (%C). MDV (CD31+ and CD105+) was evaluated as %A. All data were entered in a Microsoft Excel© spreadsheet and for statistical computation MedCalc© program was used.

**Results.** Quantitative image analysis proved to be reliable. The semi-automatic (%C) counting of Ki67 has shown an high correlation coefficient (Pearson correlation coefficient r=0,8; p<0,0001) when compared to manual counting, confirming its reliability other than its high grade of automation. MDV (CD105+) showed better results than MDV (CD31+) because of the cross- reaction with inflammatory cells in the latter. Regarding the Ki67 index, a decreasing and a paradoxical increasing were observed respectively after the treatment with B+C and B. Regarding MDV quantification (CD105+), a decreasing and a paradoxical increasing were seen respectively after the treatment with C+A and B, whereas MDV (CD31+) showed only heterogeneous results.

**Conclusions.** Quantitative image analysis allows to extract a numerical value from IHC assays, in an objective and reproducible manner, providing its possible utility in routine lab set. In particular, the %C resulted more rapid and automated than the other methods, reducing the operator-related confounding factors. Moreover, it helped us in revealing how some treatments -in our study- may reduce the proliferation index and tumor angiogenesis in breast cancer.

# QUALITÀ E SICUREZZA

## ROLE OF CYTOTECHNICIAN AT THE PASSAGE TO HPV TEST IN THE CYTOLOGY- SCREENING UNIT OF ASL BARI

V. Rubini[1], M. Russo[1], M. Iacobellis[2]

*[1] Cytotechnician; [2] Pathologist: Cytology-Screening Unit, PO Di Venere-ASL BA, Bari, Italy*

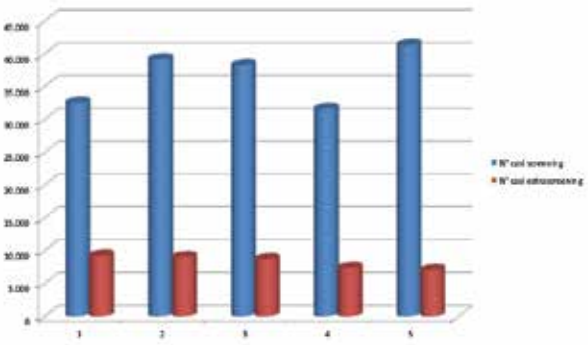
**Background.** Cervical cancer screening has traditionally been based on cervical cytology. Given the aetiological relationship between human papillomavirus (HPV) infection and cervical carcinogenesis, HPV testing has been proposed as an alternative to Pap-test [1 2]. The aim of this study is to define the cytotechnicians role in the cervical cancer screening at the passage to HPV test in ASL Bari.

**Methods.** The cytotechnicians a Biomedical Laboratory Professional with a scientific literacy in cytology and clinical skills to screen samples (he should not confuse true-negative results with false-negative results). He evaluates specimens and examines cellular samples under a microscope, looking for the presence of abnormal cells.Then he marks them and makes a preliminary diagnosis (Fig. 1).

**Results.** In the last five years the performances of Cytology and Screening Unit of ASL Bari were of 224.543 Pap test (Fig. 2, Tab. I). The cytotechnicians examined negative samples and controlled each phase (from the first analysis to the final evaluation of specimens). They worked under the direction of a pathologist who evaluated only the apparently positive samples [3]. The cytotechnicians carried out the analytical cycle with a workload range from 6 to 10 slide/hour (i.e. 36-60/day or 7,500/year) if the duty was limited to the screen only [4], but unfortunately the biggest problem of the Unit was the delay of TAT (turn around time) due to the chronic shortage of staff.

Figure 1. Work cycle of the cytotechnician.


Figure 2. Cytology Unit Performances in last five years.



Tab. I. Cytology Unit Performances in last five years.

| Year | Pap test screening | Pap test extrascreening | Total |
|---|---|---|---|
| 2013 | 32.546 | 9.276 | 41.822 |
| 2014 | 39.167 | 9.049 | 48.216 |
| 2015 | 38.282 | 8.746 | 47.028 |
| 2016 | 31.608 | 7.404 | 39.012 |
| 2017 | 41.373 | 7.092 | 48.465 |

**Discussion.** The new screening algorithm with HPV should reduce cytotechnicians workload because only women 25 to 34 years of age should be screened every three years with cytology alone (Fig. 3), while women 35 to 64 years of age should be screened every five years with HPV onlytesting (Fig. 4).

**Conclusions.** In our opinion the reduction of Pap test's number in the era of HPV testing will be an advantage for our Unit because it will allow us to provide a good quality control, such as inclusion of rapid prescreening and/or 100% rapid review of the cervical cytology examination, reducing false-negative results of routine screening. Consequently, cytotechnicians lowest workload will improve the diagnostic sensitivity and the reporting times will be shortened at the same time.

Figure 3. Cervical algorithm for women 25 to 34 years old.


Figure 4. Cervical algorithm for women 35 to 64 years old.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## NOT ONLY SCREENING: SCHOOL-WORK ALTERNATION EXPERIENCE

C. Violante[1], C. Cianci[1], M. Ciarmoli[1], M. Iacobellis[2]

*[1] Technician; [2] Pathologist: UOSVD Servizio Centralizzato Aziendale di Citopatologia e Screening, P.O. "Di Venere", ASL Bari*

**Background.** School-work alternation is an innovative teaching method, which through practical experience helps to consolidate the knowledge acquired at school and to test the attitudes of students in the field, to enrich their training and to orientate their studies and, in future work, thanks to projects in line with their study plan. The alternation between school and work, compulsory for all students of the last three years of high school, is one of the most significant innovations of law 107 of 2015 (The Good School) in line with the principle of open school. A

Figure 1.

Figure 2.

cultural change for the construction of an Italian way to the dual system, which incorporates good European practices, combining them with the specificities of the productivefabric and the Italian socio-cultural context. Starting from the 2018/2019 school year, following the entry into force of the legislative decree 13 April 2017, n. 62, the performance of the work-school alternation activity will constitute a requirement for admission to the final state exams of secondary education courses. In this regard, in fact, article 13, paragraph 2, letter c) of the aforementioned legislative decree states that "the student in possession", among others, are admitted to the state exam "carrying out work alternating school work as provided for by the study address in the second two-year period and in the last year". Article 12, paragraph 2 of Legislative Decree 62/2017 also states that "in relation to the educational, cultural and professional profile specific to each field of study, the state exam also takes into account the participation in the activities of alternation school work "summarized in the student's curriculum", which the examination committee takes into

account in the course of the interviews and which constitutes, pursuant to the following article 21, attached to the final diploma issued as a result of passing the state exam. Finally, article 17, paragraph 9, of the legislative decree 62/2017 states that "during the interview the candidate will exhibit, through a brief report and/or a multimedia project, the experience of alternating school work carried out on the path of studies".

**Materials and Methods.** The "Istituto di Istruzione Secondaria Superiore Statale Elena di Savoia-Piero Calamandrei" had instituted a particular convention with ASL Bari to permit a special training for the student attending 4th and 5th year of school. Our Unit had given hospitality to many students from 2014 to date.

**Discussion.** Last school years a group of our students toke part at a competition promoted by Bari's Camera di Commercio, on job security, and they won a cash prize. Not least, these are young people, we have the opportunity to make an extraordinary outreach operation to the prevention of HPV and an excellent advertising campaign for screening for the prevention of cervical cancer.

**Conclusions.** In our opinion school-work alternation is positive and it is an experience to be promoted more widely in health facilities.

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D. Lgs 81 9 aprile 2008.

## PROGNOSTIC VALUE OF MACROH2A IN UVEAL MELANOMA

G. Broggi[1], L. Puzzo[1], L. Salvatorelli[1], G. Li Volti[2], D. Tibullo[2], A. Russo[3], M. Reibaldi[3], A. Longo[3], Bartoloni[1], R. Caltabiano[1]

*[1] Department of Medical and Surgical Sciences and Advanced Technologies, "G.F. Ingrassia", Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Anatomic Pathology Section, University of Catania, Catania, Italy; [2] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy; [3] Department of Ophthalmology, University of Catania, Catania, Italy*

**Objectives.** Uveal melanoma (UM) is the most frequent primary intraocular neoplasm in adults. Rare cases are reported in adolescents, children, and even infants. It has been suggested that most of them arise from preexisting benign nevi. Other risk factors for UM include a fair complexion, light irides, oculodermal and ocular melanocytosis, and type 1 neurofibromatosis. Although malignant melanoma may be located at any point in the uveal tract, the choroid and ciliary body are more frequent sites [1]. UMs have been divided into three types: 1) spindle cells; 2) epithelioid cells; and 3) mixed cells. UM is biologically distinct from cutaneous melanoma (CM) by a very strong propensity to metastasize the liver. Histone variants are chromatin components that replace replication-coupled histones in a fraction of nucleosomes and confer particular characteristics to chromatin [2]. H2A variants represent the most numerous and diverse group among histone protein families; macroH2A.1 is a specific H2A variant whose expression has been studied in several neoplasms so far, including CMs. In literature it has been demonstrated that macroH2A.1 levels gradually decrease during CM progression and a high expression of macroH2A.1 in CM cells relates to better prognosis [3,4]; accordingly, the aim of our study is to evaluate for the first time macroH2A.1 as possible prognostic markers in UM.

**Materials and Methods.** Pathological reports and clinical data from 56 cases of primary choroidal and/or ciliary body melanoma treated by primary enucleation at the Eye Clinic, University of Catania, Catania, Italy, during the eight years up to May 2014, were retrospectively reviewed. The study consisted of 32 UMs without metastasis and 24 Ums with metastasis; the presence of metastasis was assessed using standard modalities, including physical examination, liver ultrasound and total body computed tomography. The macroH2A.1-staining status was identified as either negative or positive. Immunohistochemistry positive staining was defined as the presence of brown chromogen detection within the nucleus. Stain intensity and the proportion of immunopositive cells were assessed by light microscopy. Intensity of staining (IS) was graded on a scale of 0–3, according to the following assessment: no detectable staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3, as described previously. The percentage of macroH2A.1-immunopositive cells (Extent Score (ES)) was scored as a percentage of the final number of 100 cells in five categories: <5% (0); 5–30% (+); 31–50% (++); 51–75% (+++), and >75% (++++). Counting was performed at 200× magnification. The staining intensity was multiplied by the percentage of positive cells to obtain the intensity reactivity score (IRS), with a minimum value = 0 and a maximum =12; we considered IRS values ranging from 0 and 6 as low expression of macroH2A.1, and IRS values between 7 and 12 as high expression.

**Results.** 23/24 patients with metastatic melanoma showed high macroH2A.1 immunohistochemical expression (95,8%); instead, a low macroH2A.1 expression has been found in 24/32 patients without metastasis (75%). Finally, a strong association between the expression of macroH2A.1 and prognosis in UM has been observed.

**Conclusions.** A reduction of macroH2A.1 expression has been traditionally reported during tumor progression in several neoplasms, including CMs. However, due to its cell replication–stabilizing activity, an over expression of macroH2A.1 could be also observed in malignant tumors, and, in particular, in neoplasms with low proliferative index. A less aggressive biological behavior of UV might explain the different levels of expression of macroH2A.1 compared to CM. Due to its blocking role in the cell cycle, macroH2A.1 would make cells less sensitive to the action of chemotherapy, and this would suggest an additional role as a predictor of response to therapy. We first studied macroH2A.1 expression in UMs and our data seem to confirm its prognostic value in neoplasms with low proliferating activity and high metastatic potential.

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# PATOLOGIA TESTA

## LOW GRADE PAPILLARY SCHNEIDERIAN CARCINOMA: AN EMERGING SINONASAL MALIGNANT NEOPLASM

S. Carnevale[1 2], P. Morbini[1 2], G. Ferrario[1 3], F Sovardi[1 4], M Benazzo[1 4]

*[1] University of Pavia; [2] Department of Molecular Medicine; [3] PhD in Experimental medicine; [4] Otolaryngology unit*

**Introduction.** The sinonasal tract (nasal cavity and paranasal sinuses) is lined by a peculiar transitional-ciliated epithelium of ectodermal origin, the Schneiderian epithelium, which is encountered only in this site. Like any epithelium, it can develop benign and malignant neoplasms: the most known and common benign neoplasms are sinonasal papillomas, associated with either EGFR somatic mutation or HPV infection [1]; the WHO classification distinguish Scheniderian papillomas into inverted, exophitic and oncocytic types. Inverted and oncocytic variants have a low, but not trifling, risk of malignant progression to conventional squamous cell carcinoma (SCC), estimated in literature between 5% and 10% [2]. Dysplasia of surrounding squamous metaplastic epithelium is often observed near a malignant transformation of a Schneiderian papilloma [3], suggesting a multi-step pathogenesis, like other kind of malignancies. Lewis, in 2015 [3], and Jeong, in 2017 [4] reported an hitherto undescribed sinonasal tumor resembling an inverted papilloma, with bland cytologic features and granulocytic infiltrates, presenting an infiltrative pattern of invasion with bone destruction, increased mitotic activity and malignant behavior with local recurrences and, in the Lewis' case, metastatic disease. The name "low-grade papillary schneiderian carcinoma (LGPSC)" has been proposed for this entity, to highlight the absence of typical SCC features. We describe the histological, molecular and clinical features of a recent case consistent with LGPSC, and we also reconsidered a diagnosis made in our hospital in 2009. We observed two different clinical backgrounds in these cases.
**Materials and Methods.** We have analyzed hematoxylin and eosin stained sections obtained from formalin fixed paraffin embedded specimens; immunohistochemical reactions were performed on Dako OMNIS automated platform with antibodies again citocheratin 7, 19, an 34BetaE12, p53 and p16. HPV-DNA and specific genotypes were investigated with the INNO-Lipa HPV-genotyping Extra II (Fujirebio). Mutations in exons 18-21 of EGFR gene were assessed with PCR amplification and direct sequencing of the coding sequence with an Applied Biosystems 3130 Genetic Analyzer. Clinical records were reviewed to find possible risk factors or comorbidities. Cases report. The first patient was a 68 year-old male, former heavy smoker, with diabetes, who underwent nasal biopsy to confirm the clinical suspicion of Schneiderian papilloma. The second one was a 64 years-old female, active smoker, with an history of breast cancer, B-cell lymphoma (not-specified) and HCV-related chronic hepatitis, who underwent repeated resections of multiple vegetating papillary lesions of the upper respiratory tract. Both cases presented as a soft, pink-grayish papilloma with exophytic growth obstructing the upper airways. While the most recent case developed in the right nasal cavity, the former one was located at the tongue base. The lesions measured cm 1,2 and cm 1,5 respectively. Histologically, both cases showed the proliferation of epithelial basaloid cells, with complex architecture and papillary features, arranged in expansive masses, with mild or no keratinization, important granulocytic infiltrate, and increased mitotic activity (up to 5/10 HPF). The invasive growth of the lesions was hard to assess due to the fragmentation of the samples. The stroma showed no particular reaction to the infiltration. The adjacent epithelium was hard to evaluate due to sampling artifacts, but it appeared as typical schneiderian transitional epithelium with focal squamous metaplasia. Immunostains were consistent with squamous differentiation.

**Figure 1.** Case 1, HE stain, 400x. Basaloid appearance, granulocitic infiltrate and mitotic activity.

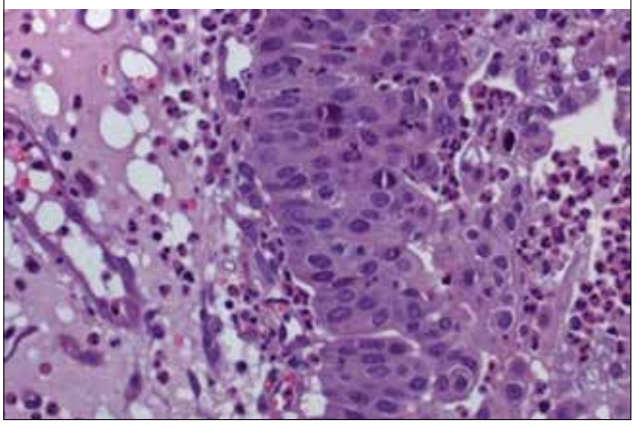


**Figure 2.** Case 2, HE stain, 20x. Complex architecture.

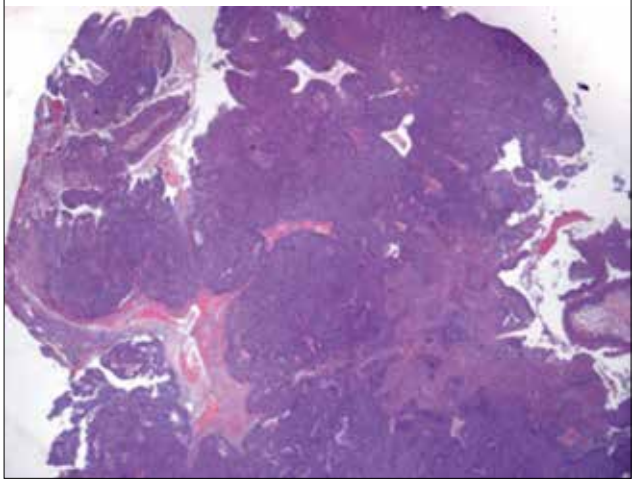
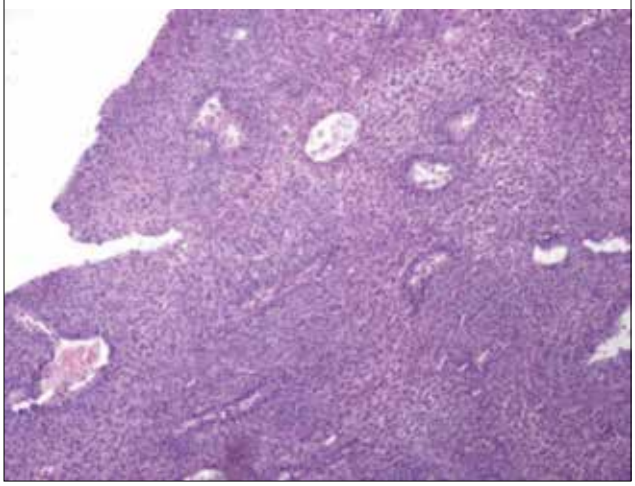


**Figure 3.** Case 1, HE stain, 20x. Papillary appearance.



p16 was positive in both cases, with a peculiar pattern (more positive in the basal layers), prompting HPV analysis: high risk HPV-52 was detected in the second case, while the first one was negative for HPV-DNA. p53 was found to be expressed partially (50-75%), with the same basal-to-upper layers gradient pattern, in both cases, resembling the other two cases in literature. Furthermore, no EGFR activating mutations were detected. Both patients are still alive and apparently disease-free at 12 and 120 months from the diagnosis.

**Discussion.** The presented cases were morphologically similar to LGPSC, described by Lewis and Jeong, even if the second one has been developed outside of the nasal cavity. This suggests that LGPSC can represent a morphological pattern, arising in different sites, and that different oncogenic pathways (including HPV) may lead to their development. Despite the evidence of recurrent EGFR mutations in Scheniderian papillomas and related SCC, EGFR mutations were not observed in our LGPSC cases, suggesting their origin is independent from benign papillomas of the Schneiderian epithelium, although the precursor lesion is still undetermined. Follow-up of our patients will be crucial for evaluating whether the surgical treatment of these lesions is appropriate to cure the early stages of the disease. It is important that head and neck pathologists distinguish this type of neoplasm from conventional benign papillomas, in order to recommend radical resection, whenever feasible, and a close follow-up given that the natural history of reported cases is characterized by a high recurrence rate, possible distant metastases and patient death [3].

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## FKBP-51 AS A RELIABLE PROGNOSTIC FACTOR AND A POTENTIAL THERAPEUTIC TARGET, IN UVEAL MELANOMA

R.M. Di Crescenzo[*1], S. Varricchio[*1], V. Napolitano[1], G. Ilardi[1], D. Russo[1], F. Merolla[2], M. Mascolo[1], A. Borzillo[1], E. Del Prete[1], R. Carandente[3], F. Tranfa[3], S. Romano[4], M.F. Romano[4], S. Staibano[1]

*[1] Department of Advanced Biomedical Sciences, Pathology Section, University of Naples "Federico II", Italy; [2] Department of medicine and health sciences, "V. Tiberio", University of Molise, Campobasso, Italy; [3] Department of Neuroscience and Reproductive and Odontostomatological Sciences, University of Naples "Federico II", Italy; [4] Department of Molecular Medicine and Medical Biotechnology, University of Naples, Federico II, Italy; * These authors equally contributed to this work*

**Introduction.** Uveal melanoma (UM) is the most frequent intraocular neoplasia. Up to 50% of patients are therapy- unresponsive and develop metastatic disease over 5 years, with a median survival of 6-12 months. Recently, several potential predictors of metastatic disease, overall survival and potential therapeutic targets have been studied [1 2] but, up to date, the results have been disappointing.

**Objective.** FKBP51 belongs to the immunophilin protein family, play a role in immune regulation and is involved in the regulation of cell proliferation, apoptosis and resistance to chemo- and radio- therapy [3]. The aim of our research project was to test the prognostic role of the immunohistochemical expression of FKBP51 in UM.

**Materials and Methods.** We evaluated the immunohistochemical expression of FKBP51 on paraffin-embedded tissue sections from 50 patients with UM surgically treated with enucleation, collected from 1984 to 2015. All patients had a long-term complete follow-up. The resulting data were analyzed for statistical significance with the SSPS softwere.

**Conclusions.** We found FKBP51 protein overexpressed only in UM cases with negative outcome, these results indicate that FKBP51 is a promising marker to identify patients with high risk of metastasis, beyond the standard prognostic parameters. The availability of specific drugs targeting FKBP51, in addition, makes this protein a very promising target for new molecular therapies against metastatic UM.

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## HER2 STATUS IN SINONASAL INTESTINAL-TYPE ADENOCARCINOMA

V. Maffeis[1], R. Cappellesso[1], A. Zanon[2], D. Cazzador[2], E. Emanuelli[2], A. Martini[2], A. Fassina[1]

*[1] Department of Medicine, Surgical Pathology & Cytopathology Unit, University of Padova, Padova, Italy; [2] Department of Neurosciences, Otorhinolaryngology Section, University of Padua, Padua, Italy*

**Objective.** Human epidermal growth factor receptor 2 (HER2) amplification/overexpression is a reliable tumor predictive marker in breast and gastric cancer (the latter more common in intestinal subtype). HER2-targeted therapies remarkably improved the overall survival of patients with HER2- positive cancer. HER2 has been found amplified with different frequencies in adenocarcinomas of various other sites [1], including the sinonasal intestinal-type adenocarcinoma (ITAC). HER2 overexpression in ITAC has been documented by immunohistochemistry (IHC), also with prognostic implications [2 3]. Nevertheless, the tumors tested with cytogenetic methods showed normal HER2 gene number [1 4], or just occasionally amplified cases (two solid type of ITAC) [5]. These contradictory results deserve further investigation. Indeed, ITAC is morphologically and immunophenotypically similar to primary intestinal adenocarcinomas and has an invariably dismal prognosis. The potential identification of a subgroup of ITAC patients that could benefit from HER2-targeted therapy justify the present investigation. Moreover, based on previous IHC/FISH studies and on ultrastructural, histochemical, and IHC similarities between ITACs and colorectal adenocarcinoma, it is conceivable that analogous oncogenes, such as HER2, could be involved in the pathogenesis of both malignancies [2]. Aim of this study was to assess HER2 status at both the protein and DNA levels in a large series of ITACs.

**Materials and Methods.** HER2 status was assessed by both IHC and chromogenic in situ hybridization (CISH) in 48 FFPE

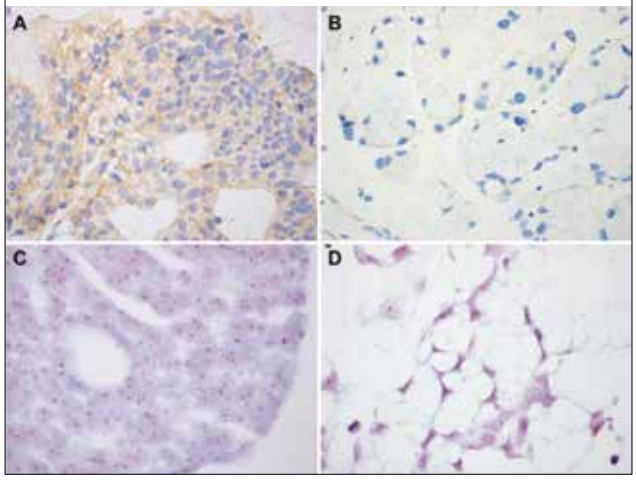


**Figure 1. A)** HER2 2+ IHC (case#41); **B)** HER2 negative IHC (case#5); **C-D)** CISH demonstrated HER2 non-amplified status.

samples of ITAC. IHC was evaluated using the four-tier score developed for gastric cancer: 0 = no reactivity or membranous reactivity in <10% of tumor cells; 1+ = faint/barely perceptible reactivity in ≥10% of tumor cells in part of their membrane; 2+ = weak-moderate lateral/basolateral/complete membrane reactivity in ≥10% of tumor cells; 3+ = strong lateral/basolateral/complete membrane reactivity in ≥10% of tumor cells.

**Results.** As for IHC, 85.4% (41/48) of ITACs were scored 0, 12.5% (6/48) 1+, and 2.1% (1/48) 2+. No HER2 amplifications were detected by CISH. Four cases were solid type, and all resulted non- amplified.

**Conclusions.** The present is the largest series of ITACs tested for HER2 status. Both techniques failed revealing HER2 overexpression/amplification. Contrary to previous studies, our findings ruled out any oncogenetic role of HER2 in ITAC pathogenesis (even in the solid type of ITAC).

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## SPANX-C IMMUNOEXPRESSION IN UVEAL MELANOMA


L. Salvatorelli[1], L. Puzzo[1], A. Russo[2], A. Longo[2], M. Reibaldi[2], M. Ragusa[3], R. Caltabiano[1]

*[1] Department of Medical and Surgical Sciences and Advanced Technologies, G.F. Ingrassia, Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Anatomic Pathology Section, School of Medicine, University of Catania, Catania, Italy; [2] Department of Ophthalmology, University of Catania, Catania, Italy; [3] Molecular, Genome and Complex Systems BioMedicine Unit, Department of Biomedical Sciences and Biotechnology, University of Catania, Catania, Italy*


**Background.** Uveal melanoma is a rare disease but it is the most common primary intraocular malignant tumor in adults with poor late prognosis. About 50% of patients will develop liver metastasis far from the enucleation within 10-15 years.. Because of this strange behavior, which still has to be better investigated, the authors suggest to search the biological causes to explain late development of liver metastasis of uveal melanoma. The SPANX multigene family includes SPANX-A1/-A2/-B/-C/-D (Human genome build 36.2). SPANX-A/D genes are made up of two exons separated by an intron of ≈650 bp and were the first SPANX genes described because of their expression in sperm cells [1]. Semiquantitative fluorescent multiplex PCR dosage analysis was carried out to identify 16 classes of SPANX-B and 13 classes of SPANX-C genes [1]. In the literature have been reported immunohistochemical studies on Spanx expression in two melanoma cell lines, VMM150 and VMM5, and in the metastatic melanoma tumor from the VMM150 cell line. These studies revealed the immunostaining at the nuclear periphery, within the nucleoplasm close to the nuclear envelope in the VMM150 cells, while only cytoplasmic staining was observed in the VMM5 melanoma cells. In addition, also the tumor cells from which the VMM150 cell line was derived were positive at the nuclear level [2]. AIM In the present study, we examined SPANX-C expression levels in 55 primary uveal melanomas, both with and without metastasis, and we evaluated their association with other high-risk characteristics for metastasis to assess if SPANX-C can be used to predict the behavior of uveal melanoma.

**Materials and Methods.** This study included a total of 55 patients with primary choroidal and/or ciliary body melanomas, treated by primary enucleation at the Eye Clinic, University of Catania, during the eight years up to October 2017. Immunohistochemical expression was assessed as positive when brown chromogen was seen in the nucleus, cytoplasm or cellular membrane. Stain intensity and proportion of immunopositive cells was assessed by light microscopy. Intensity of staining (IS) was graded in four levels: 0=no detectable staining, 1=weak staining, 2=moderate staining, 3=strong staining. The percentage of SPANX-C immunopositive cells (Extent Score, ES) was scored in five categories: <5% (0); 5–30% (+); 31–50% (++); 51–75% (+++), and >75% (++++). Staining intensity was multiplied by the percentage of positive cells to obtain the intensity reactivity score (IRS); IRS <6 was considered Low expression (L-IRS), IRS >6 was considered High expression (H-IRS).

**Results.** In the whole group (n=55) the median SPANX-C value was 4. SPANX-C expression was high in 23 (41.8%) melanomas, and low in 32 (58.2%) melanomas. In 32 primary uveal melanomas without metastasis, SPANX-C IS was strong/moderate in 11 cases (34.4%) and weak in 6 cases (18.8%). Fifteen cases (46.9%) were completely negative; ES was >50% in 10 cases (31.3%), variable between 5-30% in 7 cases (21.9%). Only 9/32 cases (28.1%) showed H-IRS, while the remaining 23 cases showed L-IRS (71.9%). (Fisher's exact test, p=0.009). In 23 primary uveal melanomas with metastasis, SPANX-C IS was strong/moderate in 19 cases (82.6%) and weak in 3 cases (13%). Only 1 case (4.3%) was completely negative. ES was >75% in 12 cases (52.2%), >50% in 3 cases (13%), 30-50% in 6 cases (26.1%), < 30% in 1 case (4.3%). 14/23 cases (60.8%) showed H-IRS, while only 9 cases (39.2%) had L-IRS (Fisher's exact test, p=0.009).

**Conclusions.** SPANX-C overexpression in cancer cells has little influence on primary tumor growth, but may be sufficient to determine an invasive phenotypes in which cells are able to change the morphology of their nucleus, adapt to vessel diameter and exit blood vessels [3]. SPANX gene overexpression could make the tumor less responsive to target therapy against the microenvironment and new therapy preventing the development of trailblazer cells could be investigated. Our results confirm

the potential role of SPANX-C in tumor behavior: we suppose that the assessment of SPANX-C expression in liver metastasis may be useful to confirm and explain our data.

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# Venerdì, 19 ottobre 2018

Sala Glass House – 9:00 - 11:30

# UROPATOLOGIA

## SMALL CELL (NEUROENDOCRINE) CARCINOMA OF THE URINARY BLADDER: ANALYSIS OF CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC FACTORS IN 30 CASES

A. D'Amuri[1], C. Gabrieli[1], V. Moretti[1], M. Filotico[1], G.C. Carluccio[2], F. Floccari[3], F. Sanguedolce[4], M. Brunelli[5]

*1Anatomic Pathology Unit, "Card. G. Panico" Hospital, Tricase (LE) - Italy ; 2Urology Unit, "Card. G. Panico" Hospital, Tricase (LE) - Italy; 3Clinic Pathology Unit, "Camillo De Lellis" Hospital, Manfredonia (FG) - Italy; 4Department of Pathology, University Hospital of Foggia, Foggia - Italy; 5Pathology Unit, Department of Pathology and Diagnostic, University and Hospital Trust of Verona, Verona - Italy*

**Introduction.** Small cell (neuroendocrine) carcinoma of the bladder (SmCC) is a rare disease, representing 0.5 to 1% of bladder tumours. It is a specific histological entity characterized by rapid metastatic dissemination and poor prognosis. Through analysis and summarization of clinicopathological features, immunohistochemical expression, pathological diagnostic criteria, prognostic and other factors in patients suffering from bladder neuroendocrine carcinoma, a better understanding of SmCC could be achieved to provide solid evidence for clinicopathology and prognosis. Until now few protocols encounter small cell neuroendocrine carcinoma with detailed clinico-pathological features among clinical trials. We reviewed the parameters visuable at morphology among a series of small cell carcinoma arising from urothelium.

**Material and Methods.** We retrospectively studied a large series of bladder SmCC from a single institution. The patients included twenty-two (22) men and eight (8) women. The ages of onset ranged from 63 to 90, with the median age being 78. The clinicopathological data of 30 cases of SmCC with up to 5-year follow-up data (median follow-up=650 days) were analyzed retrospectively based on immunohistochemical staining. Survival analyses were carried out using the Kaplan-Meier method and tested with the log-rank method. Multivariate Cox regression analysis was adopted to screen independent risk factors affecting patients survival. Histologically, referring to the WHO standard of neuroendocrine tumor classification.

**Results.** All bladder SmCC were presented at advanced stage with tumors invading the muscularis propria and beyond (n=30). SmCC was pure in 25 cases and mixed with other histologic types in 5 cases, including urothelial carcinoma (UC) (n=3), UC in situ (n=1), and squamous (n=1) features. Most SmCC expressed neuroendocrine markers synaptophysin, chromogranin, CD56 and NSE; however, they did not express UC luminal markers CK20, GATA3, and uroplakin II. Some SmCC showed focal expression of CK5/6, a marker for the basal molecular subtype. During the follow-up period, 10 patients died. The 1-, 3- and 5-year overall survival (OS) rates were 76.92%, 74.36% and 69.23%. The patient's survival was significantly associated with cancer stage but did not show significant difference between mixed and pure SmCC. Compared to conventional UC at similar stages, SmCC had a worse prognosis only when patients developed metastatic diseases.

**Discussion and Conclusions.** In conclusion, bladder SmCC is an aggressive disease which is frequently present at an advanced stage with a poor prognosis. Immunohistochemistry revealed that CD56, Syn, CgA, NSE, TTF-1, CKAE1/AE3, CK7, CK20, CK5/6, CK34Beta12, GATA3, uroplakin II, p63, HMB45, S-100 protein, p53, CD117 (c-Kit), Ki-67, and CD45 (LCA), immune markers play important roles in diagnosis, differentiation and prognosis. A fraction of SmCC show a basal molecular subtype, which may underlie its good response to chemotherapy. In some studies the inactivation of the RB1 gene may be implicated in the oncogenesis of bladder SmCC. Many factors, including the patient's age, size and shape of the tumor, operative method, perineural invasion, vascular invasion, distant organ metastasis and pathological type, show great difference in influencing OS time of patients, among which the size of the tumor, no invasion, vascular invasion and distant organ metastasis are independent risk factors affecting prognosis (survival time). Radical cystectomy is the prior alternative to treat this tumor and subsequently chemotherapy (cisplatin and etoposide). The management of neuroendocrine carcinoma of the bladder is not standardized and requires a multidisciplinary consultation. For us the recognition of this rare entity should enable better detailed tumour clustering when designing clinical trials using drugs targeting patient affected by small cell neurondocrine phenotype of urothelial carcinoma.

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## CYTOLOGICAL CRITERIA OF THE PARIS SYSTEM FOR HIGH GRADE UROTHELIAL CARCINOMA (HGUC): STRICT RULES OR USEFUL ADVICES?

V. Fiorentino, P. Straccia, T. Musarra, G. Fadda, F. Pierconti

*Università Cattolica del S. Cuore, Fondazione Policlinico "A. Gemelli", Roma*

**Background.** In late 2015 the International Academy of Cytology and the American Society of Cytopathology published the guidelines of reporting urine cytology, known as The Paris System for Reporting Urinary Cytology (TPS).

**Objectives.** After the publication of these criteria, one of the most important questions for those take care of urinary cytology is if these criteria are relevant in daily practice or if an interpretation of these cytomorphological features of malignancy are needed for identifying high grade urothelial carcinoma.
**Material** and Methods. We herein highlight all the criticism of the TPS criteria as they are reported by the recent papers present in the literature, indicating the morphological changes that cytopathologists frequently encountered when examining urine specimens and that represent the major limiting factors for fulfilling TPS criteria for HGUC.
**Results.** Vaickus et al. reported an overestimation of N/C ratio among pathologists, while Zhang et al founded an overestimation of the same parameter for intermediate values. According to Hoda et al and Orel SR and Viehl P, N/C ratio was affected by cytopreparation methods. Hang JF et al found as critical aspect the fact that N/C ratio was affected by fixation methods, while Suh J et al found that N/C ratio was affected by the type of urinary specimen. Cowan et al reported that cellular degeneration affected N/C ratio and also Pierconti et al showed that cellular degeneration after therapies affected this parameter. Renshaw AA et al. found a very low N/C ratio in "high grade urothelial carcinoma resembling umbrella cells". Lastly, Pierconti et al evaluated hyperchromasia, showing that nuclear hypochromasia was found in HGUC, while Cowan et al reported nuclear hyperchromasia in degenerated cells suggestive of HGUC. These last authors also showed that irregular nuclear membrane/ irregular coarse or clumped chromatin wasn't present in the nuclei with jet black and smooth glassy chromatin in HGUC and also that the instrumentation could affect irregular nuclear border.
**Conclusions.** Our opinion is that introducing a modified TPS classification is not needed in order to make a correct diagnosis of HGUC in urinary specimens, but it is crucial to consider that the criteria of TPS are specific but not exclusive for a diagnosis of malignancy, considering them useful advices and not strict rules. The urothelial neoplastic cells may have a combination of cytomorphological criteria wider than the four criteria indicated by TPS and the cytological diagnosis of HGUC has to rest on the assessment of the entire case, using all the nuclear and cytoplasmic features observed, considering also as important criteria clinical data, type of samples and preparation method of urinary specimens.

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# DERMOPATOLOGIA

## DIAGNOSTIC UTILITY OF CYCLIN D1 IN MESENCHYMAL BLAND-LOOKING SPINDLE CELL LESIONS OF THE SKIN: A PRELIMINARY STUDY

G. Broggi[1], G.M. Vecchio[1], G. Fuccio Sanzà[1], G. Buttafuoco[1], S. Spadola[1], G. Angelico[2], L. Salvatorelli[1], G. Magro[1]

*[1] Department of Medical and Surgical Sciences and Advanced Technologies, "G.F. Ingrassia", Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Anatomic Pathology Section, University of Catania, Catania, Italy; [2] Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi", Section of Anatomic Pathology, University of Messina,, Messina, Italy*

**Objectives.** Cyclin D1 is a G1-specific cyclin that plays a crucial role in regulating cell cycle progression in G1/S transition. It is overexpressed in several malignant tumors, including carcinomas, sarcomas, lymphomas, EWS/pPNET [1], and neuroblastomas/ ganglioneuroblastomas of children and adolescents [2]. Depending on tumor histotype, the variable expression of cyclin D1 is mainly due to different genomic alterations, including chromosomal translocation or amplification, post- transcriptional regulation or post-translational protein stabilization [3]. Mesenchymal bland-looking spindle cell lesions of the skin (dermis and hypoderm) represent a wide morphological and biological spectrum ranging from benign to malignant tumors, including tumor-like conditions such as nodular fasciitis. Differential diagnosis of these lesions may be challenging, especially if the pathologist is not familiar with soft tissue pathology. In the present study we investigated the immunohistochemical expression of Cyclin D1 to assess its utility in the differential diagnosis of the most common mesenchymal, bland-looking spindle cell lesions of the skin (dermis/ hypodermis). In this regard, cases of dermatofibroma, nodular fasciitis, dermatofibrosarcoma protuberans, neurofibromas, dermal scar, spindle cell lipoma, infantile myofibroma/ myofibromatosis, solitary circumscribed neuromas, deep benign fibrohistiocytoma, subcutaneous low-grade myxofibrosarcoma and 2 cases of dermal leiomyomas were selected and immunohistochemically stained with Cyclin D1 in order to assess as to whether this marker was differentially expressed.
**Materials and Methods.** Cases were retrieved from the pathologic files of Section of Anatomic Pathology at the University of Catania and retrospectively analyzed. Clinical data were obtained from the original pathologic reports. Tissues samples were collected from: 10 cases of dermatofibromas, 10 cases of nodular fasciitis, 10 cases of dermatofibrosarcoma protuberans, 10 cases of cutaneous neurofibroma, 5 cases of dermal scars, 5 cases of spindle cell lipoma, 4 cases of infantile myofibroma/ myofibromatosis, 3 cases of solitary circumscribed neuroma, 3 cases of deep benign fibrohistiocytoma, 2 cases of subcutaneous low-grade myxofibrosarcoma and 2 cases of leiomyomas. All lesions were negative for cytokeratins. All cases of dermatofibrosarcoma protuberans, spindle cell lipoma showed diffuse expression of CD34. Neurofibroma and solitary circumscribed neuroma were stained with S100 protein. The cases of dermal scar, nodular fasciitis and myofibroma/myofibrobmatosis were variably stained with alpha-smooth muscle actin. The cases of dermal leiomyomas were stained with alpha-smooth muscle actin, desmin and h-caldesmon. Immunohistochemical analyses were performed using the standard avidin-biotin-peroxidase method with the Dako automated immunostainer (Dako autostainer link 48, Glostrup, Denmark). All sections

were incubated with anti-Cyclin D1 (SP4, NeoMarkers) (prediluted antibody) at pH 6.0 with an incubation of 60 min at room temperature. With regard to cyclin D1 immunostaining, the percentage of positively stained cells was assessed by semiquantitative optical analysis according to a four-tiered system (<1% of positive cell = negative; 1–10% positive cells = focal staining; 11–50% positive cells = heterogeneous staining; >50% positive cells = diffuse staining). Staining intensity was graded into weak, moderate, or strong intensity.

**Results.** All cases of nodular fasciitis exhibited nuclear immunoreactivity for Cyclin D1. With the exception of one case which exhibited a heterogeneous staining (30% of stained cells), the remaining 9 out of 10 cases showed a diffuse and strong immunoreactivity (from 50% to 95% of the stained cells). A similar diffuse and strong immunostaining for Cyclin D1 was also found in 3/4 cases of infantile myofibroma/ myofibromatosis. In contrast, Cyclin D1 expression was not detected in any case of dermatofibroma, dermatofibrosarcoma protuberans, neurofibroma, dermal scar, spindle cell lipèoma, solitary circumscribed neuroma, deep benign fibrous histiocytoma, low grade myxofibrosarcoma and dermal leiomyomas. The nuclei of endothelial cells of blood vessels were positively stained with Cyclin D1 and were used as internal positive control.

**Conclusions.** With the exception of infantile myofibromas/ myofibromatosis, of which we studied only a limited number of cases, a diffuse and strong immunoreactivity for cyclin D1 was found in almost all cases of nodular fasciitis. Although there is the need of a study with a larger number of cases, a diffuse expression of cyclin D1 seems to be very helpful as a confirmatory marker of nodular fasciitis when dealing with an alpha-smooth muscle actin-positive, bland-looking spindle cell lesion. Accordingly we suggest to include Cyclin D1 in the list of the immunohistochemical panel when pathologists face a cutaneous (dermal/subcutaneous) bland-looking spindle cell proliferation.

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## SOX10: AN IMPORTANT HELP FOR THE PATHOLOGIST IN DIFFERENTIAL DIAGNOSIS OF DESMOPLASTIC MELANOMA

G. Cazzato

Desmoplastic melanoma is a variant of spindle cells neoplasm that can be difficult to diagnose both clinically and histologically. Almost 50% of desmoplastic melanoma presents as amelanotic lesions and the tumor cells can resemble fibroblasts or Schwannian cells. In addiction, although S100 usually stains desmoplastic melanoma, the neoplasm may lack expression of commons melanoma markers ( Melan- A, HMB-45, MiTF, tyrosinase,). SOX10, a nuclear trascription factor that plays an important role in Schwannian and melanocytic cell differentiation, has shown to be a sensitive and specific marker of desmoplastic melanoma. We report the case of a 54-year-old woman with a itchy non- pigmented skin lesion on the chest diagnosed as desmoplastic melanoma.

**Case.** Woman, 54 years old, with a painful and itchy non-pigmented skin papule (4x2mm) on the chest appeared for 8 months clinically diagnosed as basocellular carcinoma or squamous carcinoma. Dermoscopy: aspecific pattern.

**Methods and Results.** Excisional biopsy was sampled, formalin fixed and paraffin embedded. H&E sections showed an intradermal spinde cells proliferation with desmoplatic stroma, mast cells and tumor infiltrating lymphocytes. Nuclei was enlarged and vagely rectangular with mild atypia. Mitotic rate was <1 mm/2. Neoplastic cells showed focal neurotropism. Other sections was immunostained for S100, Melan A, HMB45 and SOX10: neoplastic cells were positive only for S100 and SOX-10 while the common markers of melanoma (Melan-A and HMB-45) were negative. Proliferant index (Ki67) was very low. We made diagnosis of desmoplastic melanoma, IV Clark level, Breslow index: 0.9mm.

**Discussion and Conclusions.** Desmoplastic Melanoma is a pernicious lesion that simulates a mesenchymal neoplasm or a fibrotic process. Clinical impression is often that of scar, dermatofibroma, or basal cell carcinoma. In some cases there is a lentigo maligna as precursor lesion. Occasionally there is history of "junctional nevus" or "Clark dyplastic nevus" excised months or years before. Lesion arises in head and neck regions and rarely in legs and other sites. At scanning magnification, desmoplastic melanoma resembles a dense spindle cell proliferation as a dermatofibroma or a scar. Clinical history of excision of a "junctional nevus" can help to think to this neoplasm. The histological features of desmoplastic melanoma are: Spindle-shaped, fibroblast-like melanocytes, with an enlarged vaguely rectangular nucleus, fold and grooves in the nuclear membrane. There are no hypercromasia and pleomorphism. Mitoses are very rare. Junctional component is absent or there is just a bland increase in single melanocytes at the dermoepidermal junction. An important feature is that, in many desmoplastic melanomas, there is a intradermal proliferation and the epidermis seems to be uninvolved. Presence of a large fibrous mass with cells that surround follicles, vessels and nerves. Presence of mast-cells. Lymphocytic nodules. Important features that may suggest demoplastic melanoma are neural differentiation and neurotropism. Neurotropism that is more frequent in advanced stage, can be demonstrated by immunostaining for p75NGFR. Desmoplastic melanomas generally lack expression of Melan- A, HMB-45, MiTF, tyrosinase, but are positive for S-100, SOX-10. SOX-10 is a nuclear antibody expressed in melanomas, neurofibromas, myoepitheliomas, astrocytomas, carcinoids and by the sustentacular cells of pheochromocytomas and paragangliomas. It's expressed also in mast cells and, unlike S-100 protein, it's negative in lymphnode dendritic cells. SOX-10 helps differential diagnosis between residual or demoplastic melanoma and excision scar because, although fibroblasts and histiocytes strongly express S-100, they are negative or only weakly positive for SOX-10. MPNSTs and DMs express both S-100 and SOX-10, but the expression seems to be different: DMs exhibit diffuse and strong immunoreactivity for both markers whereas MPTNs tend to exhibit lower rates of immunoreactivity for S-100 and SOX-10 and the expression is not diffuse or strong except for epiteliod forms. Other two markers are positive in melanomas: WT-1 and GPC3. WT-1, positive in our case, is expressed also in neurofibromas and MPNST, while there are no data about GPC3 reactivity in MPNSTs. Neurotropism highlighted by p75NGFR and S-100 and SOX-10 immunostaining are the secrets to make diagnosis of desmoplastic melanoma.

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## HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL FEATURES OF MERKEL CELL CARCINOMA: A CLINICOPATHOLOGIC STUDY OF 25 CASES

A. D'Amuri[1], F. Floccari[2], R. Filotico[3], M. Filotico[1]

*[1]Anatomic Pathology Unit, "Card. G. Panico" Hospital, Tricase (LE), Italy; [2] Clinic Pathology Unit, "Camillo De Lellis" Hospital, Manfredonia (FG), Italy; [3] Dermatology Unit, "Di Summa - Perrino" Hospital, Brindisi, Italy*

**Introduction.** Merkel cell carcinoma (MCC) is a rare, rapidly growing, highly malignant neuroendocrine carcinoma of the skin, in which a novel polyomavirus (Merkel cell Polyomavirus - MCPyV) has been implicated as a causative agent. It presents as asymptomatic, red to purple nodule in the sun exposed areas of far skin elderly individuals, hence the predilection for the head and neck region, with a poorly understood prognostic features. We evaluated histological, immunohistochemical, clinical features and the prognostic value of morphologic characteristics in primary MCC with or without divergent differentiation (DD).
**Material and Methods.** We reviewed 25 MCCs, and analyzed new sections from formalin fixed paraffin embedded specimens of all primitive skin lesions of our archival material, clinical data and morphologic features: necrosis, lymphocytic infiltrate, mitotic count (MC), cell size, lymphovascular invasion (LVI), regional lymph-node, distant metastases, and complete immunohistochemical profile. We compared DD cases with pure MCCs.
**Results.** Mean age was 80 years, about eighteen were sun-exposed. Pure MCCs were predominant in sun-exposed areas (n=17). Microscopic examination showed a poorly differentiation dermal cancerous proliferation. Neoplastic cells, rather monomorphic, have reduced and ill-defined cytoplasm, identifying sometimes rounded vesicular nucleus and mitotic. Small-cell sized tumours (n=5) had LVI, LN+, a mean MC=16/mm$^2$. Two cases had DD (squamous and sarcomatous component in one, the latter in an exposed location). Morphologically they were large-cell-sized, had LVI and MC>40/mm$^2$. None of the pure cases had these three features. Twenty cases showed necrotic areas, and ten cases had a massive lymphocytic infiltrate tumor associated. Immunohistochemical profile in all cases demonstrated paranuclear dot-like pattern of positivity for CK20, and neuroendocrine markers: NSE, chromogranin A, synaptophysin and CD56, with a high Ki-67 proliferation index. The viral infection with immunohistochemistry for MCPyV have not been studied. Differential diagnosis includes metastatic neuroendocrine carcinoma (mainly MCC or pulmonary small-cell carcinoma), melanoma or Ewing sarcoma was posed in all cases. The analysis of overall survival (OS) was 27 months on an average.
**Discussion and Conclusions.** No single or combined feature predict survival. Our preliminary data suggest that small-cell-sized and non-sun-exposed pure MCCs in younger patients represent more aggressive forms. Moreover DD-MCC morphologic features may correlate with recurrence an poor prognosis. In other studies MCPyV infection seems to be correlate to specific sites (upper extremities and trunk), massive lymphocytic infiltration and necrosis. The prognosis of these tumors is poor, marked by the frequency of early recurrences but especially the rapid invasion of adjacent structures. Their management should be multidisciplinary requiring radiotherapy followed surgery and chemotherapy.

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## CONFOCAL MICROSCOPY-HISTOPATHOLOGY COMPARISON IN INFLAMMATORY SKIN DISEASES

L. Licata[1], A.E. Verzì[2], F. Lacarrubba[2], G. Micali[2], R. Caltabiano[1]

*[1] Department G.F. Ingrassia, Section of Anatomic Pathology, University of Catania, Italy; [2] Department G.F. Ingrassia, Section of Dermatology, University of Catania, Italy*

**Aims.** Laser scanning confocal microscopy has been widely used for melanocityc lesions and recently introduced to evaluate various skin inflammatory diseases [1]. Confocal microscopy allows to study epidermal layers and papillary dermis scanning and re-building them in greyscale images. Biopsy is the gold standard method to perform an accurate diagnosis mostly in challenging cases. Clinical- hystopathological comparative study is very useful in inflammatory diseases such as Psoriasis vulgaris, Eczema, Grover's disease, Lupus discoid, Lichen Planus. Experimental comparative method between horizontal histological sections and corresponding confocal microscopy images is documented in literature [2].
**Materials and Methods**. Double punch biopsies both 5 mm diameter were performed in 22 selected patients (7 female and 15 male) after informed consent and confocal screening. Selected diseases were Psoriasis (five cases), Eczema (six cases), Grover's disease (four cases), Lupus discoid (three cases), Lichen Planus (four cases). One biopsy was treated like traditional vertical histological section, one was destinated to "horizontally inclusion" so that seriated sections can document cutaneous layers from corneous until papillary dermis. Horizontal section reproduces histologically what the confocal microscopy shows in a grayscale image.
**Results.** Histological examination of hematoxylin and eosin-stained sections and confocal images revealed reproducible match of lesions specifities. Handheld reflectance confocal microscopy (RCM) in some dermatitis indicated the presence of thick cornified layer, with intracorneus aggregates of roundish, bright and little inflammatory elements. Polygonal, nucleated brilliant structures reflect hyperparakeratosis. Compared to healty RCM images, dermal psoriasiform papillae were visible more superficial, dilated and full of tortuous capillaries.

RCM images of granular and spinous layers in eczematiform epidermis showed dark hypo-reflective among clearer areas corresponding to fluid exudates collections. Interface dermatitis RCM images showed up ipergranulosis, damaged basal layers and apoptotic keratinocytes alike bulgy hyperreflective elements. Worm hole aspect of cutaneous papilla can be distinguished in RCM images. Follicolar plugging is most suggestive RCM image, with dark opening clugged by hyperreflective material overlapping to same histological colored image. Acantholitic disease horizontal sections showed epidermal fragmentation visible in RCM images like dark hypo-reflective spaces, in which inflammatory elements and acantholytic cells floated.
**Conclusions.** Comparative study of histological vertical and horizontal sections with RCM images is useful to dermatologist to have a better understanding of confocal microscope images. Possibility to create a panel of morphologycal matches can improve the choice of sampling area making pathologist's work easier. This method applied to diseases such as Micosis Fungoides could avoid in the first stage the need to repeat biopsy for diagnostic purposes.

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## CYTOPLASMIC EXPRESSION OF WT1 IS HELPFUL FOR CONFIRMING THE DIAGNOSIS OF DERMATOFIBROSARCOMA PROTUBERANS


E. Piombino, G.M. Vecchio, F. Motta, G. Broggi, A. Giorlandino, L. Salvatorelli, G. Magro

*Department of Medical and Surgical Sciences and Advanced Technologies, G.F. Ingrassia, Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Anatomic Pathology Section, School of Medicine, University of Catania, Italy*


**Aims.** WT1 gene encodes a zinc-finger transcription factor first identified as a tumor suppressor gene playing a key role in Wilms' tumor, but also involved in proliferation and apoptosis, depending upon the cellular context [1]. The cellular localization of the WT1 protein has been a matter of debate over the last two decades. The antibodies against the N-terminal portion (clone 6F-H2) revealed both cytoplasmic and nuclear expression, in contrast to the exclusive nuclear localization previously visualized by antibodies against the C-terminal portion (WT C-19 polyclonal antibody). There is increasing evidence showing the presence of WT1 protein within the cytoplasm in several tumors, suggesting its complex regulator activity in transcriptional/translational processes [2]. Interestingly, diffuse and strong WT1 cytoplasmic staining has been observed in both benign and malignant vascular tumors, infantile-type fibromatosis and fibrosarcoma, rhabdomyosarcoma, some neuroblastic tumors, benign and malignant peripheral nerve sheath tumors, gastrointestinal stromal tumors (GISTs), leiomyosarcomas, and epithelioid cell myofibroblastoma of the breast [3-5]. The diagnosis of bland-looking spindle cell lesions of the skin is often challenging. The use of a basic immunohistochemical panel, including cytokeratins and S100 protein, is mandatory to exclude epithelial or peripheral nerve sheath/melanocytic lesions, respectively. CD34 is usually used as marker of dermatofibrosarcoma protuberans. The aim of the present study was to investigate immunohistochemically the expression and distribution of WT1 (clone 6F-H2) in 62 cases of mesenchymal bland-looking spindle cell lesions occurring primarily in the dermis- hypodermis to assess the potential differential diagnostic utility of this transcription factor. Among these lesions, the distinction between dermatofibrosarocma protuberans and dermatofibroma/deep benign fibrohistiocytoma was emphasized.
**Materials and Methods.** The cases were retrieved from the pathology files of the section of Anatomic Pathology at the University of Catania. Clinical data were obtained from the original pathology reports. Tissues samples were collected from: 25 cases of dermatofibrosarcoma protuberans, 15 cases of dermatofibroma (classic type and cellular variants), 5 cases of dermal scars, 5 cases of spindle cell lipoma, 3 cases of deep benign fibrohistiocytoma, 3 cases of dermatomyofibroma, 2 cases of nodular fasciitis, 2 cases of leiomyomas, and 2 cases of solitary fibrous tumors. All lesions were negative for cytokeratins and S100 protein. All cases of dermatofibrosarcoma protuberans, spindle cell lipoma and solitary fibrous tumor showed diffuse expression of CD34. The two cases of solitary fibrous tumors were also stained with STAT6. The cases of dermal leiomyomas were stained with alpha-smooth muscle actin, desmin and h-caldesmon. Dermal scars, dermatomyofibromas and the cases of nodular fasciitis were variably stained with alpha-smooth muscle actin. Immunohistochemical analyses were performed using the standard avidin-biotin- peroxidase method (Dako autostainer link 48, Glostrup, Denmark). The antibody against the N- terminal portion of WT1(clone 6F-H2, from Dako) was used. With regard to WT1 immunostaining (both nuclear and cytoplasmic staining), the percentage of positively stained cells was assessed by semi-quantitative optical analysis according to a four-tiered system (<1% of positive cell = negative; ; 1–10% positive cells = focal staining; 11–50% positive cells = heterogeneous staining; >50% positive cells = diffuse staining). Staining intensity was graded into weak, moderate, or strong intensity.
**Results.** Neither nuclear nor cytoplasmic staining was obtained in all cases of dermatofibroma, spindle cell lipoma, dermatomyofibroma, nodular fasciitis, leiomyomas and solitary fibrous tumor. In contrast all cases of dermatofibrosarcoma protuberans exhibited a diffuse (70-90% of neoplastic cells) and strong staining for WT1, restricted exclusively to the cytoplasm of the neoplastic cells. In all cases examined, WT1 was detected in the cytoplasm of endothelial cells of intra- and extra-lesional blood vessels and this staining served as internal control.
**Conclusions.** The present study first shows a diffuse and strong cytoplasmic expression of WT1 in dermatofibrosarcoma protuberans, whereas its potential morphological mimickers, especially dermatofibroma and deep benign fibrohistiocytoma, are negative. Based on these findings, we confirm that a diffuse and strong cytoplasmic expression of WT1 protein is of complementary diagnostic value to the CD34 in confirming the diagnosis of dermatofibrosarcoma protuberans.

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# EMATOPATOLOGIA

## PRIMARY CENTRAL NERVOUS SYSTEM DIFFUSE LARGE B CELL LYMPHOMA WITH CORTICOID MITIGATED HISTOPATHOLOGIC FEATURES: A DIAGNOSTIC CHALLENGE FOR THE PATHOLOGIST

S. Fraticelli1 2, F. Capuano1 2, A. Bonometti1 2, R. Riboni1 2, M. Lucioni2 3, D. Bongetta3 4, L. Arcaini1 3 5, M. Paulli1 2 3

*[1] University of Pavia; [2] Anatomic Pathology; [3] IRCCS Policlinico S. Matteo, Pavia; [4] Neurosurgery; [5] Hematology;*

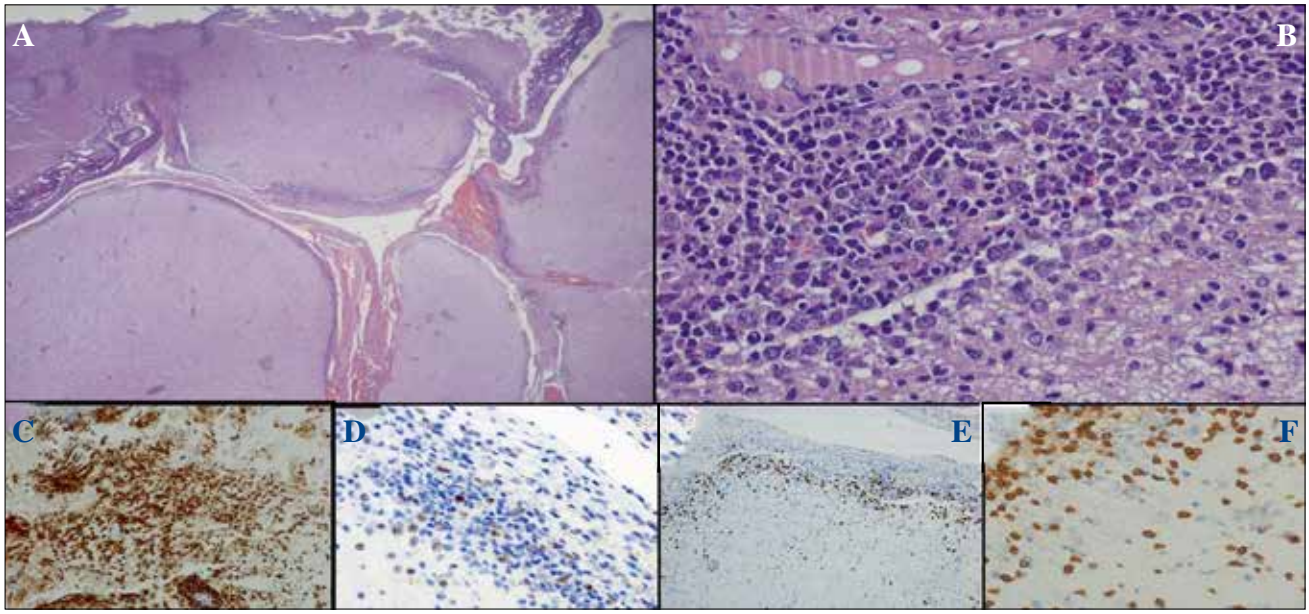
**Aims.** Primary central nervous system lymphoma (PCNSL) is a rare form of extranodal non Hodgkin lymphoma with an aggressive course [1]. Its annual incidence is 0,47 cases/100.000 [2] and it is increasing in the lasts decades [3]. The median age of presentation is 60 years [4]; most of patients are immunocompetent even tough immunodeficiency is one of the main risk factors [3]. The precise pathogenesis of PCNSL is still unknown [5]. Clinical presentation is often non-specific with a large numbers of symptoms (headache, seizures, vomiting, cognitive decline, etc.) [2 5 6]. Biopsy is required to achieve a final diagnosis of lymphoma. The aim of this report is to describe a case of PCNSL with atypical clinical presentation and peculiar histopathologic feature, related to prebioptic corticosteroid treatment.

**Methods.** We report the case of a 47-year-old man with an history of chronic hypertension, who was referred to our University Hospital in March 2018. He arrived in our ER with a 15-days history of dysarthria and confusion. At physical examination he was wakeful and he had no sensitive and visive deficit. Laboratory tests for EBV, HIV, HSV, VZV, HBV, HCV were negative. The cranial-contrast- enhanced-CT and the MRI confirmed multiple lesions in various areas of the brain. The clinicians suspected an autoimmune encephalopathy and they started a corticosteroid therapy. Two months later he came back to the ER for a head injury, since then he was confused and he could not keep the upright station. The physicians decided to conduct a cerebral biopsy.

**Results.** On gross examination there were multiple fragments of cerebral tissue (between 0.2 cm and 2.5 cm). Histological examination documented aspecific-inflammatory alterations with a mild infiltrate of reactive T lymphocytes (CD3+) (Fig. 1F) and histiocytes (CD68+), admixed with a minority of large atypical cells with centroblastic/immunoblastic morphology growing in a perivascular pattern and infiltrating leptomeninges (Figs. 1A, 1B). No angioinvasion was found. At the immunohistochemistry atypical cells were: CD20+ (Fig. 1C), CD79a+,c-myc+/-,bcl6-/+ (Fig. 1D), bcl2-/+, CD10-, MUM1+/- ("non-germinal-center B cell" histogenesis). ISH for EBV was negative. The proliferation rate (Mib1/Ki67) (Fig. 1E) was high in the atypical cells. The pathological features of atypical cells were consistent with a high grade large B-cell lymphoma. Based on the scarcity of neoplastic cells and on the recent corticosteroid therapy, we made the diagnosis of "DLBCL corticoid- mitigated lymphoma". As of today, the patient is still treated with chemio and radiotherapy at our Hematology division.

**Conclusions.** Primary central nervous system lymphoma (PCNSL) is a rare entity and it is a diagnostic challenge especially after corticosteroid treatment [4]; up to 95% of these neoplasm are diffuse large B cell lymphomas (DLBCL sec. WHO 2017) [2 3 7]. This type of lymphoma is morphologically indistinguishable from its systemic counterpart [3]. It is positive for mature B cells markers, most express bcl6 and MUM1/IRF4, while CD10 is often negative (90% of cases). Proliferative rate is usually high (70-90%) [2 4]. Among PCNSL, the so-called "DLBCL corticoid- mitigated lymphoma" was firstly described by Brück W. et al. in 2013 [8]. Typically, PCNSL presents as a

**Figure 1.** **(A)** HE 2x; **(B)** HE 40x; **(C)** CD20 20x; **(D)** 4bcl6 40x; **(E)** Ki 67 10x; **(F)** CD3 40x.



single lesion (60%) and there may be an meningeal involvement. The symptoms are aspecific and they can mimic different type of disease [6]. Usually PCNSL shows microscopic features of a highly cellular tumor. The lymphoid cells infiltrate the blood vessels and from these "perivascular cuffs" permeate the neural parenchyma. The neoplastic cells resemble centroblasts or immunoblasts. In addition, there is a reactive bystander infiltrate of small T and B lymphocytes, macrophages, activated microglial cells and reactive astrocytes [2 4]. On the contrary, the use of corticosteroid drugs before performing diagnostic biopsy is a clinical problem because the neoplastic cells are highly susceptible to steroid induced apoptosis. For this reason at histological evaluation the neoplastic cells may be present only in a small number or absent [2 4 8]. In addition after corticosteroid treatment, at least in PCNSL, an important reactive infiltrate may be observed, including T cells and macrophages exhibiting an high proliferative index, thus, raising the differential diagnosis with T -cell lymphoma or histiocytic neoplasm [4 8]. Others differential diagnosis to be considered are autoimmune disorders, infections and other primary and secondary CNS neoplasms (systemic NHL, glial tumors etc.) [4]. In conclusion, the corticoid treatment can mask the typical histologic features of PCNSL, making the diagnosis a challenge even for the experienced pathologist. Therefore it is important to take in consideration a stereotassic biopsy before starting any therapy. PCNSL has a worse outcome than systemic DLBCL. Nowadays, the prognosis has improved thanks to polychemotherapy and radiotherapy. Most protocols report median progression free survival of about 12 months and an overall survival of 3 years. Unfortunately, the long term survivors have an higher risk to develop delayed neurotoxicity [2 3 6 7].

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# PATOLOGIA MAMMARIA

## A RARE CASE OF A PRIMARY HIGH-GRADE ANGIOSARCOMA OF THE BREAST IN A YOUNG WOMAN WITH BREAST IMPLANTS


M.R. Campanino[1], M. Fuggi[1], D. Russo[1], F. De Rosa[1], M. Cieri[1], M. Menkulazi[2], V. Tammaro[2], M. Santangelo[2], L. Insabato[1]

*[1] Pathological Anatomy Unit, Department of Advanced Biomedical Sciences, University of Naples "Federico II", Italy; [2] General Surgery and Transplant Unit, Department of Advanced Biomedical Sciences, University of Naples "Federico II", Italy*


**Aims.** Mammary sarcomas are fairly rare neoplasms, they comprise less than 1 percent of all malignant tumors of the breast. Vascular lesions of the breast comprise a heterogeneous group that includes a variety of benign, atypical, and malignant lesions. Angiosarcoma is a rare soft tissue tumor of the breast. It occurs in both a primary form without a known precursor, and a secondary form that has been associated to a history of irradiated breast tissue. Available literature reports many papers focusing on post-radiation angiosarcomas, however only case reports or small series of patients affected by primary angiosarcoma were reported [1 2]. Association between mammary angiosarcoma and implants is excedingly rare. Very recently a small series of 11 primary angiosarcomas of the breast have been reported, however none showed association with mammary implants [3]. Angiosarcomas are aggressive and tend to have a high risk of local and metastatic recurrence [1]. We present a rare case of a primary angiosarcoma that developed in a young woman with breast implants.

**Materials and Methods.** Between January 2000 and August 2018 seven cases of angiosarcoma of the breast were retrieved from the files of the Department of Pathology, University of Naples Federico II, Italy. Six cases were post-radiation therapy angiosarcoma with a median age of 65.5 years (56-84 years) and one case was a primary angiosarcoma aged 31 years. We have focused our attention on this last patient. Two years ago she underwent in office practice to breast augmentation. One year later she complaining of pain and swelling in the left breast and an antibiotic treatment was made, however without benefit. Then, she was treated with several cycles of antibiotics, alternating with cortisone, anti-inflammatory and antifungal drugs for about six months, with slight improvement. Fine-neede aspiration was negative. She had no history of breast cancer in her family. On July 2018 she was admitted to the Breast Unit of "Federico II" Hospital of Naples-Italy, and underwent a wide left breast excision biopsy of cm 11x5x1.5. On section it appeared whitish with reddish areas and elastic consistency. Microscopically it showed breast tissue with a widespread proliferation of anastomosing vascular channels dispersed in a dense collagen stroma of perilobular and intralobular areas. Foci of cellular solid areas, composed of spindle-cells, with focal intravascular papillary formations were seen. There were some highly cellular areas with endothelial cells with well- defined nucleoli and high mitotic index (about 8 mitosis x10HPF), and some "blood lakes". Immunohistochemical analysis showed a highly expressed antinuclear antibody ERG (Erythroblast transformation-specific [ETS]-related gene) in the tumor cells. The proliferative activity, assessed with ki-67/MIB-1, was about 20%, focussing 75%. A diagnosis of a primary high-grade angiosarcoma of the breast was made.

**Conclusions.** We report a rare case of a high-grade angiosarcoma in a young woman with breast implants. Angiosarcomas are exceedingly rare and highly aggressive breast tumors. We comment the possible relationship between silicona's implants and angiosarcoma's genesis. To the best of our knowledge in the scientific literature only three cases related to this association have been described: 1. A high- grade angiosarcoma associated with ruptured breast implants; 2. A case of chest wall angiosarcoma associated with breast implants; 3. A breast angiosarcoma in a patient with multiple surgical procedures and breast implant [4-6]. Trought a review of the literature the adverse effects of silicone implants and angiosarcoma histogenesis were reviewed. It has been known that implanted materials can induce inflammation, blood vessel proliferation, and the possible development of angiosarcoma. The rarity of primary angiosarcoma suggests that even if there was a multifold increase for this cancer among women with breast implants, the resulting data would not have enough power to show a statistically significant change. The low incidence of this particular tumor, including a possible increase caused by implanted materials, would not affect overall breast cancer rates but is a complication of which clinicians should be aware.

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## DIAGNOSTIC ROLE OF IMMUNOHISTOCHEMISTRY IN THE EVALUATION OF METASTATIC BREAST CARCINOMA

A. D'Amuri[1], M. Filotico[1], F. Floccari[2], F. Sanguedolce[3], M. Brunelli[4]

*[1] Anatomic Pathology Unit, "Card. G. Panico" Hospital, Tricase (LE), Italy; [2] Clinic Pathology Unit, "Camillo De Lellis" Hospital, Manfredonia (FG), Italy; [3] Department of Pathology, University Hospital of Foggia, Foggia, Italy; [4] Pathology Unit, Department of Pathology and Diagnostic, University and Hospital Trust of Verona, Verona, Italy*

**Introduction.** Up to one third of patients with breast cancer will show evidence of metastatic spread over their course of disease. Common sites of metastasis in breast cancer, in addition to axillary and supraclavicular lymph nodes, include bone, liver, lung, pleura (with associated pleural effusion), brain, skin, gynecologic organs, and gastrointestinal tract, although any anatomic site can be the potential target. Therefore, pathologists often face the challenging task to confirm the diagnosis of a clinically suspicious metastatic breast cancer, to evaluate the possibility of breast origin in the workup for a metastatic tumor of unknown primary, or to distinguish a metastatic breast lesion from a new second primary carcinoma. Correct distinction in the latter scenario is critical not only for the different treatment the patient will receive but also for the different prognostic implication the diagnosis will confer. Although less frequently, non-breast primary tumors can disseminate to the breast. When evaluating metastatic lesions, clinical history, radiologic findings and review of prior slides are most important and more helpful than any special studies in distinguishing breast from non-breast tumor. However, the clinical history may not be revealing, and the prior slides may not always be available for review. In such situations, selected use of immunohistochemistry (IHC) markers may provide an invaluable diagnostic adjunct in elucidating the primary site. The aim of our study is to describe few unusual sites of metastatic breast cancer and provide an insight to IHC markers that aid in the identification of breast primary. Material and methods. We performed a retrospective analysis of metastatic breast cancer in our Institution, with particular location, diagnosed between 2016 to 2018. To determine the correct origin and the type (ductal vs lobular) of the tumours, immunohistochemical stains with mammaglobin, GCDFP-15, GATA-3, CKAE1/AE3, CK7, ER, PR, E-Cadherin, PAX-8, WT-1, CK20, CEA, CDX-2 were performed.
**Results.** We identified two patients with unusual sites of metastatic breast carcinoma. The first case was an incidental lobular breast carcinoma metastatic to an uterine endometrial polyp (tamoxifen-associated). The second case was a metastatic lobular carcinoma presented as a gastric- fundus lesions and bilateral ovary mass. Information of the patient's history of breast cancer were not noted. Immunohistochemical analysis revealed an immunophenotype consistent with breast lobular carcinoma. The tumour cells were positively stained for ER, PR, GCDFP-15, mammaglobin, GATA-3, CKAE1/AE3 and CK7; while negative for E-Cadherin, CK20, CDX-2, CEA, PAX-8 and WT-1.
**Discussion and Conclusions.** Metastatic breast cancer in unusual sites (gynecological and gastrointestinal) is possible, even uncommon, and pathologists must consider it in their practice, as a primary manifestation or as a late dissemination of disease, many years after the diagnosis of primary tumour.

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## DIAGNOSIS AND MANAGEMENT OF BREAST LYMPHOMA: A CASE SERIES OF 15 PATIENTS

V. Fiorentino, T. Musarra, G. Angelico, D. Arciuolo, M. Valente, F. Inzani, A. Santoro, F. Cacciatori, A. Carlino, A. Mulè, G.F. Zannoni

*Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy*

**Background.** Breast lymphoma (BL) is a rare form of extranodal lymphoma (ENL). BLs have been categorized into primary breast lymphoma (PBL) and secondary breast lymphoma (SBL) types. SBLs are more frequent than PBLs. Although mimicking clinical presentation and radiological features of primary breast cancer (BC), prognosis and treatment management are different. Therefore, differential diagnosis relies on pathological examination and biopsy is mandatory.
**Objectives.** We conducted a retrospective observational study with the aim to report prevalence, features and management of BLs at our institution.
**Material and Methods.** The database of our pathology institute was retrospectively searched for breast neoplasms coded as lymphoma from January 2000 to July 2018. The assessed data were: gender, age at diagnosis, laterality, stage (primary or secondary), histopathological diagnosis and grading (high vs. low) treatment, recurrence and survival status. Progression-free survival (PFS), overall survival (OS) and 5-year recurrence rate were estimated. An exploratory analysis comparing survival (PFS, OS and 5-year recurrence rate) between high- and low-grade histotypes and primary and secondary stages was performed.
**Results.** Fifteen patients were included in the analysis. All patients were affected by B-cell type lymphomas; the most frequent subtype was diffuse large B-cell lymphoma (DLBCL). Patients affected by high-grade lymphomas were treated with polychemotherapy followed in about half of cases by consolidative ipsilateral breast radiotherapy. Patients affected by low-grade lymphomas were treated with local treatment only (surgical resection +/– radiotherapy). No patient received radical surgery or axillary dissection. At a median follow up of 9 years, four patients had relapsed (all affected by high-grade lymphomas) with central nervous system (CNS) involved in half cases.

Both median PFS and OS were not reached, but a slight trend toward a better survival was noticed in favor of primary breast lymphoma (PBL), while a clearer survival benefit was observed in the low-grade cohort. The estimated 5-year survival rate was 77% within the whole population, 78% vs. 66% for primary vs. secondary and 100% vs. 66% for low-grade vs. high-grade BL.
**Conclusions.** Our results are in accordance with previously reported evidences concerning epidemiology, clinicopathological features and management of BLs. Indeed, we reported a 5-year survival rate of 77% in the whole population, which is in accordance with 40–80% rate previously showed. Moreover, relapses occurred in half cases within CNS. This is concordant with previous studies, where, despite administration of optimal treatment, extranodal progression has frequently been reported, involving mainly CNS and breast. Thus, given the high risk of CNS recurrence and the poor prognosis after such event, some authors hypothesized the inclusion of CNS-directed prophylaxis in the initial management of specific high-grade BLs.
Despite median PFS and OS were not reached, a slight trend toward a better survival was noticed in favor of PBL vs. SBL and a clearer survival benefit was observed in the low-grade vs. high-grade cohort. Moreover, all patients whose disease recurred were affected by high-grade histotypes. Thus, our data corroborate the evidence that low-grade BLs display an indolent trend and allow hypothesizing that PBLs present a better prognosis compared with SBLs.
Although being a rare disease, histological characterization of BLs is crucial in order to guarantee a tailored management. Indeed, high-grade BLs should receive a combination of chemotherapy and involved-field radiation therapy, whereas low-grade BLs require loco-regional treatment only.

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## DETECTION OF HUMAN PAPILLOMAVIRUS DNA IN BASAL-LIKE SUBTYPE OF INVASIVE BREAST CARCINOMA: A CASE SERIES OF 36 PATIENTS

T. Musarra, V. Fiorentino, G. Angelico, D. Arciuolo, M. Valente, F. Inzani, A. Santoro, F. Cacciatori, A. Carlino, A. Mulè, G.F. Zannoni

*Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy*

**Background.** Oncogenic HPVs are involved in breast cancer but their role in breast carcinogenesis is still debated. The involvement of a virus in carcinogenesis is demonstrated after its integration in host DNA with consequent expression of oncogenic proteins. It is well established that specific subtypes of HPV are a major cause of human cancers. HPVs are a group of host specific DNA virus with a remarkable epithelial cell specificity. More than 120 different HPV genotypes have been identified and almost 45 subtypes, isolated from the low genital tract, have been grouped into high- and low- risk HPV types, considering their risk potential to induce an invasive cancer. Although data about HPV prevalence in breast cancer, risk factors, genetic pathway and molecular pathogenesis are still very contrasting and dissenting, recent studies have proved the HPV presence in few breast carcinomas, by identifying the classic coilocytic alterations and defining the causal role of the virus in breast carcinogenesis. It is well known that HPV16 and HPV18 are able to promote the neoplastic transformation of ductal epithelial cells. Numerous Authors have identified some oncogenic HPVs, as well as HPV16/33/35, in invasive and/or metastatic carcinomas. Several studies have emphasized that in breast parenchima the favourite host cells for HPV infection are the basal cells of the mammary ducts, expressing membranous receptors as caveolin 1, CD44, CD151, integrin alfa2beta4, laminin, the same molecular markers identifying the basal-like phenotype of the breast cancer, which represents the 15-20% of the all breast cancers. Morphologically, breast basal type carcinomas are mammary malignant high grade neoplasias with sheets of small basaloid cells and large polygonal cells. Histologically, BLC have typical pushing margins, wide areas of comedonecrosis, interstitial lymphoid stroma and scant desmoplasia. The classical immunophenotype corresponds to the basal layer/myoepithelial cells.
**Objectives.** Aims of this work are to detect HPV DNA in basal-like breast cancer, perform genotyping and demonstrate the p16 overexpression in neoplastic cells in order to prove the possible carcinogenic HPV role in this specific tumoral type and to help the development of therapy and prevention for this aggressive group of breast carcinoma.
**Material and Methods.** 36 cases of formalin fixed-paraffin embedded breast basal-like carcinomas were studied by immunohistochemistry for p16 and by DNA PCR (L1-type specific primers).
**Results.** 28 cases out 36 showing p16 immunohistochemical overexpression were selected for molecular biology analysis. Amplification of DNA samples showed single HPV DNA infection in 10 cases (HPV16 in 4 cases; HPV18 in 2 cases; HPV45 in 4 cases). Single HPV DNA infection has also been observed in 4 controls of G3 ductal carcinoma NOS and double infection has been reported in only one control (HPV16/HPV18). The overall HR-HPV prevalence was 35,7% in breast basal like carcinoma.
**Conclusions.** Basal-like carcinomas account for 12-24% of all breast cancer, and almost all of them are triple-negative cancers (ER-, PR-, HER2-). There is a great overlapping between basal-like and HPV related squamous cell carcinomas of other sites (younger patients, same risk factors, same histological phenotype, aggressiveness, high histological grade) and in addition, several studies indicate a possible role for HPV in breast cancer, so that a common viral aetiology could be suggested. Several studies have emphasized that high risk HPVs, by transforming own circular dsDNA in a linear genetic fragment, can get into DNA of the host cell and, here, rule interactions between E6 viral oncoprotein and p53 tumour suppressor gene, and between HPV E7 and pRB tumour suppressor product. HPV-positive cancers show loss of p53, retinoblastoma RB pathway inactivation, and p16 upregulation. These steady molecular bonds lead to a progress in the cell cycle, an increased cell proliferation and a decreased apoptosis, contributing to carcinogenesis. By identifying among basal-like carcinomas, a subgroup characterized by HPV infection (35,7%), this study has shown that high risk HPV could contribute to breast carcinogenesis. Therefore, the new interesting approach to the study of the breast cancer should be based on the understanding of its molecular viral background and on the detection of over-expressed viral oncoproteins. The

investigation of oncogenic gene expression in HPV-related basal-like breast cancer and the study of its potential value as a predictor of neoplastic progression and clinical outcome could allow to characterize a possible evolutive morphological profile of breast malignancies. Finally, the employment of biomarkers improves the current diagnostic tools but also can contribute indirectly to therapeutics as a predictor of choice for the correct clinical management.

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## MOLECULAR SUBTYPING AND LYMPH NODE STATUS IN BREAST CANCER

M.I. Pastena[1], S. De Summa[2], F.A. Zito[1], S. Diotaiuti[3], E. Mattioli[1], A. Marzano[1], M. Siciliano[1], S. Tommasi[2], A. Paradiso[4]

*[1] UOC Anatomia Patologica, IRCCS-Istituto Tumori "Giovanni Paolo II"; [2] USD Diagnostica Molecolare e Farmacogenetica, IRCCS-Istituto Tumori "Giovanni Paolo II"; [3] Chirurgia ad indirizzo senologico, IRCCS-Istituto Tumori "Giovanni Paolo II"; [4] Oncologia Medica Indirizzo Sperimentale & Direzione Biobanca, IRCCS-Istituto Tumori "Giovanni Paolo II"*

**Aims.** The purpose of this study is to verify, in this single-center consecutive series of breast cancers treated at the IRCCS-Istituto Tumori "Giovanni Paolo II" of Bari, the correlation between molecular subtype classification of breast carcinomas according to St Gallen indications, involvement of axillary lymph nodes and sentinel lymph node (SLN) status.
**Materials and Methods.** The present survey was conducted in a consecutive series of 2002 cases of early breast cancer diagnoses classified molecularly (ER, PgR, Ki67, HER2/neu) according to the 5 categories of St Gallen 2011. These categories were then related to clinical node status or SLN status at time of primary surgery.
**Results.** 64,78% of cases resulted clinically positive for malignant nodes in axilla, while the remaining 35,22%, classified as clinically node-negative, received SLN biopsy. All cases were classified as: Luminal A (52.24%), Luminal B HER2/neu negative (17.03 %), Luminal B HER2/ neu positive (11.23 %), HER2 positive (not luminal) 8.89 %, Triple negative 10.59 %. The category with the greatest probability of finding clinically positive axilla is the Luminal B/HER2 positive with 76% of the cases, while the Luminal A has the lowest risk with 58.6%

Figure 1.

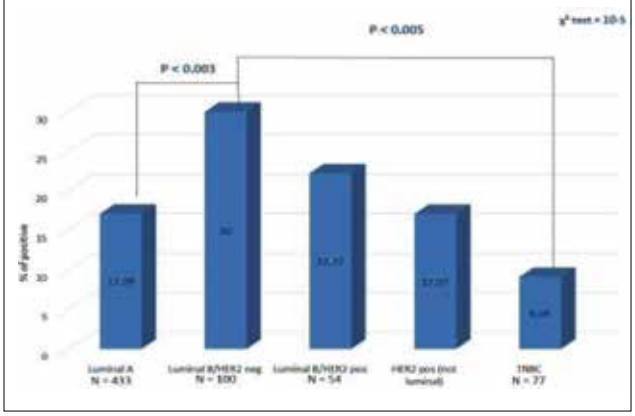



Figure 2.

MULTIVARIATE LOGISTIC REGRESSION

| | Odds ratio (95% CI) | p-value |
|---|---|---|
| **Genomic profile** | | |
| Luminal A | 0.82 (0.52÷1.29) | 0.39 |
| Luminal B/HER2 negative | 1.73 (0.94÷3.09) | 0.06 |
| Luminal B/HER2 positive | 1.63 (0.75÷3.28) | 0.18 |
| HER2+ (not luminal) | 0.76 (0.25÷1.87) | 0.58 |
| Triple negative | 0.58 (0.24÷1.21) | 0.18 |

of the cases (p<0.001). Below is the graph displaying the frequencies of molecular subtype and positive SLN. In addition, we performed a multivariate logistic regression, adjusted for tumor size and age, with SLN status as dependent variable. Results confirmed the increased risk to have a positive sentinel lymph node in the Luminal B/HER2 negative subset of patients (p=0.06), as shown in Figure 2.
**Conclusions.** Breast cancer molecular subtyping is able to predict clinical status of axillary nodes of patients candidate to primary surgery. However, such classification has a limited usefulness for prediction of sentinel lymph node status, probably due to the use of immunohistochemical surrogates to determine molecular subtypes. Biological parameters have been also used as continuous variables to perform statistical analyses and, in particular, to build up a decision tree; the results of this study will be presented later.

## ANDROGEN RECEPTOR, FORKHEAD BOX A1 EXPRESSION AND CLINICAL SIGNIFICANCE OF TUMOR-INFILTRATING LYMPHOCYTES IN TRIPLE NEGATIVE BREAST CANCER

C. Saponaro[1], M. Centonze[1], G. Opinto[1], F. Fanelli[1], R. Sozio[2], M. Zambetti[2], M.I. Pastena[2], Popescu[2], A. Mangia[1]

*[1] Functional Biomorphology Laboratory; [2] Pathology Department, IRCCS Istituto Tumori "Giovanni Paolo II", Viale Orazio Flacco 65, 70124 Bari, Italy*

**Background.** Triple negative subgroup of breast cancer (TNBC) still lacks targeted treatment options, despite in recent years great successful in the breast cancer (BC) management have been achieved [1]. Androgen receptor (AR) is a promising therapeutic target for BC even if its prognostic value is not clearly defined in TNBCs [2]. It regulates transcriptional activity by different transcription factors, including Forkhead box A1 (FOXA1). Further, tumor infiltrating lymphocytes (TILs) have been reported as prognostic marker in TNBC [4], even if little is known about their interaction with both AR and FOXA1. Herein we explored the potential correlation between the expression of these biomarkers and their possible role in TNBCs.
**Methods.** Expression of AR and FOXA1 was evaluated by immunohistochemistry in 124 TNBC patients with a long follow-up. For evaluation of AR mRNA expression a commercial kit (RNAscope® 2.5 High Definition (HD)-BROWN Assay, Advanced Cell Diagnostics, Newark, CA) has been used. Tissue sections were examined under a standard bright field microscope at 20-40X magnification. Positive signals were visible as brown punctate dots. TILs were performed in full-face hematoxilyn and eosin sections, strictly adhering to the criteria proposed of the International TILs Working Group 2014 [5].

Figure 1. **A)** Androgen Receptor I) negative and II) positive immunohistochemical protein staining and the corresponding III) negative and IV) positive mRNA expression detected by RNAscope. Scale bars = 20 µm. **B)** Representative images of I) negative and II) positive immunohistochemical staining for FOXA1 protein. **C)** A representative tissue samples with I) low TILs and II) high TILs density. TILs was performed in full-face H&E sections. Scale bars = 50 µm.

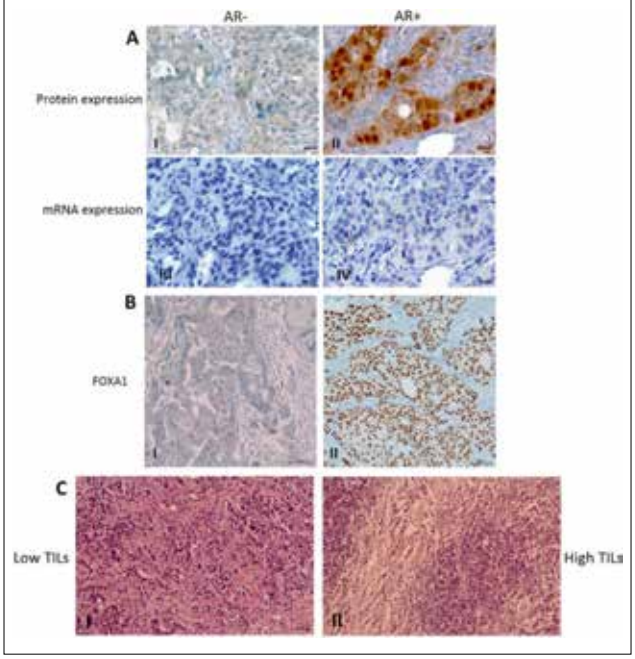
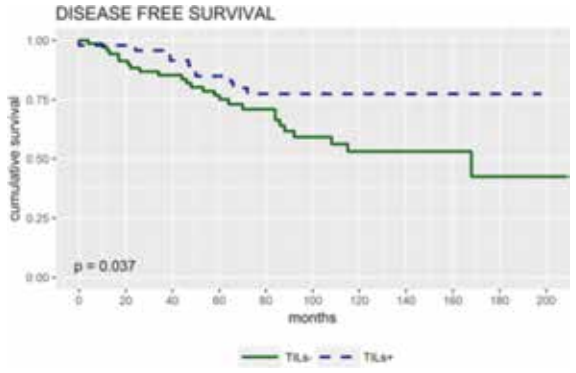



Figure 2. Survival curve for patients with TILs+ versus TILS- presence (p=0.037).




**Results.** AR was present in 87% (108/124) of tumors and the 14.8% (16/108) of these tumors were AR+. The RNAscope assay confirmed the immunohistochemistry data, showing AR mRNA expression in the same tumor samples (Fig. 1A). FOXA1 was present in 91.1% (113/124) of tumor cells and it was overexpressed in 32.7% (37/113) of them (Fig. 1B). AR expression was significantly associated with FOXA1 (p=0.007). Stromal TILs were present at a low level in 59.8% of the tumors (Fig. 1C) and we observed that tumors with positive FOXA1 expression presented low levels of TILs (p=0.028). Multivariate analysis identified the TILs as an independent prognostic marker for DFS (p=0.045). Further, the subgroup of patients with high TILs had a better DFS compared to patients with low TILs (p=0.037) (Fig. 2).

**Conclusions.** Our preliminary data show that TILs were the only independent prognostic factors in TNBCs and their presence or absence might affect also patient's clinical outcome. Moreover, the association between AR and FOXA1 suggest the useful of a combined evaluation of these biomarkers to identify a specific subgroup of patients.

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## VACUUM ASSISTED BIOPSY (VAB) IN BREAST PATHOLOGY: THE PREDICTIVE ROLE OF B3 CLASSIFICATION. A PROSPECTIVE MONOINSTITUTIONAL ANALYSIS ON 648 CONSECUTIVE VAB BREAST BIOPSIES OBTAINED UNDER TOMOSYNTHETIC GUIDANCE.

G. Musci, M. Bonzanini, E. Barresi, N. Mirabassi, D. Bernardi, V. Sabatino, M. Pellegrini, L. Resta, A. Pennella, M. Barbareschi

**Aim.** To evaluate the predictive value of B3 classification on VAB obtained under tomosynthetic guidance to define the best clinical management of non-palpable breast lesion.

**Introduction:** The management of B3 lesion is still controversial and the correct clinical management of patients depends upon different approaches. The guidelines of the Azienda Provinciale per i Servizi Sanitari (APSS) in Trento provide a grid for management of these patients, according to radiological and pathological parameters. These guidelines allow a dichotomous approach, consisting in surgical excision of B3 lesions with atypical features (atypical ductal hyperplasia ADH, flat epithelial atypia FEA, mucocele-like lesions) or strict follow-up for non-atypical lesions (radial scar/complex sclerosing lesions, papilloma without atypia). These guidelines were based on a previous retrospective study of ours performed on a series of 769 cases evaluated on the basis of stereotactic VAB breast biopsies using an 11 Gauge needle (Bernardi et al. Tumori. 2012 Jan-Feb;98(1):113-8).

**Material and Methods.** The series includes 652 nonpalpable breast lesions (microcalcification, distortion, opacity) observed from January 2013 until December 2015; 25 cases (male patient, lymphoma or women without follow-up data) were excluded. All patients with B3 diagnosis were followed for at least 12 months; patients which did not receive surgery were invited to repeat mammographic/senologic exam at 12 month intervals. All biopsies were obtained with tomosynthetic guidance using a 9 Gauge needle. Radiological and pathological classification were done according to European guidelines (Perry, European guidelines for quality assurance in breast cancer screening and diagnosis, 2006). B3 lesions were subdivided in two groups with and without atypia, according to the local guidelines (Percorso diagnostico terapeutico del carcinoma della mammella; https://www.apss.tn.it/percorsi-procedure-protocolli). For statistical purposes LN (ALH, CLIS classic type) was considered as risk factor and not included as positive in the evaluation of positive predictive value (PPV); B3 cases which were not surgically treated, but were free of disease at follow-up, were considered to have benign lesions. The series included 37 (6%)

Tab. I.

| | | Diagnosis on surgical excision | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N. | | Negative follow-up | Negative | Atypia | In situ carcinoma | Invasive carcinoma | PPV in situ carcinoma | PPV invasive carcinoma | PPV carcinoma |
| VABB | 148 | **Total B3** | 34 | 59 | 47 | 7 | 1 | **4,73** | **0,68** | **5,41** |
| | 11 | Papilloma | 7 | 3 | 1 | 0 | 0 | 0,00 | 0,00 | 0,00 |
| | 36 | Radial Scar | 16 | 18 | 2 | 0 | 0 | 0,00 | 0,00 | 0,00 |
| | 15 | Lobular Neoplasia | 5 | 2 | 8 | 0 | 0 | 0,00 | 0,00 | 0,00 |
| | 62 | **Total B3 without atypia** | 28 | 23 | 11 | 0 | 0 | **0,00** | **0,00** | **0,00** |
| | 35 | FEA | 3 | 12 | 17 | 2 | 1 | 5,71 | 2,86 | 8,57 |
| | 50 | ADH | 3 | 23 | 19 | 5 | 0 | 10,00 | 0,00 | 10,00 |
| | 1 | Mucocele-li ke lesions | 0 | 1 | 0 | 0 | 0 | 0,00 | 0,00 | 0,00 |
| | 86 | **Total B3 with atypia** | 6 | 36 | 36 | 7 | 1 | **8,14** | **1,16** | **9,30** |

cases classified as B1, 201 (32%) B2, 148 (23,6%) B3, 15 (2,4%) B4, 226 (36%) B5.

**Results.** Out of 37 B1 cases, 35 were followed clinically without evidence of disease and 2 underwent surgery (one of these proved benign and one showed LN). The positive predictive value (PPV) was 0. Out of 201 B2 cases, 197 were followed clinically with evidence of neoplastic disease only in one case after a follow-up of one year (after review of slides and mammograms, which proved to be negative, this case was considered as an interval cancer) and 3 underwent surgery (two of these proved benign, one showed radial scar and one showed a FEA). PPV was 0,5%. Out of 148 B3 cases, 114 underwent surgical excision; 34 were followed clinically and all were considered free of disease at a median follow-up of 3,6 years. Table shows the results of follow-up and surgical excisions and the PPVs for the whole category B3, for B3 lesions with and without atypia and for each histologically defined lesion. All of 15 B4 cases underwent surgery. PPV: 60% (9 cases were confirmed as in situ/invasive cancer, 3 cases were ADH, 1 FEA+LN, 1 LN, 1 negative). All of 221 B5 cases underwent surgery. PPV: 91,15%. The PPV B5 cases is influenced by the fact, that in 20 cases the lesion was very small and completely excised by the 9G VABB procedure, and the surgical specimens showed only the residual scar. All B5 cases with negative surgical specimen were re-evaluated and the diagnosis confirmed by an experienced breast pathologist.

**Conclusions.** Our study confirms that in our organization non-palpable breast lesions with a B3 diagnosis on VABB specimen should be: a) treated conservatively adopting an integrated clinico-radiological approach if the histological diagnosis is lobular neoplasia, radial scar or papilloma without atypia; b) should underwent surgery if the diagnosis includes any atypical feature (FEA, ADH, papilloma with atypia, mucocele-like lesions, low grade phylloid tumors). This approach can be adopted only if the health organization is able to provide a dynamic multidisciplinary diagnostic approach and an accurate follow-up of the patients. In fact, as far as the management and outcome of patients is greatly influenced by a variety of parameters (type of radiologic guidance, needle diameter, multidisciplinarity, …) each institution must verify the clinical outcome and define its own procedures.

## MOLECULAR AND HISTOPATOLOGIC PARAMÈTERS PREDICTIVE OF AXILLARY LVMPH NODE INVOI,VEMENT IN BREAST CANCER: AN ITALIAN MULTICENTRIC STUDY

C.B. Spigariolo[1], R.L. Boldorini[1], T. Addati[2], L. Ambrosiani[3], S. Bianchi[4], T. Caporusso[5], M. Castelvetere[6], D. Di Clemente[2], M. Feltri[3], S. Funes[7], G. Ficarra[8], C. Giardina[5], P. De Giuli[9], P. Graziano[6], D. lnchingolo[2], E. Macrì[1], A. Maiorana[8], R. Nenna[2], E. Pescarmona[10], I. Terrenato[1], M.C. Vivaldo[9]

*[1] AOU Maggiore della Carità (Novara); [2] Ospedale L. Bonomo (BT); [3] Ospedale Vaduce (Como); [4] AOU Careggi (Firenze); [5] Policlinico di Bari; [6] Casa Sollievo della Sofferenza (San Giovanni Rotondo); [7] Ospedale San Martino (Belluno); [8] Policlinico di Modena; [9] Ospedale San Lazzaro (Alba); [10] Regina Elena - IFO (Roma)*

**Background and Endpoints.** Breast cancer is the most frequent carcinoma-in women. Axillary lymph node involvement is ·one of the most relevant breast cancer negative prognostic factors. Curtently, sentinel lymph node biopsy (SLNB) is considered the gold standard for the diagnosis [1-3]. Presence of micro or macro-metastasis in sentinel lymph node (SLN) is related to the risk of non-sentinel node metastases ranging respectively from 20 to 50%. Therefore, most patients underwent unnecessary axillary lymph node dissections. Nowadays, axillary dissection [4] is indicated only in presence of macro-metastasis in SLN, detected by conventional histology/immunohistochemistry or by one-step nucleic acid amplification (OSNA). The first end-point of this multicentric retrospective study was to identify the main predictive factors of axillary non-sentinel node metastasis by developing a mathematical model that predict the probability of lymph node metastasis. The second aim was to validate this mathematical model on a wider retrospective population. The presence of independent factors of non-sentinel node metastasis ih the micro-metastasis subgroup was investigated as a surrogate end-point.

**Methods.** Histologic and molecular features of 282 breast cancers from two hospitals (Novara and Modena) were recorded. The patients enrolled underwent breast and axillary surgery after diagnosis of metastasis in sentinel lymph node, evaluated with OSNA method. The following parameters were·collected: turnar (T) size, grading (G), histologic type, lymphatic/vascular invasion (LVl), ER PgR status, HER2neu status, Ki67, molecular classification (Luminal A, Luminal B, HER-2 Like,

**Tab. I.** Results of invariate analysis of 282 patients.

| | Parameters | OR | P-value |
|---|---|---|---|
| Histology | Lobular vs Dutcal | 1, 15 | 0,639 |
| Grading | G2 vs GI<br>G3 vs GI | 1,60 6<br>2,02 | 0,398<br>0,215 |
| LVI | Sì vs No | 1,56 | 0,096 |
| ER | Pos vsNeg | 1,12 | 0,805 |
| PgR | Pos vs Neg | 1,11 | 0, 752 |
| HER2neu | Pos vs Neg | 0,77 | 0,451 |
| Ki67 | ≥ 20 vs < 20 | 1,14 | 0,588 |
| Molecular type | Luminal B vs Luminal A<br>Triple Negative vs Luminal A<br>HER2 vs Luminal A | 1,00<br>1,925<br>0,77 | 0,999<br>0,481<br>0,631 |
| T Size | | 1,039 | 0,001 |
| CK19 | Macro vs Micro | 5,7 | 0,000 |

**Figure 1.** ROC curve of number of CK19 mRNA.

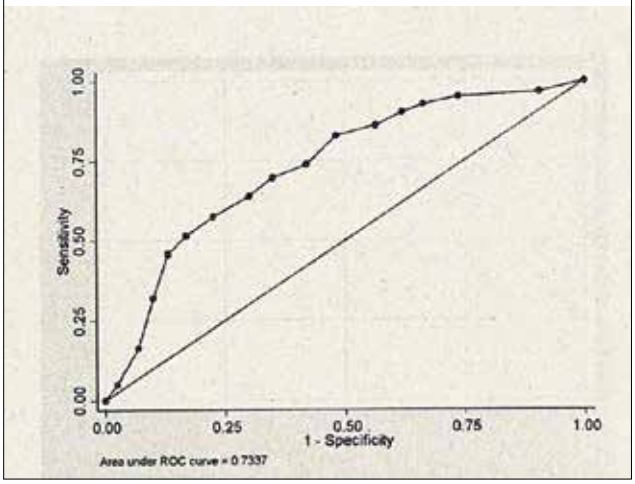



**Figure 2.** Variation in sensivity and specificity in relation to the risk cut off (903 patients).

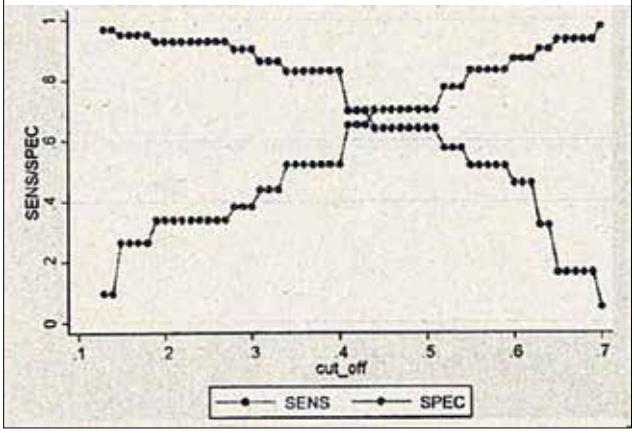



**Figure 3.** Variation in FP and FN in relation to the risk cut off (903 patients).

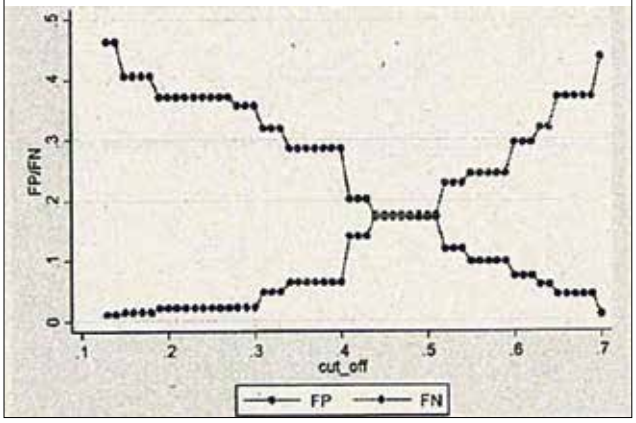



**Figure 4.** Variation in PPV and NPV in relation to the risk cut off (903 patients).

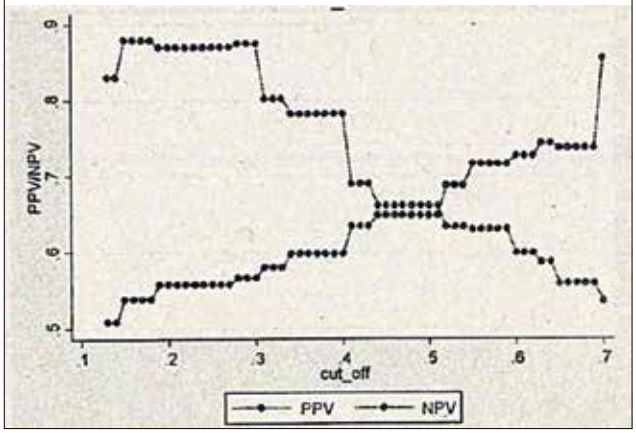
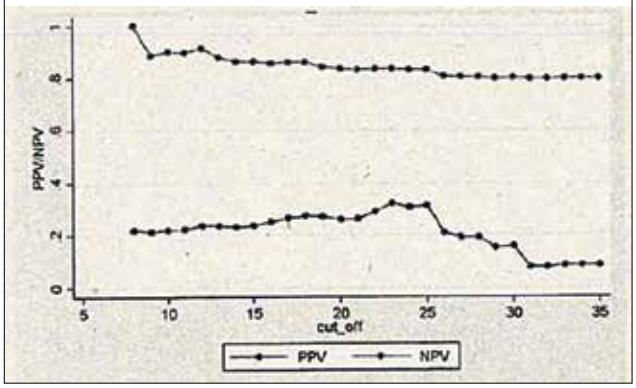



**Figure 5.** Variation in PPV and NPV in relation to the risk cut off in the micrometastasis subgroup (903 patients).




Triple negative), number of NSNs removed, number of positive NSNs removed, cytokeratin 19 (CKl 9) mRNA copy number of positive sentinel nodes. Firstly, an univariate analysis was conducted to define which ofthe above-listed parameters was statistically significant to predict non sentinel lymph node involvement. Secondly, the predictors were included in a multivariate analysis. Then the identified predictors were collected from all' Italian OSNA centers. 903 patients were totally recruited from ten hospital institutions. Statistic analysis were conducted with STATA 13 software.

**Results.** Univariate analysis in the first validation group of 282 patients indicated that only turnar size (p-value < 0,001) and mRNA copy number of CKl 9 (p-value < 0,001) were statistically significant independent predictive parameters (Tab. I).

The predictors were included in a multivariate analysis. From the coefficients of the multivariate analysis, a mathematical model which predicts the probability to-have axillary node metastasi was created. Both of the parameters were stratified in quartiles. The discrimination of the model quantified with the area under the receiver operating characteristics (ROC) curve (AUC) was 0,73 (Fig. 1). The risk cut off 0,34, obtained through the analysis of 903 patients, guarantees a good compromise between false positive (FP=28,6%) and false negative (FN=6,3%); sensitivity and specificity were respectively 87,1% and 44,2% (Figs. 2, 3, 4). After demonstrating that only the turnar diameter and mRNA copy number ofCKl 9 were statistically significant independent predictive parameters, it was exam ined whether a specific turnar size was predicfive of metastasis in the micro-metastasis subgroup (N=223) without reaching a statistically significant result.

**Conclusions.** This study indicated that the risk of metastasis in NSNs is related to both CKl 9 m.RNA copies, as evaluated by OSNA method and the size of turnar. Since these parameters are available during surgery, this mathematical model can be applied easily to suggest the need to perform or exclude the axillary dissection.

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## CD8/FOXP3 RATIO IN TRIPLE-NEGATIVE BREAST CANCER: CORRELATION WITH CLINICOPATHOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT


V. Tsvetkova[1], E. Orvieto[2], F. Miglietta[1], G. Griguolo[1], M. Lo Mele[3], L. Albertoni[3], S. Bonaguro[1], V. Guarneri[1 4], P. Conte[1 4], M.V. Dieci[1 2]

*[1] Department of Surgery, Oncology and Gastroenterology- DISGOG, University of Padova, Italy; [2] Department of Pathology, Azienda ULSS 18, Ospedale Santa Maria della Misericordia, Rovigo, Italy; [3] Department of Pathology, Azienda Ospedaliera; Padova, Italy; [4] Medical Oncology 2, Istituto Oncologico Veneto IOV-IRCCS; Padova, Italy*


**Background.** Triple-negative breast cancer (TNBC) is a subtype of breast cancer with aggressive clinical behavior. The main purpose of our research is to investigate heterogeneity of the immune microenvironment of TNBC and to correlate immune parameters with survival.

**Methods.** Our study population includes samples from 199 TNBCs patients all treated with neoadjuvant and/or adjuvant chemotherapy between 2000 and 2014 at the Istituto Oncologico Veneto of Padua. Immunohistochemistry was performed for CD8 (Monoclonal Mouse Anti-Human CD8, Clone C8/144B, Dako Cytomation, Glostrup, Denmark), FOXP3 (Monoclonal Mouse Anti-Human FOXP3, Clone 236A/E7, Abcam, Cambridge, MA, USA) and MNF116 (Monoclonal Mouse Anti- Human cytokeratin, Clone MNF116, Agilent DAKO, Denmark). The expression of CD8 and FOXP3 was evaluated across the whole histological section within tumor stroma. The analysis was performed on digital slides by using a specifically developed Visiopharm® software application. The software aligned, for each sample, three consecutive sections with the three immunoistochemical stains, to allow the correct evaluation of CD8 and FOXP3 on stroma, excluding MNF116 positive areas. CD8/FOXP3 ratio was calculated. TILs were assessed according to available consensus guidelines [1]. Metastasis-free survival (MFS) was defined as the time from diagnosis to distant relapse or death. Statistical analysis was performed by applying IBM SPSS Version 24.

**Results.** Most of the patients were diagnosed with ductal invasive carcinoma (91.4%) of grade 3 (87.2%) and high Ki67 >/=30% (83.9%). Median value of CD8/FOXP3 ratio was 4.28 (Q1-Q3 2.36-7.86); 37% of the patients had TILs >/=30%. Increased CD8/FOXP3 ratio was observed in case of low Ki67 (median CD8/FOXP3 ratio 6.65 and 3.98 in Ki67<30% and Ki67>/30%, respectively, p=0.037), G1-2 (median CD8/FOXP3 ratio 6.88 and 3.91 in G1-2 and G3, respectively, p=0.051) and high TILs (median CD8/FOXP3 ratio 5.33 and 3.56 in TILs>/=30% and TILs<30%, respectively, p=0.002). Median follow up was 74 months. No statistically significant association was observed between CD8/FOXP3 ratio and metastasis free survival (MFS). MFS rate at 3 years was 84.4% for patients with CD8/FOXP3 ratio > median vs 81.8% for patients with CD8/FOXP3 ratio < median. (HR 1.33, 95% CI 0.72-2.43, p=0.361). Patients with TILs>/=30% showed a trend towards a better MFS as compared to low TILs patients (MFS rate at 3 years: 90% vs 81%, respectively, HR 0.51, 95%CI 0.23-1.14, p=0.098).

**Conclusions.** In this large cohort of TNBC patients, we observed high levels of CD8/FOXP3 ratio. Tumors with high grade and high ki67 showed lower CD8/FOXP3 ratio, possibly reflecting cancer-mediated efforts to counterbalance the antitumor immune activation in biologically aggressive tumors. No association between CD8/FOXP3 ratio and MFS was observed. Based on this study, CD8/FOXP3 ratio may not be considered a useful parameter to refine prognosis prediction in TNBC.

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# PATOLOGIA ENDOCRINA

## IMMUNOCYTOCHEMICAL AND MOLECULAR DIAGNOSIS OF TALL CELL VARIANT OF PAPILLARY THYROID CARCINOMA ON LIQUID-BASED CYTOLOGY: A PRELIMINARY STUDY


C. Brunelli[1], P. Straccia[1], M. Curatolo[1], M. Dell'Aquila[1], S. Sfregola[1], A. Santoro[2], G.P. Petrone[1], M. Raffaelli[3], C.P. Lombardi[3], E.D. Rossi[1], G. Fadda[1]

*1 Division of Anatomic Pathology and Histology; 2 Div. of Gynecological and Breast Pathology; 3 Div. of Endocrine Surgery, Catholic University, Fondazione Policlinico "A. Gemelli", IRCCS, Rome, Italy*


**Background.** Tall cell variant (TCV) of papillary thyroid carcinoma (PTC) has been recognized as a form of PTC showing an aggressive biological behaviour. There are considerable controversies regarding the definition, clinical and pathological features of TCV because of its rarity and difficulties in its diagnosis. The American thyroid association guidelines task force

on thyroid nodules and differentiated thyroid cancer in 2015 redefined the tall-cell variant as a tumour consisting of over 30% of cells that are two or three times as tall as they are wide. However, thresholds for clinical significance of a TCV are not univocal in different studies. Our aim was to study the cytological features of TCV and to define the criteria for a preoperative diagnosis of such aggressive variant of PTC.

**Methods.** We included in our study 36 patients (27 women and 9 men) from January 2012 until June 2018 with a cytology preoperative diagnosis of PTC, processed with Thin Prep technique, and with a histological diagnosis of tall cell variant of PTC. The mean age of our group was 47 years (range: 26-94). Since our series included cases prior to the 2015 redefinition of TCV, PTCs were identified as TCV when that component constituted 50% or more of the neoplasm. Tall cells were defined as cells with their height at least twice their width, with an eosinophilic cytoplasm with the characteristic nuclear features of PTC (i.e., nuclear irregularity, clearing, overlapping, grooves, and pseudo inclusions). Cytological findings and FNAC diagnosis of each case were compared with the histopathological picture.

**Results.** Tumours with tall cell characteristics had a strong association with an older age at presentation, larger tumour size, high frequency of extra-thyroid extension, and BRAF mutation regardless of the percentage of tall cells. Among the 36 cases, 24 were preoperatively diagnosed as PTC (66.7%), 10 (27.8%) as suspicious for PTC (TIR 4) and 2 as follicular neoplasms (5.6%) No case was diagnosed as low-risk indeterminate lesion (TIR 3A) or benign (TIR 2). Five cases – three TIR 5 and two TIR 4 – resulted positive for both HBME-1 and galectin-3, no case resulted to be negative for the same panel. BRAF was found to have the V600E mutation in 7 out of 10 cases tested (70%).

**Conclusions.** TCV of PTC is usually large in size, it has a higher prevalence in older patients and it is more likely to extend to extrathyroidal tissues than classic PTC. TCV has also a greater tendency for local recurrencies and extranodal metastasis, and it is associated with higher mortality than classic PTC. TCV is usually diagnosed on histology after the surgery has been already performed but a cytological diagnosis of clear-cut PTC with a positive immunohistochemical panel for HBME-1 and galectin-3 and a mutation of BRAF V600E should prompt for an accurate evaluation of the neoplastic elements for the presence of tall cells.

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## TALL CELL VARIANT OF PAPILLARY THYROID CARCINOMA AND ATA RISK STAGING: A PRELIMINARY STUDY

T. Musarra[1], A. Rizzo[2], C. Brunelli[1], E.D. Rossi[1], A. Pontecorvi[3], C.P. Lombardi[4], M. Salvatori[2], G. Fadda[1]

*1 Division of Anatomic Pathology and Histology; 2 Div. of Nuclear Medicine; 3 Division of Endocrinology; 4 Div.of Endocrine Surgery, Catholic University, Foundation "Agostino Gemelli" School of Medicine,Rome, Italy*

**Introduction.** Papillary thyroid cancer (PTC) is the most common type of thyroid cancer, generally characterized by a good prognosis. However, some histological variants of PTC pursue a more aggressive course with the possibility of incomplete response after administration of radioactive iodine (RAI) and a higher risk of cancer recurrency according to the ATA Guidelines for the management of differentiated thyroid carcinoma. In particular, the tall cell variant (TCV) of papillary carcinoma represents the most frequent of these aggressive variants and it should be reported not only when it is the predominant pattern but indeed when only 10% of its features are present in an otherwise usual PTC. The aim of this study is to evaluate the correlation of the different rates of TCV with the clinical response.

**Methods and Materials.** From January 2016 to July 2017, 46 patients submitted to total thyroidectomy were diagnosed as having a TCV pattern of PTC at the histological examination at the Division of Anatomic Pathology and Histology of the Catholic University, Foundation Agostino Gemelli Hospital of Rome. The rate (%) of TCV was evaluated on the histological material and the patients, after the RAI treatment, were followed at our institution for at least one year with serum thyroglobulin assay and sonographic examination. Among these patients 24 presenting stage T1 were excluded from the study. The remaining 22 patients were classified according to the ATA risk guidelines and underwent radioiodine treatment. Clinical responses after treatment were classified as complete response vs incomplete response (incomplete biochemical response or structural incomplete response).

**Results.** Out of these 22 patient submitted to total thyroidectomy, 15 had a complete response to iodine administration (68,2%) and 7 had an incomplete response (31,8%). Among the patients with complete response 5 patients were T2 (33,3%), 9 were T3 (60% ) and 1 was T4 (6,7%). The average rate of TCV was 37,3 % with a median value of 30%. Among the patients with incomplete response, the TCV rate was 42,9% with a median of 40%; 1 patient resulted T2 (14,3%), 2 were T3 (28,6%) and 4 were T4 (57,1%). To analyze our data, we used the Student's t test with a t value of 0.562 and a p value of 0.581 which are statistically significant.

**Conclusions.** Although the series is still limited, our data shows a statistically significant difference between the rates of TCV in the two populations of patients under investigation. These preliminary results confirm the importance of evaluating the exact rate of TCV in papillary carcinoma since it may predict the prognostic outcome of these tumors and affect the clinical management of these patients.

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### BENIGN AND MALIGNANT DOMINANT NODULE IN HASHIMOTO'S THYROIDITIS: A CLINICO-PATHOLOGIC STUDY ON A SERIES OF 342 PATIENTS

L. Salvatorelli[1], A. Zappalà[2], F. Motta[1], P. Amico[3], A. Torrisi[4], M. Castaing[4], G.M. Vecchio[1], G. Magro[1]

*[1] Department of Medical and Surgical Sciences and Advanced Technologies, G.F. Ingrassia, Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Anatomic Pathology Section, School of Medicine, University of Catania, Catania, Italy; [2] Department of Surgical Oncology, Azienda Ospedaliera di Rilievo Nazionale di Alta Specializzazione "Garibaldi Nesima", University of Catania, Catania, Italy; [3] Umberto I Hospital, Enna, Italy; [4] Integrated Cancer Registry (RTI) of Catania-Messina-Siracusa-Enna, Department of Hygiene and Public Health G.F. Ingrassia, University of Catania, Catania, Italy.*

**Background.** Hashimoto's thyroiditis (HT) is an autoimmune disorder which represents the most common inflammatory condition of the thyroid. HT occurs more frequently (up to 95% of cases) in women with age ranging from 30 to 50 years. It usually presents as a diffuse, firm, non-tender enlargement of the thyroid, due to the presence of micronodules imparting gland a resemblance to a hyperplastic lymph node. Some patients affected by HT may develop one or more nodules (> 1 cm), clinically evident or detected by ultrasonography, for which the term "dominant nodule" (DN) has been proposed. Due to the high rate of association between papillary thyroid carcinoma (PTC) and HT [1], the detection of a DN larger than 1 cm is so alarming for clinicians that fine needle aspiration cytology (FNAC) is highly advised.
**Aim.** To investigate: i) the clinicopathologic features of DN in HT; ii) the association between HT, DNs and PTC; iii) the predictive value of DN for a concurrent PTC elsewhere in the thyroid gland.
**Materials and Methods.** We selected a series of 342 patients with histologically proven HT (i.e. diffuse lymphocytic infiltration of the stroma with formation of lymphoid follicles and diffuse oxyphilic changes of the follicular epithelium). DN was defined as a nodule grossly or histologically detected, measuring ≥ 1 cm in its greatest diameter. The morphological features of "DN" were statistically correlated with clinical parameters. Benign or malignant tumors, especially PTC, were carefully searched on histological examination and correlated with clinical parameters, as well as with the occurrence of DN.
**Results.** We found that 48.5% of patients with HT had a "DN" ranging in size from 1 to 4.5 cm. In most cases (92.2%) DN was single, while in 7.8% at least two nodules were identified. Histologically most DNs (78.3%) resulted to be benign, namely "hyperplastic follicular lesions (HFLs)". In contrast only 7,9% of DNs resulted to be PTC (malignant DN). Surprisingly 65% of benign DNs lacked inflammation and 55% were composed of non-Hurthle cells. Among the HFLs lacking inflammation, 55.6% were composed of Hurthle cells, while the 72.7% exhibited follicles lined by non-Hurthle cells. Notably, PTC was found in 25.7% (88 out of 342) of patients with HT. The majority of PTC (53.4%; 47 out of 88) were represented by microPTC (<1cm), as areas >1 cm (15.9%; 14 out of 88), or in the form of a DN (7.9%; 27 out of 342). In three patients with two DNs, histological examination showed that both nodules were PTC, with a total of 30 PTC presenting as DN.
**Conclusions.** Our findings show that most DNs (78.3%) in HT are histologically benign, namely HFLs, and not PTCs or follicular-derived neoplasms as commonly believed [2]. Unexpectedly a significant number of benign DNs, despite their cellular composition (Hurthle or non-Hurthle cells), lacked any inflammatory component. Accordingly, pathologists should be aware of this possibility to avoid a misdiagnosis of follicular neoplasms in HT, either pre-operatively (TIR3) or post- surgically. In addition our study first show that the detection of a benign DN is a protective factor for the occurrence of PTC.

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## Venerdì, 19 ottobre 2018

Sala Cassiopea – 9:00 - 12:30

# PATOLOGIA FETOPLACENTARE

### SHOULD THE PATHOLOGIST TALK TO THE PATIENT? FETAL POST AUTOPSY INTERVIEW: TOOL OF THERAPEUTIC MANAGEMENT, GRIEVING PROCESS AND RISK CONTROL

C.M. Biatta[1], G.Scaglione[1], M.Paudice[1], C. Trambaiolo Antonelli[1], B. Spina[2], F. Buffelli[3], E. Fulcheri[3], V.G. Vellone[1 4]

*[1] Anatomia Patologica, Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate (DISC), Università degli Studi di Genova; [2] UOC Anatomia Patologica, Ospedale Policlinico San Martino, Genova; [3] UOSD Patologia Feto-Placentare e Ginecologica, Istituto G. Gaslini, Genova; [4] UOC Anatomia Patologica Universitaria, Ospedale Policlinico San Martino, Genova*

**Background.** Clinical-pathological autopsies are decreasing all over the world, with the exception of fetal post mortem examinations which, according to Italian law, are mandatory from the twelfth week of pregnancy.
**Objectives.** We analyzed the experience of parents in relation to abortion providing, through post mortem examination and following interview, clinical and emotional care and offering critical information for the consideration and planning of a subsequent pregnancy.
**Materials and Methods.** All fetal post autopsy interviews carried out at Ospedale Policlinico San Martino in the period January 1, 2013 – July 1, 2018 have been included in the retrospective study. Bereaved parents, sometimes advised by clinicians, asked to meet the gynecopathologist, specialized in fetal, placental and perinatal field, who performed post mortem examinations. All autopsies have been reported with a standardized check-list based protocol including macro and microscopic analyses, together with placental examination. A standardized check-list based record followed every pathologist-parent meeting.
**Results.** Among the 214 post mortem fetal examinations (gestational age 12 – 42 weeks), 74 pathologist-parent interviews have been requested (34.6%), following both miscarriages (62.2%) and induced abortions (37.8%). Mothers, usually pregnant for the first time (mean age 34 years old), often brought along a family member, being the partner most of the time (56.8%). Although the mean mother age is nearly the same and the pathologist approach doesn't differ in both the miscarriage and abortion courts, women who experienced a miscarriage late in pregnancy asked for a post autopsy interview to obtain information to face with

the mourning and for medicolegal issue. Miscarriage etiology is more frequently placental, while abortions are often induced due to fetal pathologies.

**Conclusions.** Fetal autopsy and placenta examinations are critical in miscarriage and abortion investigation, providing information for an effective prevention. Gynecologist and genetist have a crucial role in the planning of a subsequent pregnancy especially in miscarriages and abortions, respectively. The management of polymalformative genetic syndromes emphasizes the importance to work in a multidisciplinary team, collaborating with gynecologist, radiologist and genetist. The pathologist, meeting the parents, offers a clear explanation about fetal post mortem examination and may help them in coping with bereavement and post mortem decision-making, preventing potential unfounded medicolegal issues. It is imperative that the pathologist remains impartial throughout the meeting with the patient, but, at the same time, is able to offer the sympathy that may be called for in grieving process. Overall, in our experience, pathologist-parent interaction has been positively perceived, with sporadic difficulty due to sociodemographic barriers and previous psychiatric disorders.

# GINECOLOGIA

## ATYPICAL ENDOMETRIAL HYPERPLASIA AND EARLY ENDOMETRIAL CANCER: A NOVEL FERTILITY-SPARING TECHNIQUE AND THE ROLE OF PATHOLOGY

P. Giampaolino[1], A. Di Spiezio Sardo[1], A. Mollo[2], A. Raffone[2], M.R. Campanino[3], A. Travaglino[3], F. Zullo[2], L. Insabato[3], G. Bifulco[2]

*[1] Department of Public Health, University of Naples Federico II, Naples, Italy; [2] Department of Neuroscience, Reproductive Sciences and Dentistry, University Federico II, Naples, Italy; [3] Department of Advanced Biomedical Sciences, University Federico II, Naples, Italy*

**Aim.** Atypical endometrial hyperplasia (AEH) is a preneoplastic diseases characterized by an irregular proliferations of crowded endometrial glands with evident cytologicatypia. AEH is the precursor of endometrioid-type endometrial cancer EEC [1]. The treatment of choice for AEH and early EEC should be total hysterectomy [2 3]. However, these lesions are often diagnosed in young women with desire of pregnancy, and a fertility-sparing approach is required. To date, progestins are widely used as a conservative treatment for AEH and well-differentiated EEC at stage FIGO IA without invasion of the myometrium [3]. Among the several different fertility-sparing treatments, the levonorgestrel-releasing intrauterine system (LNG-IUS) is considered the most effective one [2 4]. Our aim was to evaluate safety and effectiveness of the combination of hysteroscopic endometrial focal resectionwith LNG-IUS for AEH and EEC in young women to preserve their fertility, highlighting the importance of pathological diagnosis.

**Materials and Methods.** Our study was designed as a retrospective case series (Canadian Task Force Level II-3). The medical records of 69 consecutive patients from 2007 to 2017 with diagnosis of AEH (n=55) or EEC (n=14) meeting inclusion criteria were reviewed. All patients were treated at the Obstetrics and Gynecology Unit, University Federico II, Naples, Italy. On patients with AEH, hysteroscopic resection preserving the basal layer of the endometrium was performed. On patients with EEC, hysteroscopic resection in three steps according to Mazzon'stechnique [5] was performed as follows: 1) removal of the exophytictumor (specimen 1); 2) removal of 4-5 mm of adjacent endometrium (specimen 2); 3) removal of 3-4 mm of underlying myometrium (specimen 3) (Fig. 1). The histologic examination of the specimens was performed at the Anatomic Pathology Unit, University Federico II, Naples, Italy. In order to include women in the fertility-sparing treatment, specimen 1 should be diagnosed as endometrioid type, well differentiated endometrial cancer without lymphovascular space invasion; specimen 2 should be cancer-free, indicating that the lesion was focal; specimen 3 should be cancer-free as well, demonstrating that the myometrium was not infiltrated. Subsequently, LNG-IUS was inserted and maintained for at least 12 months. Patients were followed for a total of 24 months with serial hysteroscopic biopsies.

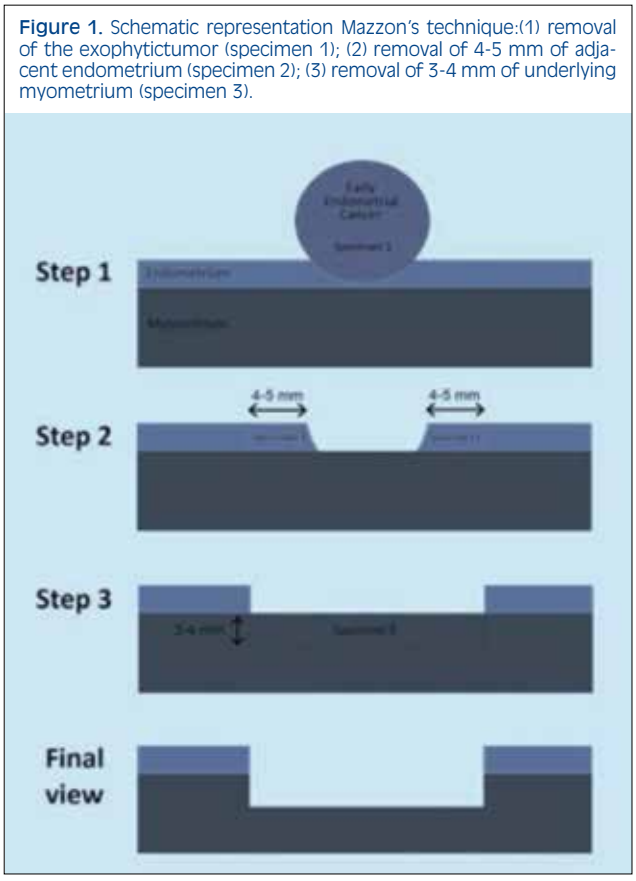


Figure 1. Schematic representation Mazzon's technique:(1) removal of the exophytictumor (specimen 1); (2) removal of 4-5 mm of adjacent endometrium (specimen 2); (3) removal of 3-4 mm of underlying myometrium (specimen 3).

**Results.** Rates of response, live birth and recurrence were assessed. Out of 55 patients with AEH, 51 (92.7%) achieved a complete response, 2 of whom (3.9%) had subsequent relapse, 3 (5.5%) showed partial response, while only 1 (1.8%) was non-responder with stable disease. Out of 14 patients with EEC, 11 (78.6%) achieved a complete response, 2 of whom (18.2%) had subsequent relapse, 1 (7.1%) showed partial response, while 2 (14.3%) were non-responders (1 stable disease and 1 progression). Among 25 patients who had removed LNG-IUS, 10 (40%) gave birth after natural conception in the last twelve months of follow-up.

**Conclusions.** The combination of hysteroscopic resection with LNG-IUS as fertility-sparing treatment of AEH and EEC showed similar response and live birth rates compared to those reported in literature for progestins alone, but a considerably lower relapse rate [6]. We advocate the use of this combined approach as an alternative fertility-sparing option for AEH and ECC, highlighting the importance of pathological diagnosis to avoid both under- and over-treatment.

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## LOSS OF BCL-2 PROTEIN EXPRESSION IN ENDOMETRIAL HYPERPLASIA APPEARS AS A SPECIFIC MARKER OF PRECANCER AND MIGHT BE A NOVEL INDICATION FOR TREATMENT: A SYSTEMATIC REVIEW AND META-ANALYSIS

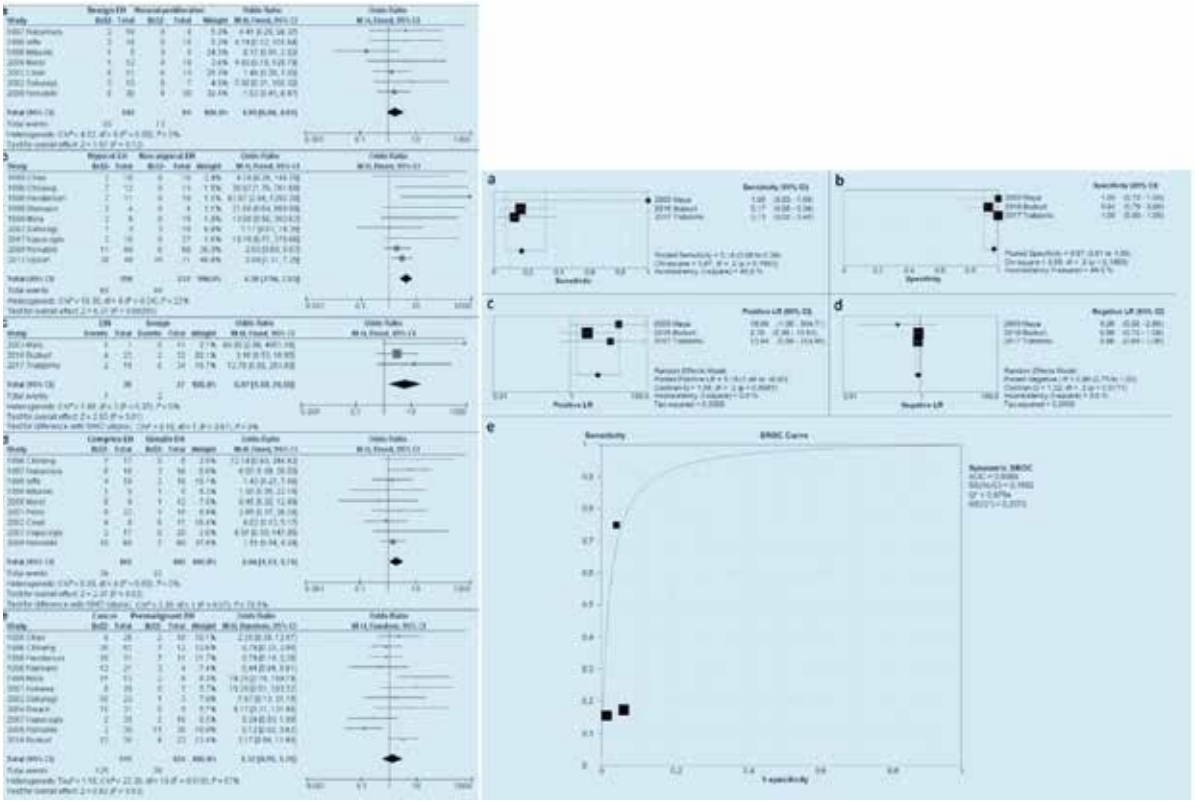
A. Travaglino[1], M.R. Campanino[1], A. Raffone[2], G. Saccone[2], A. Mollo[2], F. Zullo[2], L. Insabato[1]

*[1] Department of Advanced Biomedical Sciences, School of Medicine, University Federico II, Naples, Italy; [2] Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University Federico II, Naples, Italy*

**Introduction.** Endometrial hyperplasias (EH) includes both benign proliferations, caused by unopposed estrogens action, and premalignant disease. These two conditions are differentiated by two possible histologic classifications: the World Health Organization (WHO) classification, based on cytologic atypia, disregarding glandular complexity, and the endometrial intraepithelial neoplasia (EIN) classification, based on three morphologic parameters (glandular crowding, lesion diameter>1mm, cytology different from adjacent endometrium) and a careful exclusion of benign mimics and cancer [1]. B-cell lymphoma 2protein (Bcl-2) is an antiapoptotic protein that is upregulated by estrogens. While Bcl-2 acts as an oncogene in several tumors, it has been observed to be down-regulated in endometrial carcinomas [2 3]. Therefore, loss of Bcl-2 protein expression has been studied as phenotypical marker to improve the differential diagnosis between benign and premalignant EH [4]. We aimed to evaluate: 1)Bcl-2 protein loss as a marker of endometrial precancer, by assessing it in the proliferative endometrium, benign EH, premalignant EH and endometrial cancer; 2) diagnostic accuracy of Bcl-2 in the differential diagnosis between benign and premalignant EH; 3) if the accuracy of Bcl-2 changes by using EIN classification, rather than WHO, as reference standard to define premalignancy.

**Materials and Methods.** Electronic databases were searched from their inception to March 2018. Studies assessing Bcl-2 im-

Figure 1. (A) Forest plots reporting graphically odds ratio for Bcl-2 loss of expression in: a) benign hyperplasia vs normal proliferative endometrium; b) atypical vs non-atypical hyperplasia; c) EIN vs benign hyperplasia; d)simple vs complex hyperplasia; e) premalignant hyperplasia vs cancer. Odds ratio was calculated for each study and as pooled estimates with 95% CI. (B) Forest plots reporting graphically diagnostic accuracy of immunohistochemical loss of Bcl-2 expression in differentiating between benign and premalignant hyperplasia as defined by EIN criteria: a) sensitivity; b) specificity; c) positive likelihood ratio; d) negative likelihood ratio; e) area under the curve (AUC) on SROC curves.



munohistochemistry in endometrial specimens were included. The association of Bcl-2 protein loss with the different histologic categories was assessed by using odds ratio (OR), with a significant p-value<0.05. Diagnostic accuracy was assessed as sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) and area under the curve (AUC) on SROC curves.

**Results.** Twenty observational studies assessing 1,278 specimens were included. Bcl-2 loss rates were not significantly different between proliferative endometrium and benign EH (p=0.12), and between premalignant EH and endometrial cancer (p=0.53). Among EH, Bcl-2 loss was significantly associated with premalignancy, according to both WHO (OR=4.39; p<0.00001) and EIN classification (OR=6.07; p=0.01), and also with architecture complexity (OR=2.06; p=0.02) (Fig. 1A). Using the WHO classification, Bcl-2 protein loss showed low diagnostic accuracy in detecting premalignant hyperplasia (AUC=0.708), with sensitivity=0.41, specificity=0.81, positive likelihood ratio=3.22, and negative likelihood ratio=0.69. Using the EIN classification, accuracy was high (AUC=0.938), with sensitivity=0.18, specificity =0.97, positive likelihood ratio=5.16, negative likelihood ratio =0.86 (Fig. 1B).

**Conclusions.** Bcl-2protein loss is a marker of endometrial precancer, with high specificity and high diagnostic accuracy if EIN classification is used. Bcl-2 loss in EH might be a novel indication for treatment, even in absence of overt precancerous features (e.g. cytologic atypia) at histologic examination. Bcl-2 loss better correlates with EIN classification than WHO, highlighting the importance of glandular complexity as precancerous feature.

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## MORPHOLOGICAL CHANGES IN LEIOMYOMAS TREATED WITH ULIPRISTAL ACETATE


A. De Leo[1 2], D.D. Di Nanni[1], E. Fontana[3], A.M. Perrone[3], P. De Iaco[3], B. Corti[1], C. Ceccarelli[2], D. Santini[1]

*UO Anatomia e Istologia Patologica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia; Dipartimento di Medicina specialistica, diagnostica e sperimentale, Università di Bologna, Bologna, Italia; Oncologia Ginecologica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia*


**Objective.** Ulipristal acetate (UPA) is an oral selective progesterone receptor modulator (SPRM) and it is considered a hormonally active drug effective in the management of uterine leiomyomas (ULMs). UPA inhibits the proliferation of leiomyoma cells and induces apoptosis reducing vascularization, cell proliferation and cell survival. The histological endometrial changes associated with PRM treatment have been described as PAEC (PRM Associated Endometrial Changes), but the morphological features of ULMs treated with PRMs are currently not well-defined. The aim of the study was to describe and evaluate the PRM-associated changes in ULMs.

**Material and Methods.** We studied 12 consecutive patients treated with UPA for ULMs, starting from January 2016 to December 2017. An UPA cycle consisted in a daily oral administration of 5 mg of UPA for 3 months. The clinical data, gross and histologic features were retrospectively reviewed. The mitotic count was evaluated in each case using the phosphohistone-H3 (PHH3) and hematoxylin and eosin (H&E)-stained sections. Ki67 proliferation index was also assessed. Statistical analysis was performed using SPSS.

**Results.** The patients ranged in age from 39 to 51 (mean and median, 45) years. The median number of treatment cycles was 1.5. In all patients ULMs were multiple, grossly softened and paler than the usual leiomyoma. Marked cyst appearance was present in lesions greater than 5 cm (66 % of cases). Vascular alterations (thick-walled blood vessels, lymphangioma-like appearance of the vessels, intravascular thrombi, contraction of the muscular wall of the vessels), were evident in all cases. The edema percentage ranged from 0 to 50% (on average 19.6%). 5 cases (45%) showed foci of ischemic-type necrosis. In 7 cases (63%) the lesions exhibited areas with diffuse pyknotic and vacuolated cells. Endometrial changes (PAEC) were present in 81.8 % of cases: diffuse PAEC was found in 6 (66%) cases, while focal PAEC occurred in 3 (33%) cases. In 75% of cases, the fallopian tube showed a benign epithelial hyperplasia (type I secretory cell outgrowth) and a statistically significant association between tubal alterations and PAEC was found. PHH3 identified easily-missed mitosis by conventional mitotic count. Indeed, PHH3 accurately found hot-spots with a mean of mitoses of 0.4 per 50 high-power fields (ranging from 0.1 to 0.9). On the contrary, the mean traditional mitotic rate calculation on H&E- stained sections was 0.06 per 50 high-power fields. The average Ki-67 proliferation index was 2.7% and it was mainly expressed in the vessels associated to ULMs.

**Conclusions.** Pathologists should be aware of PRM-associated morphological changes in ULMs that may represent a response to the treatment. PAEC and tubal alterations should not alarm the pathologists because they are related to UPA treatment. Furthermore, these unusual features and cystic changes could explain the initial unmarked reduction in size of the lesions that clinicians may interpret as treatment failure, leading to an unnecessary hysterectomy.

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## PATHOLOGIC FINDINGS IN RISK-REDUCING SALPINGO-OOPHORECTOMY (RRSO) IN WOMEN WITH BRCA1/ BRCA2 MUTATIONS: A SINGLE CENTER EXPERIENCE

A. De Leo[1 2], D.D. Di Nanni[1], C. Ceccarelli[1 2], F. Conte[2], D. Turchetti[4], M. Taffurelli[5], P. De Iaco[3], M. Perrone[3], D. Santini[1]

*[1] U.O. Anatomia e Istologia Patologica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia; [2] Dipartimento di Medicina specialistica, diagnostica e sperimentale, Università di Bologna, Bologna, Italia; [3] Oncologia Ginecologica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia; [4] U.O. Genetica Medica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia; 5 U.O. Chirurgia della mammella, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia*

**Objective.** Bilateral salpingo-oophorectomy offers the greatest risk reduction for ovarian cancer in high-risk women with an identified BRCA germline mutation. The aim of this study was to define the incidence of precursor lesions and cancer after RRSO.

**Materials andMethods.** We retrospectively reviewed 71 BRCA-mutated patients who underwent RRSO from 2012 to 2017. All cases were examined according to the protocol for Sectioning and Extensively Examining the FIMbriated End (SEE-FIM).

**Results.** The median age at RRSO was 49.9 years. BRCA 1 mutation was detected in 46/71 (64.8%) patients, BRCA2 mutation in 25/71 (35.2%) patients. 50/71 (70.4%) patients had breast cancer. BRCA1 breast cancer was more frequently high grade (G3) and showed a strong association to triple negative phenotype (p=0.001), high TILs (tumor-infiltrating lymphocytes), and aberrant expression of p53 (p=0.01). Occult invasive gynecological cancer was detected in two patients: a tubal high grade serous carcinoma in a BRCA2+ patient and an ovarian low-grade endometrioid carcinoma in a BRCA1+ patient. 5 (7%) STIC (Serous Tubal Intraepithelial Carcinoma), 5 (7%) STIL (Serous Tubal Intraepithelial Lesion) and 13 (18.3%) SCOUT (Secretory Cell Outgrowth) were detected. 10 patients had endometriosis and benign ovarian lesions were found in 17 cases. The detection rate of STIC/STIL or invasive cancer was 16.9% (12/71). Tubal lesions were more common in BRCA2 mutation carriers (68% vs. 39%).

**Conclusions.** In our institution, a rigorous surgical protocol with meticulous pathologic review at RRSO yielded an overall detection rate of 9.8% for occult gynecological carcinoma in BRCA mutation carriers.

## OSNA ASSESSMENT OF LYMPH-NODES METASTASES IN EARLY STAGE ENDOMETRIAL CARCINOMA: AN INSTITUTIONAL EXPERIENCE

V. Fiorentino, M. Valente, T. Musarra, G. Angelico, D. Arciuolo, F. Inzani, A. Santoro, A. Mulè, G.F. Zannoni

*Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy*

**Background.** Endometrial cancer (EC) is the sixth most common gynecologic malignancy in women worldwide with around 300,000 new cases reported annually. The prognostic value of lymphadenectomy for patients with early stages is a matter of debate. Despite this fact, presence of nodal metastasis is an important element to determine the appropriate adjuvant management. Since 2015, the National Comprehensive Cancer Network (NCCN) guidelines proposed to perform the sentinel lymph node (SLN) mapping in selected early stage EC patients: it reduces the intraoperative as well as the long-term morbidity of a systematic lymphadenectomy, and, thanks to the ultra-staging analysis, improves the detection rate of the micro-metastasis and the isolated tumor cells. Histology and IHC are burdensome, time consuming and not suited for rapid, intraoperative diagnoses, so that patients could be considered for systematic lymphadenectomy and adjuvant therapy only in the post-surgical evaluation. To overcome this limit, Sysmex Corporation (Kobe, Japan) has developed an innovative molecular method, the One Step Nucleic Acid Amplification (OSNA) reaction, that in combination with the reagent "Lyno-amp BC", allows the rapid and accurate detection of cytokeratin 19 (CK19) mRNA in metastatic lymph nodes of breast, gastric and colon cancer patients.

**Objectives.** The principal aim of the current study is to confirm these data in a consecutive series analyzing the micro- and macro-metastases detection rate of OSNA assay compared to frozen section examination and subsequent ultra-staging examination in early stage endometrial cancer (EC).

**Materials and Methods.** From March 2016 to July 2018, data of 40 consecutive FIGO stage I EC patients were prospectively collected in an electronic database. The sentinel lymph node mapping was performed in all patients. All mapped nodes were removed and processed. Sentinel lymph nodes were sectioned and alternate sections were respectively examined by OSNA and by frozen section analysis. After frozen section, the residual tissue from each block was processed with step-level sections (each step at 200 micron) including H&E and IHC slides.

**Results.** Sentinel lymph nodes mapping was successful in 29 patients (72.5%). In the remaining 11 patients (27.5%), a systematic pelvic lymphadenectomy was performed. OSNA assay sensitivity and specificity were 87.5% and 100% respectively. Positive and negative predictive values were 100% and 99% respectively, with a diagnostic accuracy of 99%. As far as frozen section examination and subsequent ultra-staging analysis was concerned, we reported sensitivity and specificity of 50% and 94.4% respectively; positive and negative predictive values were 14.3% and 99%, respectively, with an accuracy of 93.6%. In one patient, despite negative OSNA and frozen section analysis of the sentinel node, a macro-metastasis in 1 non-sentinel node was found.

**Conclusions.** Despite the limit of this study, mainly represented by the small sample size and the low number of metastatic nodes, we confirmed that the OSNA assay measuring CK19 mRNA copy numbers could be used as a novel tool for the analysis of SLN in early stage EC patients. We can conclude that the combination of OSNA procedure with the sentinel lymph node mapping could represent an efficient intra-operative tool for the selection of early-stage EC patients to be submitted to systematic lymphadenectomy. Waiting for the results of the ENDO-OSNA prospective multicenter validation study, further studies should define the prognostic impact of molecular assessment of SLN and the non-SLNs management in EC patients.

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## PRESACRAL MALIGNANT SOLITARY FIBROUS TUMOR: A CASE REPORT

V. Fiorentino, T. Musarra, G. Angelico, D. Arciuolo, M. Valente, F. Inzani, A. Santoro, A. Mulè, G.F. Zannoni

*Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS - Università Cattolica del Sacro Cuore, Rome, Italy*

**Background.** Solitary fibrous tumor (SFT), is a spindle cell neoplasm with an uncertain clinical behaviour which occurs most often in the pleura. However, a wide variety of extrapleural sites also have been noted, including orbit, nasal cavity, salivary glands, upper respiratory tract, thyroid, peritoneum, retroperitoneum and pelvis, genitourinary system, and soft tissue. It is reported that 30% to 40% of SFTs are located at extra-pleural regions. Retroperitoneal malignant SFT is rare, with only few cases reported in the literature. Histopathological diagnosis is very challenging and is mainly based on STAT-6 immunoreactivity along with the characteristic "patternless" growth of the neoplastic spindle cells.
**Case presentation.** In the present study, we report a case of a retroperitoneal presacral mass of a 44 year old Caucasian woman. On radiologic examination the lesion was described as a solid expansive mass measuring 7.2x7.5x8.6 cm strongly adherent to the sacrum. Grossly the lesion appeared as a solid greyish nodular mass with scattered areas of necrosis (15% of the tumoral mass). Histological examination showed mesenchymal neoplasia composed of spindle cells with high cellularity and mild atypia. Necrotic area was mainly of vascular type, even though some tumoral necrosis foci were present. Mitotic rate was 9/10HPF. Neoplastic cells were immunoreactive for STAT-6, CD34, CD99, p16, ER (1+, 15%) and PR (1+, 3%) and negative for ASMA, caldesmon, desmin, TLE1, DOG1, CD117, HMB45, S100, Melan-A and MITF. Ki 67 was 20%. This immunophenotype and the histological findings are typical of solitary fibrous tumor; the high cellularity, the mitotic index over 4/10HPF and the presence of necrosis are pathologic aspects correlated to more aggressive clinical behavior in terms of local recurrence and metastasis, even several years after the primary resection.
**Discussion.** World Health Organization (WHO) classifies SFT as intermediate fibroblastic or myofibroblastic tumors along with hemangiopericytomas, which means that SFTs are considered tumors that rarely if ever metastasize. Histopathological diagnosis is challenging and mostly based on a "patternless pattern" on microscopic examination. This pattern is a storiform arrangement of spindle cells combined with a "hemangiopericytoma-like appearance" and increased vascularity of the lesion. Differential diagnosis includes other spindle-cell neoplasms such as leiomyoma, inflammatory myofibroblastic tumor, angiomyolipoma, and gastrointestinal stromal tumor. Immunohistochemistry is very helpful, and SFTs are positive for Bcl-2, vimentin, and CD99, as well as CD34. Negative expression of S100, cytokeratin, EMA, SMA, CD117, CD31, and desmin is the norm and adds to the correct diagnosis. The combination of positive Bcl-2 and CD34 is guiding histopathologically towards the diagnosis of SFT, since 75% of extrapleural SFTs positively express these two markers. SFTs are considered malignant when histopathological examination shows high cellularity, high mitotic activity (more than 4 mitoses per 10 HPF), pleomorphism, necrosis, and hemorrhagic changes. In our case, the tumor had the capacity for a high degree of proliferation as a malignant tumor. On the basis of these criteria, the specimen in our case was histologically considered to be a malignant SFT. SFTs usually have a favorable prognosis after complete local excision, but they can hold the potential for recurrence or metastasis as well. Although the relationship between morphology and outcome with SFTs is poor, the pathologic findings should not be ignored when considering further therapy. The basic treatment principle for soft tissue tumors including SFTs is resection with sufficient clear margins. In our case, the tumor was completely resected macroscopically (simple complete resection), but exhibited pathologic malignancy and the capacity for a high degree of proliferation. Due to the rarity of SFTs, especially in the retroperitoneum, studies to define the best management approach are lacking and adjuvant treatment options are based on case reports and observational studies. Even benign cases are reported recurring locally or at distant sites, indicating unpredictable behavior of this rare neoplasm, with malignant transformation potential. The latter is the basis of follow-up with tomographic imaging.
**Conclusions.** In summary, we presented an unusual case of malignant retroperitoneal SFT: the histologic features and immunohistochemistry were helpful for the diagnosis. SFT in the retroperitoneum should be managed aggressively with primary surgery and careful clinical long-term follow-up is necessary. Uncertain clinical behavior and lack of management guidelines confuse clinicians and multidisciplinary team approach is essential.

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## CEREBELLAR METASTASIS FROM OVARIAN CLEAR CELL CARCINOMA WITH PIK3CA ACTIVATING MUTATION: A CASE REPORT

T. Musarra, V. Fiorentino, G. Angelico, D. Arciuolo, M. Valente, F. Inzani, A. Santoro, A. Mulè, G.F. Zannoni

*Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy*

**Background.** Ovarian clear cell carcinoma (OCCC) is an intriguing histologic subtype of epithelial ovarian cancer (EOC) that demonstrates a clinical behavior different from that of other epithelial cancers. OCCC, in fact, it frequently presents as a large pelvic mass, and rarely occurs bilaterally, and it is often associated with endometriosis [1]. It is often accompanied by a thromboembolic complication, hypercalcemia, and is frequently observed at early stages. Several studies have discussed the prognosis of OCCC without much agreement, although there is general acceptance of OCCC's insensitivity to conventional platinum-based chemotherapy followed by poor prognosis. Radiation therapy may have an under-recognized role in the management of OCCC [2].
**Objectives.** We herein report an unusual case of solitary

cerebellar metastasis in a woman affected by an early stage (FIGO IC) OCCC. To the best of our knowledge, this is the first reported case of cerebellar metastasis from ovarian clear cell carcinoma. In presence of neurologic symptoms, both clinicians and pathologists must be aware of this rare possibility to assure the patient a correct management and really effective therapeutic options.

**Material and Methods.** Surgical specimen was submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques, embedded in paraffin, cut to 2.5µm, and stained with hematoxylin and eosin. Mutational status analysis of hot spot regions in KRAS, NRAS, BRAF and PIK3CA genes was performed using Pyrosequencing approach, with the PyroMark Q24TM system and the PyroMark TM- Q24 software (Qiagen GmbH, Hilden, Germany) for data analysis, according to the manufacturer's protocol, starting from 2.5µm sections of formalin-fixed paraffin- embedded tissue of both primary OCCC case and related brain metastasis.

**Results.** A 50-year-old woman presented at our observation with a four months history of headache, raised intracranial pressure and vertigo. Three years earlier she had undergone total abdominal hysterectomy with bilateral salpingo-oopherectomy for a right-side OCCC, without surface involvement but with positive ascitic fluid and no extra-ovarian spread (pT1c, FIGO stage IC). The patient had received 3 cycles of carboplatin and paclitaxel-based chemotherapy. Histopathological evaluation of the resected specimen revealed a malignant neoplasm showing a predominantly solid pattern of growth, with occasional tubular and glandular formations; however, papillary structures were not appreciable. The neoplastic cells were separated by a delicate fibrovascular stroma and displayed a rounded or polyhedral morphology with abundant clear to eosinophilic cytoplasm and enlarged, oval nuclei. Nuclear atypia and pleomorphism were not encountered and the mitotic activity ranged from 2 to 5 mitoses per 10 high power fields. Immunohistochemical stains performed on the specimen showed the same profile of the previously diagnosed ovarian tumor. In detail, neoplastic cells showed positive stain for PAX8, cytokeratin 7, EMA and Napsin A and negative stain for WT1, p53, cytokeratin 20, estrogen and progesterone receptors. Sequencing analysis revealed the presence of the typical activating point mutation in exon 9 of PIK3CA, p.E545K, (c.1633G>A) in both primary and metastatic lesion. Surprisingly, six months after surgery, the patient is well with complete disappearance of symptoms and no evidence of recurrence, maybe due to the presence of PIK3CA activating mutation in exon 9 that, as described in literature data, may be associated to a better prognosis.

**Conclusions.** In our case, the patient is surprisingly in good general conditions, with complete disappearance of symptoms and no evidence of recurrence. This outcome could be due to the presence of PIK3CA activating mutation in exon 9 [3]. In conclusion, female genital tract cancers, including EOCs, are considered "neurophobic" since brain metastases from these types of cancers are occasional and usually develop as part of a widespread disseminated disease. The present paper emphasizes the importance to suspect a brain metastasis in patients with a previous diagnosis of EOC, who develop neurologic symptoms such as headache, gait difficulty, vertigo, nausea and vomit. Generally, the prognosis of EOC patients with brain metastases is poor; however, PIK3CA mutations can be used as prognostic indicator in OCCC, to determine the possibility of a better patient outcome.

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## THOLOGIC FEATURES OF UTERINE SMOOTH MUSCLE TUMORS TREATED WITH PROGESTERONE RECEPTOR MODULATORS: A CASE SERIES


T. Musarra, V. Fiorentino, G. Angelico, D. Arciuolo, M. Valente, F. Inzani, A. Santoro, A. Mulè, G.F. Zannoni

*Department of Woman and Child Health, Fondazione Policlinico Universitario A. Gemelli IRCCS - Università Cattolica del Sacro Cuore, Rome, Italy*


**Background.** Hysterectomy has long been considered the treatment of choice for symptomatic uterine leiomyoma (ULM), despite the consequences on fertility in women of reproductive age. Nowadays, minimally invasive surgical approaches such as power morcellation and hormonal therapy with gonadotropin releasing hormone (GnRH) analogues and Ulipristal Acetate (UPA) are more often proposed in women who wish to preserve their fertility. Currently, UPA is emerging as a novel therapeutic approach indicated for intermittent, long term and preoperative treatment of symptoms related to uterine fibroids. The reversible blocking of the progesterone receptor induced by UPA inhibits the proliferation of leiomyoma cells and also induces an inhibition of the ovulation reducing, thus, the heavy menstrual bleeding related to ULM. However, about 5% of patients do not benefit from this treatment and the possible explanations for this phenomenon are still debated. Recently, some authors emphasized the presence of unsuspected leiomyosarcoma (LMS) as a possible cause of poor response to UPA.

**Objectives.** We herein present the clinical, pathological and therapeutic implications of UPA treatment in a series of twelve young patients affected by uterine smooth muscle tumors which were treated conservatively with Ulipristal Acetate and morcellation.

**Material and Methods.** Tissue samples analyzed in the present study were collected from patients receiving 5 mg/d of UPA for 12 weeks. For pathological examination of resected specimens, sections (4-5 mm in thickness) were cut from paraffin blocks. The sections were stained with haematoxylin and eosin for general morphological characterization. The histological diagnosis of leiomyosarcoma was rendered on the basis of nuclear atypia, increased mitotic activity (>10 mitoses/10 high power fields), atypical mitotic figures and tumor necrosis.

**Results.** The mean age of patients was 42. From twelve patients analyzed we observed 9 benign smooth muscle tumors (leiomyoma), one smooth muscle tumor of uncertain malignant potential (STUMP) and two leiomyosarcomas. All patients with a diagnosis of leiomyoma experienced a reduction in tumor size and an improvement in symptoms related to the mass (bleeding and abdominal pain). By contrast, patients affected by STUMP and Leiomyosarcoma experienced a worsening of symptoms (persistent bleeding) after the first 3 months of treatment with UPA. Pathological examination of malignant morcellated specimens revealed the unsuspected diagnosis of leiomyosarcoma and STUMP based on the presence of severe nuclear atypia, tumor necrosis and increased mitotic activity. Unfortunately after six month of follow-up one patient with

the diagnosis of leiomyosarcoma died for multiple peritoneal recurrences and lung metastases. The other two patients are still alive after 6 month of follow-up and show no local recurrences or metastases.

**Discussion.** Ulipristal acetate is a selective progesterone receptor modulator that has recently been approved for intermittent, long term, and preoperative treatment of moderate-to-severe symptoms associated with ULM in adult women of reproductive age. The reversible blocking of the progesterone receptor induced by UPA explains its anti-proliferative, anti-fibrotic and pro-apoptotic effects which are responsible of the reduction of ULM size. Moreover, due to its interaction with endometrial progesterone receptors, UPA induces amenorrhea reducing thus the severe bleeding related to ULM. Moreover, a considerable small percentage of patients (4.8%) has been reported to not benefit from UPA treatment and the possible motivations for this inefficacy are still debated. In this regard, a recent study identified the following predictive parameters of UPA treatment failure: young age (<35 ys), absence of previous pregnancy and the size of the dominant fibroid ≥ 80 mm. Hence, the persistence of heavy bleeding and pelvic pain during UPA treatment is not to be considered specific for the diagnosis of LMS, but the suspect of an unexpected LMS must be taken into consideration as a cause of inefficacy of this hormonal therapy. Similarly, our reported LMS cases were preoperatively misdiagnosed as benign smooth muscle tumors (ULM). Therefore, the treatment with UPA revealed to be ineffective or only partially beneficial, so that both patients experienced a rapid increase of symptomatology. To the best of our knowledge, a total of 6 cases of LMS treated with UPA for suspected ULM have been reported in the literature.

**Conclusions.** Our reported cases emphasize that the poor or absent response to Ulipristal Acetate treatment in addition to the instrumental evidence of a single mass may be indicative of the presence of an unsuspected leiomyosarcoma clinically and radiologically misdiagnosed as leiomyoma. The awareness of this possibility would avoid a delay in the diagnosis as well as unuseful and potentially dangerous treatments such as morcellation.

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## MULTIVARIATE ANALYSIS OF HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROGNOSTIC FACTORS IN ENDOMETRIAL CARCINOMA

M. Paudice[1], L. Peñuela[2], C.M. Biatta[1], G. Scaglione[1], L. Valle[1], B. Spina[3], S. Ferrero[4], V.G. Vellone[1 5]

*[1] Anatomia Patologica, DISC, Università di Genova; [2] UOSD Patologia Feto-perinatale, IRCCS Istituto Giannina Gaslini, Genova; [3] UO Anatomia Patologica, IRCCS Ospedale Policlinico San Martino, Genova; [4] Ostetricia e Ginecologia, DiNOGMI, Università di Genova; [5] UO Anatomia Patologica Universitaria, IRCCS Ospedale Policlinico San Martino, Genova*

**Objective.** Endometrial carcinoma (EC) is the most common malignant disease of the female genital tract. It is the fifth cancer in women and the seventh cause of cancer death in North Europe. We investigated the prognostic role of some histopathological and immunohistochemical factors in EC in term of disease free survival (DFS) and overall survival (OS).

**Method.** Out of the total number of patients who had surgery for EC (S. Martino Polyclinic Hospital, Genoa, Italy) over the period 1.1.2013-1.7.2016, we considered only those with available clinical and radiological follow-up data after hysterectomy. Patients treated with neoadjuvant therapy were ruled out. All surgical specimens have been routinely processed to obtain 3-µm thick histological sections, finally stained with Hematoxylin and Eosin. As histopathological features, we considered histotype, stage at diagnosis, type of infiltration (infiltrative/expansive), desmoplasia (presence/absence), intratumoral necrosis (presence/absence), Tumor Infiltrating Lymphocytes (TIL; absent/mild or moderate/severe), lymphatic and blood vessels invasion (presence/absence). For each case the percentage of staining (%) of a panel of immunohistochemistry markers (IHC) including ER$\alpha$, PR, Ki67, p53, $\beta$-catenin, E-cadherin, BCL-2 and Cyclin D1 has been investigated. Clinical, pathological and IHC data were entered in a Microsoft Excel© spreadsheet. Discrete and continuous variables were compared respectively using the $\chi 2$ test and Kruskal-Wallis test. Survival univariate analysis was studied with Kaplan- Meier survival curves, while survival multivariate analysis with Cox-Models. The significance was confirmed with the Log Rank Test. For statistical computation MedCalc© and OriginPro® programs were used.

**Results.** Out of 99 cases eligible for our purposes, we found 69 low-grade endometrioid (LGEC), 8 high-grade endometrioid (HGEC) and 22 other high-grade endometrial carcinomas (OHEC); the latter consisted of 6 serous, 4 carcinosarcoma, 2 clear cell, 8 mixed and 2 undifferentiated histotypes. The DFS multivariate analysis showed a strong positive correlation between poor prognosis and advanced stage (p=0.0042). The OS multivariate analysis revealed a positive correlation between poor prognosis and advanced stage (p=0.0003), presence of desmoplasia (p=0.04) and high Ki67 proliferative index (of borderline significant, p=0,052). Other factors - that resulted significantly correlated with the prognosis in univariate analysis - lost their role after the multivariate analysis. In univariate analysis, OHEC histotype was positively correlated with a worse prognosis when compared to endometriod type (OS: p=0.005; DFS: p=0.002). In the same way the low expression of PR was positively correlated with a poor prognosis (OS: p=0.017; DFS: p=0.014). Basically, some of the proposed prognostic factors seem just to well correlate with the stage of disease but they lose their independence in multivariate analysis.

**Conclusions.** In our small but representative case study, the prevalence of different EC histotypes are in line with the scientific Literature. The multivariate analysis confirmed the central role of the disease staging as the main important prognostic factor in EC both in term of OS and DFS. Moreover the presence of desmoplasia and the Ki67 proliferative index have demonstrated to be significantly correlated with OS.

## ENDOCANNABINOID SYSTEM IS DIFFERENTLY EXPRESSED IN OVARIAN EPITHELIAL TUMORS ACCORDING TO THE DUALISTIC MODEL OF OVARIAN CARCINOGENESIS

A. Ronchi[1], F. Grauso[2], M. Montella[1], I. Panarese[1], G. Toni[1], G. Roccuzzo[1], M. Stilo[1], F. Zito Marino[1], R. Sabetta[1], E. La Mantia[1], E.M. Messalli[2], R. Franco[1]

*[1] Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy; [2] Department of Woman, Child and General and Specialized Surgery, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy*

**Objectives.** The endocannabinoid system (ECS) is a complex endogenous signaling system that influences multiple metabolic pathways essential for the homeostasis of the organism. ECS is composed of transmembrane endocannabinoid receptors (Cannabinoid Receptor Type 1 CB1R and Cannabinoid Receptor Type 2 CB2R), endogenous ligands and enzymatic system involved in biosynthesis, transporting, degradation and signaling (Fatty Acid Amide Hydrolyse FAAH). Recent studies demonstrated that ECS may affect cell survival and cell proliferation, suggesting a correlation between ECS and cancer. In a previous study, we reported that malignant epithelial ovarian tumors (EOT) showed an increased expression of CB1R compared to benign and borderline EOT. Our current study aims to confirm the expression of the ECS in the human EOT, assessing the trend of CB1R and FAAH expression according to histological type and grading.
**Materials and Methods.** This study included 118 patients affected by EOT (36 benign tumors, 34 borderline tumors and 48 malignant tumors), consecutively treated during a decade in our Department of "Women, Child and General and Specialized Surgery", University of Campania "Luigi Vanvitelli" (Naples, Italy). All cases were revised by two different pathologists, which evaluated histological type and grading of the neoplasms. Three tissue micro arrays (TMAs) were realized. Immunohistochemical expressions of CBR1 and FAAH were evaluated taking into account both the percentage of positive cells and intensity of expression.
**Results.** Concerning CB1R immunohistochemical expression, 22/118 (19%) cases resulted negative, 44/118 (37%) showed a weak expression, 38/118 (32%) showed a moderate expression, 14/118 showed a strong expression. In particular, in the malignant cases, the expression resulted negative in 20/48 (42%), weak in 15/48 (31%), moderate in 7/48 (15%) and strong in 6/48 (12%). FAAH expression resulted negative in 41/104 (39%) cases, weak in 39/104 (38%) cases, moderate in 17/104 (16%) cases and strong in 7/104 (7%) cases. Concerning CB1R expression, Kurman's Type I tumors showed weak expression in 2/14 (14%) cases, moderate expression in 5/14 (36%) cases, strong expression in 6/14 (43%) cases. 1/14 (7%) Type I tumor resulted negative. Kurman's Type II tumors showed weak expression in 13/34 (38%) cases, moderate expression in 2/34 (38%) cases. 19/34 (56%) cases resulted negative, and none showed strong expression. Concerning FAAH expression, Kurman's Type I tumors resulted negative in 9/14 (64%) cases and showed weak expression in 3/14 (22%) cases, moderate expression in 2/14 (14%). Strong FAAH expression was not observed in Kurman's Type I tumors. Instead Kurman's Type II tumors showed weak expression in 8/34 (23%) cases, moderate expression in 4/34 (12%) cases. 22/34 (65%) cases resulted negative and none showed strong expression.
**Conclusions.** The present study confirmed a variable expression of the ECS in human EOT. Kurman's Type I tumors showed a moderate-strong expression of CB1R (79% - 11/14), while Kurman's Type II tumors showed a negative-weak expression (94% - 32/34), with statistically significant difference ($p<.01$). Further studies are necessary to define the cellular and molecular mechanisms of ECS pathway in EOT and to evaluate the prognostic significance of ECS expression.

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## IMMUNOHISTOCHEMICAL ANALYSIS OF STEROID HORMONE RECEPTORS IN ENDOMETRIOSIS RELATED TO DIFFERENT ANATOMIC SITES

G. Scaglione[1], C.M.Biatta[1], M. Paudice[1], N. Boveri[2], V. Remorgida[2], E. Fulcheri[3], V.G. Vellone [1 4]

*[1] Anatomia Patologica, DISC, Università degli studi di Genova; [2] Ostetricia e Ginecologia, DiNOGMI, Università degli studi di Genova; [3] U.O.D.S. Patologia Feto-perinatale, IRCCS Istituto Giannina Gaslini, Genova; [4] UO Anatomia Patologica Universitaria, IRCCS Ospedale Policlinico San Martino, Genova*

**Background.** Endometriosis is a chronic inflammatory disease, characterized by the presence of endometrial implants at ectopic sites (extrauterine), dysmenorrhea, chronic pelvic pain and infertility. Endometriosis occurs in 2-10% of women in reproductive age and approximately in 50% of infertile women. Hormonal receptors are implicated in pathogenesis, progression and maintenance, particularly an excessive sensibility to estrogens and a progesterone lack or resistance.
**Objectives.** Our purpose is to analyze the different expression of the steroid receptors related to different anatomic sites, particularly androgens, still little investigated. Furthermore we aim to validate the pathogenetic hypothesis concerning the influence of sexual hormones in the maintenance of endometriosis.
**Materials and Methods.** The expression profile of Estrogen Receptor (ER Alfa), Progesterone Receptor (PR) and Androgen Receptor (AR) was investigated by immunohistochemistry (IHC) in selected paraffin blocks. For every receptor was valued the percentage of expression (distribution score DS), the intensity (intensity score IS) both in the gland and in the stroma. The two scores were combined for getting the histologic score (Hscore =ISxDS), a semi-quantitatively assessment of receptor in both components.
**Results.** 59 surgical specimens were selected from 40 patients with diagnosis of endometriosis carried out at Ospedale Policlinico San Martino from 2015 to 2018 (mean age 38,74 years). We selected only cases with glandular endometriosis. The endometriosis location were: single focus in 25 patients, two foci in 11 patients, three foci in 4 patients. Involved sites: left ovary (n.16), right ovary (n.9), Douglas' pouch (n.9), bowel (n.7), uterine ligament (n.7), addominopelvic wall (n.6) and urinary tract (n.5). Epithelial cells, in the 59 total lesions, revealed the following hormone expression: 48 ER+, 2 ER–, 9 not evaluable; 54 PR+, 0 PR –, 5 not evaluable; 35 AR+, 21 AR–, 3 not evaluable. Stromal cells results: 50 ER+, 0 –, 9 not evaluable; 54 PG+, 0 –, 5 not evaluable; 46 AR+, 10 AR–, 3 not evaluable. The ER resulted broadly express in both components, in

the glands (71,2% +/– 233) and in the stroma (75,8 +/- 141); the PR was mainly positive in the stroma (77,2% +/– 1826) compared to the glands (57,6% +/- 32,3); the AR was less positive in the glands (14,8% +/– 17,1) compared to the stroma (38,2%+/–26,3). No difference ER and AR expression were observed in the various anatomic sites both in the glands that in the stroma, while a significant lower expression of PR in the Douglas' pouch ($p<0.05$).

**Conclusions.** The ER results diffusely and strongly expressed in all examined sites, while a marked variability is observed in the expression of the PR, in particular in Douglas' pouch. Altogether the AR results low expressed with a marked variability for intensity and distribution. In conclusion the results obtained confirm the importance of estrogens in the maintenance and progression of lesions. The variability of PR expression justifies the observation of resistant cases to the medical therapy.

## HIGH GRADE LEIOMYOSARCOMA OF THE UTERUS WITH HUMAN CHORIONIC GONADOTROPIN PRODUCTION

G. Satta[1], V. Marras[1], M.A. Fedeli[1], A.C. Camboni[1], M. Trombetta[1], F. Tanda[1], G. Ruiu[2], F. Pili[3], M. Contini[3], P. Cossu Rocca[1 3]

*[1] Department of Medical, Surgical and Experimental Medicine, Unit of Pathology, University of Sassari, Sassari (Italy); [2] Gynecology and Obstetrics Unit, "Giovanni Paolo II" Hospital, ASSL Olbia, ATS Sardegna, Olbia, Italy; [3] Department of Diagnostic Services, Pathology Unit, "Giovanni Paolo II" Hospital, ASSL Olbia - ATS Sardegna, Olbia, Italy.*

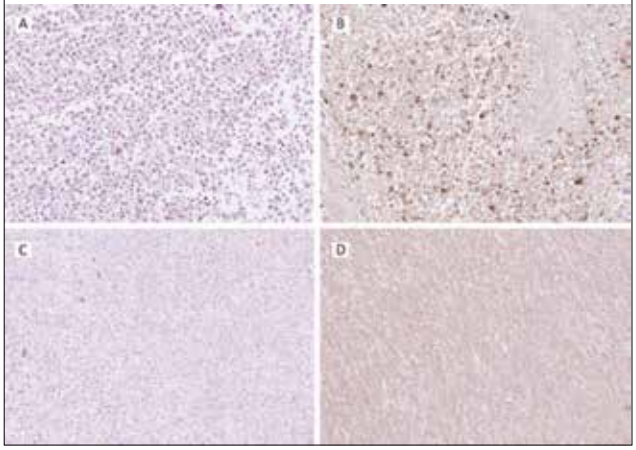
**Background.** Human chorionic gonadotropin (b-HCG) is a well-known diagnostic tumor marker for germ cell tumors with choriocarcinomatous features, although elevate levels of b-HCG have also been associated with a variety of non-germ cell tumors, such as pulmonary, gastric and pancreatic cancer. So far, very few cases of malignant soft tissue tumors with anomalous b-HCG production have been reported in the literature [1]. The aim of the present study is to describe a rare case of b-HCG producing high grade uterine leiomyosarcoma.

**Materials and Methods.** A 52-years-old woman with a 4-month history of menometrorrhagia and pelvic pain was admitted to the Gynecology Unit at "Giovanni Paolo II" Hospital, Olbia, Italy. Transabdominal ultrasound examination revealed an enlarged uterus (150 x 88 x 70 mm) with a poorly demarcated and hypervascular area characterized by a mixed echogenic and anechogenic features, suggestive of uterine intramural leiomyoma, measuring 90 mm in the largest diameter. Blood laboratory tests showed high levels of β-HCG (96/mlU/ml); alpha-fetoprotein, CA125, and CA15.3 were within normal limits. Urine pregnancy test was positive. Based on the clinical, laboratory and radiological data, a total hysterectomy was planned, which was entirely submitted to intraoperative consultation to rule out malignancy. Frozen section evaluation of the uterine mass displayed morphologic features consistent with a poorly differentiated infiltrative neoplasm, with extensive necrosis, mainly composed of large epithelioid cells with pleomorphic, atypical nuclei; neoplastic invasion of the cervix was also appreciable. Following the intraoperative pathologic diagnosis, the patient underwent a wider resection of vaginal fornices and bilateral salpingo-oophorectomy, with full staging procedures. Surgical procedures were uneventful. Representative tissue samples of the uterine tumor were formalin fixed, paraffin embedded, sectioned at 3 µm, and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed on the automated Ventana BenchMark ULTRA platform.

**Results.** At gross examinations, the uterine corpus was remarkably distorted due to the presence of a soft round mass measuring 12 cm in the greater diameter. The lesion showed a gray-yellowish color and wide necrotic areas. It appears to infiltrate the full thickness of uterus wall and focally to bulge into the uterine cavity. No macroscopic abnormalities where found within the residual uterine mucosa. On histology, the mass appeared to be unencapsulated and poorly defined, and to be composed of barely-cohesive large epithelioid cells with clear cytoplasm, and pleomorphic, highly atypical nuclei with scanty nuclear pseudoinclusions. High mitotic rate and extensive coagulative necrosis were also appreciable. Among the epithelioid proliferation, minor areas characterized by the presence of atypical spindle cells organized in bundles, reminiscent of classical leiomyosarcoma, were discernible. The neoplasm infiltrated the myometrium and reached the serosal surface of the uterus. Features of lymphovascular invasion were also found. As described at gross examination, the residual uterine mucosa was preserved, showing only edema and focal adenomyosis. Immunohistochemical staining was performed, confirming the double differentiation of the lesion. The epithelioid component showed diffuse immunoreactivity for vimentin and CD10; moderate positivity for β-HCG; EMA, AE1/AE3, ER, Smooth Muscle Actin, Muscle Specific Actin, Desmin, CD30, PLAP, α-fetoprotein (AFP), α-Inhibin, S100 and HMB45 were negative. The spindle cell component showed diffuse positivity for vimentin, Smooth Muscle Actin and Muscle Specific Actin and negativity for all the previously reported markers (Fig. 1). Based on these findings, the lesion was identified as a poorly differentiated malignant mesenchymal neoplasm, indicative of a high grade leiomyosarcoma with "dedifferentiated" features and abnormal b-HCG production. The patient is presently alive and well, with no evidence of disease progression or recurrence, seven months after surgery.

**Conclusions.** Malignant soft tissue tumors with ectopic production of b-HCG have rarely been described. So far, b-HCG expression has been documented in a few cases of osteosarcoma, and single case reports of chondrosarcoma, liposarcoma, or unclassified high grade sarcoma. b-HCG producing leiomyosarcomas are very scarce, and mainly located in extrauterine sites, as intracranial, intrascrotal, retroperitoneal and intestinal. Only a single case report of a uterine high grade leiomyosarcoma with b-HCG production has been previously published

Figure 1. Representative images of the tumor, highlighting the "dedifferentiated" epithelioid cell component (a. H&E; b. immunostain for beta-HCG) and the spindle cell component (c. H&E; d. immunostain for Smooth Muscle Actin).



in the literature [2]. The question of whether β-HCG production in malignant soft tissue tumors may contribute as an additional unfavorable prognostic indicator is still a matter of debate. Nevertheless, in these patients, serum β-hCG could be suitable as a marker of treatment response and disease monitoring.

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# PATOLOGIA MOLECOLARE

## CTC AR-V7 PCR ASSAY AND ITS ROLE IN CASTRATION RESISTANT PROSTATE CANCER PROGRESSION


A. Caniglia[1], B. Pilato[2], S. Tommasi [2], C. Carella[3], A. Lasorella[2], K. Danza[2], I. Vallini[4], A. Campanella[2], S. De Summa[2], E. Naglieri[3]

*[1] Anatomopathology Unit, IRCCS Istituto Tumori "Giovanni Paolo II" Bari, Italy; [2] Molecular Diagnostics and Pharmacogenetics Unit, IRCCS Istituto Tumori "Giovanni Paolo II" Bari, Italy; [3] Medical Oncology Unit, IRCCS Istituto Tumori "Giovanni Paolo II" Bari, Italy; [4] Bird srl, R&D facility, Rezzato, Italy*


Castration resistant prostate cancer (CRPC) represents the most aggressive status of this neoplastic disease, also characterized by the absence of biomarkers predictive of clinical outcome. Despite the advantages of the second-generation androgen deprivation therapy (ADT), as abiraterone or enzalutamide, resistance mechanisms, primitive or acquired, often develop. An example of acquired resistance is the expression of androgen receptor (AR) splice variants (AR-Vs), in particular AR-V7, detected in circulating tumor cells (CTCs). In this field, hot topics are the methodology used to isolate CTC and the assay for AR-V7 measurement. Our study aims to standardize a procedure to detect AR-V7 biomarker and to confirm its prognostic role in CRPC patients.

**Materials and Methods.** We realized cell based Reference Sample as Standardized Quality Control tool for CTC-AR-V7 assay and developed standardized operating procedure to detect the biomarker. Forty-four castration resistant prostate cancer patients have been consecutively enrolled in the outpatient clinic of IRCCS Istituto Tumori of Bari, after signing the informed consent approved by the Institutional Review Board. Patients were treated with the standard of care abiraterone/enzalutamide or taxol. Median follow-up was 20,5 months.

**Results.** In our series 27% of patients presented the splicing form AR-V7. Overall PSA response rate, defined as PSA reduction ≥ 50% from the baseline, was respectively 33%, 44% and 28% in patients treated with abiraterone/enzalutamide/ormono and chemotherapy. When considering the presence of AR-V7 in the overall series and in patients treated with abiraterone or enzalutamide in first line, in both cases there was an inverse relationship between PSA reduction and AR-V7 presence. In particular, only 3 patients, positive for AR-V7 had PSA reduction ≥ 50%, while 72,7% of AR-V7 patients had no PSA response both considering the overall series and the patients specifically treated with abiraterone/enzalutamide (Fig. 1). Moreover, 75% (6/8) AR-V7+ patients were hormone-resistant to abiraterone/enzalutamide treatment, whilst 57% (8/14) AR-V7- patients were hormone sensitive, even if no statistical significance had been reached, due to the small size of the cohort. In the overall cohort median PFS was 6 months (95% CI, 7 to NA) in AR-V7 + patients and 18 months (95% CI, 7 to NA) in AR-V7 - patients (p=0,054) (Fig. 2).

**Figure 1.** Waterfall plot depicting best PSA response according to AR-V7 status in patients treated with Abiraterone or Enzalutamide in first line: we detected 50% (10/20) AR-V7+ patients of which 7 had no PSA response.

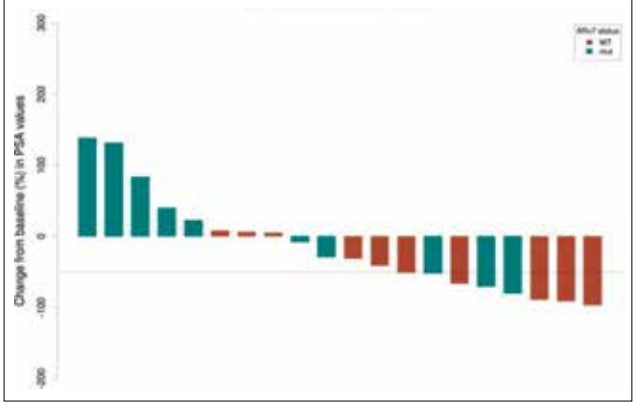
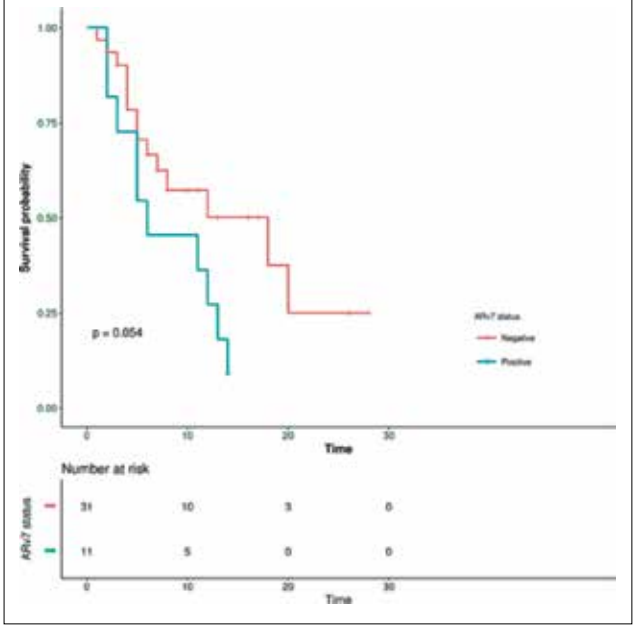



**Figure 2.** Kaplan-Meier curves related to PFS of the overall series: AR-V7+ patients (blu line) had a poor PFS (p=0,054). Red line: AR-V7 - patients




**Conclusions.** The standardized procedure has a high sensitivity and specificity and permitted to confirm the prognostic role of AR-V7.

## ROLE OF CANONICAL AND NON CANONICAL SPLICING MUTATION IN PATIENTS WITH LYNCH SYNDROME


D. Della Libera, A. D'Urso

*UOC Anatomia Patologica - Ospedale S. Maria del Prato, Feltre - ULSS1 Dolomiti.*


**Introduction.** Hereditary cancer syndromes can predispose

affected individuals and their relatives to the early development of neoplasias belonging to the corresponding spectrum of tumours. Identification of the causal mutation is essential for a correct diagnosis. Splicing mutations represent a considerable percentage of disease-causing tumours [1 2]. Although the pathogenic role of 5' and 3' canonical splice site variants is already evident, the potential impact of exonic mutations on RNA splicing, by altering exonic splicing enhancer (ESE) and exonic splicing silencer (ESS) sequences, is still underestimated [2]. Published data document that MLH1, a mismatch repair (MMR) gene involved in Lynch syndrome (LS), the most common form of hereditary colorectal (CRC) and endometrial cancers (EC), shows a high degree of exon mutations leading to splicing aberrations 1. Based on these observations, we aimed to review the molecular characterisation offered to the cohort of patients analysed at the Anatomic Pathology Unit of Feltre Hospital (ULSS1 Dolomiti) for LS, focusing on mutations not presently listed in the LOVD-InSiGHT database, evaluating their potential implications in splicing processes.

**Materials and Methods.** Tumours were subjected to immunohistochemical (ICH) staining for MMR gene products (Roche/Ventana, Rotkreuz, Switzerland), microsatellite instability (MSI) (HNPCC kit; Experteam, Venice, Italy), and BRAF gene mutation analysis (Diatech Pharmacogenetics, Jesi (AN) Italy) according to the manufacturers' instructions, and MLH1 promoter methylation analysis as described previously [3]. Direct Sanger sequencing was used to analyse MMR genes. The multiplex ligation- dependent probe amplification (MLPA) technique was used to evaluate large genome rearrangements (LGRs). RNA analysis was conducted using Extrazol and RT Plus kits (ELITech Group, Puteaux, France) according to the manufacturer's instructions.

**Results.** From 2012 until now, 170 cases of CRC were screened by ICH for MMR genes, MSI and BRAF mutation analysis, and MLH1 promoter hypermethylation. Candidate patients were subsequently referred to the second level of molecular testing for MMR genes. The analysis revealed seven cases harbouring LGRs involving MLH1 and MSH2 genes [4] and eight cases characterised by a small mutation affecting the MLH1, PMS2 and MSH6 genes. Among the latter, four are not listed in the LOVD-InSiGHT database. The first mutation, c.1558+1G>A [5], involves a canonical 5' splice site consensus sequence of intron 13 of MLH1. Subsequent analysis conducted on patient- derived RNA demonstrated that it causes exon 13 skipping, clarifying its pathogenic role. The other three mutations—c.1281delT, c.576dupT and c.1550_1151delTT—affect the PMS2 gene. These are frameshift mutations that give rise to a premature stop codon and a truncated protein. It is interesting to note that, although these are exonic mutations, in silico analysis conducted using Human Splicing Finder [6] revealed that c.1281delT creates a new ESS site and alterates an ESE one, c.576dupT alterates an ESE site, while the third, c.1550_1151delTT, does not seem to involve splicing regulatory elements. Given the availability of c.1281delT carrier-derived RNA, we performed further analysis to clarify how the exonic PMS2 mutation could impact on splicing processes. Tests revealed the presence of multiple aberrant transcripts, the most abundant of which harbour exon 11 skipping while the minority form is characterised by skipping in exons 11 and 12, confirming the in silico results.

**Discussion.** Published data suggest that one-third of disease-associated alleles alter gene splicing [7]. In genomes, canonical splice sequences are an essential component of the exon splicing process, providing a specific molecular signal for the RNA splicing machinery to identify precise splice points. The c.1558+1G>A mutation we found involves the canonical donor site consensus sequence in intron 13 of the MLH1 gene and causes exon 13 skipping. It is interesting to note that the LOVD-InSiGHT database reports variant c.1558+1G>T found by five different authors [8-12]. In particular, RNA analysis conducted by Benatti et al. revealed how the c.1558+1G>T mutation gives rise to an aberrant transcript longer than the wild-type form, including the first 108 bp of intron 13 because of the use of a cryptic acceptor splice site [9]. Our characterization contributed to clarifying how the newly identified c.1558+1G>A variant that involves the same position in the MLH1 sequence of the mutation mentioned above determines a different splicing defect, probably because of different base substitution. While it is well established how variants in the consensus donor (5') and acceptor (3') splice site regions can abolish or diminish the strength of canonical splice sites, the potential impact of exonic variants on RNA splicing is not always considered. Our data emphasise the possible implication of exonic mutations in MMR genes other than MLH1 in splicing disruption. In particular, RNA analysis of the PMS2 c.1281delT carrier shows that the exonic mutation, by creating a new ESS site and alterate an ESE one, gives rises to a predominant aberrant transcript with exon 11 skipping. A previous review of MMR gene transcripts underlined the existence of several naturally occurring MMR alternative transcripts with no associated mutations, nine of which derive from PMS2 gene [13]. No exon 11 skipped transcript was listed between the naturally occurring alternative mRNA sequences of PMS2, strengthening the pathogenic role of c.1281delT in disrupting the normal splicing process. In conclusion, the universal screening strategy for diagnosis of LS, gradually introduced from 2012 until now at our unit has allowed us to identify 8% of LS-related CRCs. The molecular analyses we conducted proved to be particularly useful in delineating the pathogenetic role of MMR gene variants not previously described, especially in PMS2 mutation carriers. In fact, both technical difficulties associated with the presence of pseudogenes that hamper molecular testing, and the low penetrance of the PMS2 mutation, could give rise to underestimation of its mutation rate, especially in a clinical setting in which patient selection is made considering only a strong family history for LS-related tumours. This explains why data on the proportion of PMS2-driven LS and detailed molecular characterisation of its variants are scarce. Furthermore, our molecular characterisation, consistent with published reports, underline the potential implication of exonic mutations in disruption of the splicing process, clarifying one of the possible mechanisms of MMR gene variant pathogenicity, allowing the formulation of an accurate diagnosis and subsequent personalised clinical management and surveillance of patients with LS.

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## MOLECULAR EVALUATION OF CELL-OF-ORIGIN (COO) IN DIFFUSE LARGE B- CELL LYMPHOMAS

E. Forti, S. Manfredini, L. Liguori, M. Gozzoli, S. Pozzi, A. Bari, S. Sacchi, A. Maiorana, S. Bettelli

*Università degli Studi di Modena e Reggio Emilia (UNIMORE), Dipartimento di Scienze Mediche e Chirurgiche Materno-Infantili e dell'Adulto (SMECHIMAI)*

**Background/Aims.** Diffuse large B-cell lymphoma (DLBCL) is one of the most frequent and aggressive B-lymphomas. It represents an heterogeneous group in regard to morphological features, genetic alterations and clinical behaviour [1]. Two distinct molecular subtypes, called Activated B- Cell -like (ABC) and Germinal Center B-cell -like (GCB), have been defined using gene expression profiling (GEP) on the basis of Cell-Of-Origin (COO). This classification acquires a prognostic and therapeutic value and therefore it is important for patient stratification [2]. In standard practice, COO is defined by the immunohistochemistry (IHC)-based Hans algorithm carried-out on formalin-fixed paraffin-embedded (FFPE) tissue [3]. Recent technological improvements allow to apply GEP directly on RNA extracted from FFPE tissue. Aim of this study is to validate the GEP-approach comparing data obtained with the IHC method.

**Materials and Methods.** A cohort of 83 DLBCL patients was collected for this study. Archival FFPE samples were obtained from the Department of Pathology-AUO Policlinico di Modena. IHC stainings were performed under an automated standardized protocol (Ventana Benchmarks; Ventana, Tucson, USA) using antibodies against CD10, BCL6 and MUM1. The IHC profiling was obtained in accordance with the Hans algorithm. mRNA from representative FFPE sections was extracted with an automatic method (Maxell, Promega) and quantified with Expose (Trinean). 50- 500 ngr of total RNA were used for GEP in a NanoString platform (Nanostring Tecnologies, Seattle, USA) with application of the Lymph2Cx panel that includes a 20 gene-code set, as described by Scott et al. [4]. Data analysis was performed following the manufacturer's protocol.

**Results.** Application of Hans algorithm on IHC sections allowed to identify 46 ABC (46/83, 55%) and 37 GBC (37/83, 44%) whereas 1 case was Unclassified (1/83,1%). Nanostring Lymph2Cx algorithm identified 26 ABC (26/83, 31%), 43 GCB (43/83, 52%) and 13 Unclassified DLBCL (13/83, 16%). Among the 83 cases, only one failed to meet the required RNA quality for the test. Comparison between IHC- and Lymph2Cx-results showed 57 concordant cases (57/69, 83%) and 12 discordant cases (12/69, 17%), after exclusion of the Failed and molecularly Unclassified samples. In particular, 24 cases were defined as ABC and 33 as GBC by both IHC and Lymph2Cx. Among the 12 discordant results, 10 cases were identified as ABC by IHC examination whereas they were defined as GCB by Nanostring analysis. Moreover, Hans algorithm identified 11 ABC and 2 GBC among the 13 Nanostring Unclassified DLBCL.

**Conclusions.** In summary, our study demonstrates a good degree of correlation between the IHC- based approach and GEP by Nanostring Platform. From a technical point of view, Lymph2Cx allows to assign the DLBCL subtype using low amounts of RNA, standardized protocols and rapid turn-around-time. Nanostring platform and Lymph2Cx panel could be useful tools for DLBCL patients' stratification.

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## TRANSCRIPTOMICS LANDSCAPE OF NECROPTOSIS GENES IS ASSOCIATED WITH DENDRITIC CELLS INFILTRATION: A PAN-CANCER STUDY OF 5,451 PRIMARY SOLID TUMORS

Lorenzo Nicolè[1*], Tiziana Sanavia[2*], Rocco Cappellesso[1] and Ambrogio Fassina[1]

*[1] Department of Medicine (DIMED), Surgical Pathology Unit, University of Padua, Padua, Italy; [2] Department of Biomedical Informatics, Harvard Medical School, Boston, MA, USA; [*] Equal contribution*

**Objective.** Necroptosis (NPC) is a form of programmed cell death that culminates with the rupture of the cell membrane followed by the releasing of cellular elements. Evidence showed that tumors with high expression of NCP-related genes are associated with high cytotoxic CD8+ T-cell infiltrates, mediated by signaling from Dendritic (DC) and CD4+ T-cells. This study shows a pan-cancer view of the relationship between NCP and immune infiltration and their prognostic relevance across 24 cancer types from The Cancer Genome Atlas (TCGA).

**Materials and methods.** Gene expression RNA-seq data from 5,451 primary solid tumors were considered, excluding cases with treatments before surgery and with residual tumor. A deconvolution algorithm was used to estimate the level of tumor-infiltrating immune cells in each RNA- seq sample, considering the populations: B-cells, CD4 T-cells, CD8 T-cells, Macrophages and DC. For each immune population, the relative infiltration score was dichotomized at low and high infiltration using the 25th and 75h percentiles, respectively. Logistic regression and likelihood ratio test were applied to 163 genes belonging to Necroptosis pathway from KEGG database to test whether they are significantly associated to the infiltration of a specific immune population. FDR-adjusted p-values <0.05 were considered statistically significant. The prognostic relevance of the NCP genes significantly correlated with the infiltration was evaluated by Cox regression and log-rank test.

**Results.** DC and CD4+ T-cells showed the highest number of cancer types (8) reporting more than half genes of NCP pathway significantly correlated with their infiltration. CD8+ T-cell infiltration correlated with >50% of NCP genes in 5 of these 8 cancer types: Kidney-Renal, Breast, Prostate, Pancreatic and Thyroid tumors. DC also showed the highest number of NCP genes (69) correlated with their infiltration in more than half of the analyzed cancer types, including the main genes involved in NCP execution: RIPK1, RIPK3, MLKL and CFLAR. 60 and 58 of these genes showed a prognostic relevance ($p<0.05$) for overall and disease-free survival in at least one cancer type, respectively.

**Conclusions.** NCP has a relevant role in eliciting immune response against tumor through DC- mediated immunity in specific cancer types. In the new incoming era of immunotherapy, immune profiling of tumor from high throughput-derived transcriptomics and genomics data, holds a great potential in order do define specific biomarkers for prognostic or predictive purposes.

## EGFR EXON 19 DELETION SWITCH AND DEVELOPMENT OF P.L792Q MUTATION AS A NEW RESISTANCE MECHANISM TO OSIMERTINIB: A CASE REPORT

P. Pisapia, F. Pepe, C. De Luca, R. Smeraglio, M. Cieri, C. Bellevicine, U. Malapelle, G. Troncone.

*Department of Public Health, University of Naples "Federico II", Naples, Italy.*

**Aim.** The identification of EGFR gene mutations plays a key role in the management of Non Small Cell Lung Cancer (NSCLC) patients, in order to administrate tyrosin kinase inhibitors (TKIs) [1-4]. However, after treatment with a first or a second generation EGFR TKI, different mechanisms of resistance can arise. The most common resistance mechanism is represented by the exon 20 p.T790M point mutation [5]. To date, these patients have the possibility to be treated with a third generation TKI (osimertinib) [5]. Also in this instance, there are possibility of several mechanism of acquired resistance, the most common is represented by the exon 20 p.C797S [6].

**Material and Methods and Results.** A 68 years-old man with a metastatic NSCLC and an EGFR exon 19 deletion, identified on a CT-guided Fine Needle Aspiration (FNA) on a liver lesion sample, was treated in first line with gefitinib and, after progression and the identification of the resistance point mutation p.T790M with the concomitant initial deletion on a liquid biopsy sample with a next generation sequencing (NGS) approach, with osimertinib in second line. Another liquid biopsy was requested by the oncologist after disease progression and with a NGS approach we identified a different EGFR exon 19 deletion (p.L747_A750>P), and the uncommon point mutation in EGFR exon 20 (p.L792Q), without the evidence of p.T790M.

**Conclusions.** we reported an uncommon mechanism of resistance to osimertinib, identified with a validated NGS approach.

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## COMPARISON BETWEEN THE BETHESDA AND HAMELIN PANEL FOR THE ANALYSIS OF MICROSATELLITE INSTABILITY IN COLORECTAL CANCER PATIENTS

C. Caprera[1], M. Corsi[1], G. Ramelli[2], M. Boergesen[3], R.K. Petersen[3], F. Perillo[2], P. Spina[2], L. Mazzucchelli[2], U.B. Christensen[3], S. Ascani[1], M. Frattini[2]

*[1] University of Perugia, Institute of Pathologic Anatomy "Santa-Maria", Terni, Italy; [2] Institute of Pathology, Locarno, Switzerland; [3] PentaBase Aps, Odense, Denmark*

**Background.** Microsatellite instability (MSI) analysis has become very important in the last years especially in the light of the possibility of immunotherapies administration. Moreover, the determination of the MSI status is used as molecular marker for assessing prognosis and chemotherapeutic decisions in colorectal cancers (CRC) patients. There are many panels, available on the market, used to establish the microsatellite status, but the only one approved by international authorities remains the Bethesda assay, which is based on the analysis of 2 mononucleotide and 3 dinucleotide repeats. However, there are emerging data showing that other assays can better describe the microsatellite status, but comparative studies are still missing. Therefore, we decided to compare the results of the Bethesda panel with those obtained with the second most diffused panel, the Hamelin's assay.

**Materials and Methods.** After DNA extraction (QIAamp DNA FFPE Tissue Kit, Qiagen, Chatsworth, CA, USA), we characterized the MSI status of 116 patients affected by CRC using the Bethesda and the Hamelin panel. The Bethesda (BAT25, BAT26, D2S123, D5S346 and D17S250) and Hamelin (BAT25, BAT26, NR-21, NR-22 and NR-24) loci were amplified with a multiplex ready-to-use PCR mix (Pentiplex®, Pentabase, Odense, Denmark) approach and the products were subjected to capillary electrophoresis using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The same rule was used for both panels: a patient is classified as microsatellite stable (MSS) if none of the analyzed loci are instable. If 30% of the analyzed loci are instable the patient is classified as microsatellite instable low (MSI-L) and if more than 30% of the markers are mutated a sample is considered as microsatellite instable high (MSI-H).

**Results.** The results obtained with the Bethesda panel showed that 28 patients were classified as MSI-H (24.1%), 15 as MSI-L and 73 as MSS. With the Hamelin's assay 26 samples were classified as MSI-H (22.4%) and the remaining 90 as MSS. No MSI-L cases were detected using the Hamelin's panel. Indeed, all the Bethesda MSI-L samples were classified as MSS with the Hamelin's panel and displayed a normal expression of the proteins of the mismatch repair (MMR) system. All the Bethesda MSI-H patients were also classified as MSI-H by the Hamelin's assay with the exception of 2 cases: these discrepant cases were characterized by stability of the mononucleotide loci and instability of the dinucleotide loci of the Bethesda panel, by stability of all mononucleotide loci of the Hamelin's panel, by a normal expression of the MMR system proteins, by absence of BRAF mutations and by absence of MLH1 promoter methylation. Moreover, an unusual pattern was observed in the healthy tissue of 21 samples (18.1%) using the Hamelin's panel. In these cases, the normal mucosa showed normal expression of all proteins of the MMR system.

**Conclusions.** Based on the results we obtained, the Hamelin's panel seems to better represent the MSI analysis, especially for cases classified as MSI-H by the Bethesda panel with instability identified only in the dinucleotide loci. Moreover, even if

the Hamelin's panel should not require the analysis of healthy tissue, the identification of unusual patterns resembling instability, that can therefore lead to an incorrect evaluation of the case if the evaluation is limited to the tumor tissue, strongly suggests to add the analysis of normal mucosa also for the Hamelin's panel. Finally, more comparative studies among the different panels assessing microsatellite instability are advisable.

## IDYLLATM ASSAY AND NEXT GENERATION SEQUENCING: AN INTEGRATED EGFR MUTATIONAL TESTING ALGORITHM

C. De Luca[1], A.G. Rappa[2], G. Gragnano[1], U. Malapelle[1], M. Barberis[2], G. Troncone[1]

*[1] Department of Public Health, University of Naples Federico II, Naples, Italy; [2] Division of Pathology and Laboratory Medicine, Istituto Europeo di Oncologia, Milan, Italy*

**Aim.** To date, any predictive molecular laboratory analysing non-small cell lung cancer (NSCLC) specimens for the molecular status assessment of the different predictive biomarkers needs to implement and careful validate different molecular methodologies [1]. Updated international guidelines by College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology (CAP/IASLC/AMP) recommend the next generation sequencing (NGS) as the initial procedure to perform [2]. However, in a relevant subset of cases, we performed problems regarding library generation or amplicon coverage [3 4]. In these cases with a NGS 'invalid' result, the IdyllaTM system may represent a valid alternative for the rapid assessment of the epidermal growth factor receptor (EGFR) molecular status [5].
**Material and methods.** We retrospectively analyzed N = 68 archival DNA samples previously genotiped by Ion Torrent NGS assay. In this setting, N = 43 (63%) cases (including N = 24 EGFR mutant samples) had an adequate NGS result, whereas N = 25 (37%) did not generate adequate library or had an insufficient amplicon coverage. All samples were re-analized by directly pipetting the DNA inside the EGFR IdyllaTM assay cartridge.
**Results.** IdyllaTM confirmed, in the 43 cases with an adequate NGS result, the correct EGFR mutational assessment. Of interest, all (24/24; 100%) mutation in EGFR identified by NGS were confirmed by IdyllaTM. In the other 25 cases with an inadequate result with NGS, a high percentage of cases (20/25; 80%) were adequately processed by IdyllaTM. In particular, in this setting in 4/25 (16%) of cases, IdyllaTM detected actionable EGFR mutations.
**Conclusions.** IdyllaTM assay represents a very useful tool to rapidly process cases for which NGS does not allow genotyping.

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## MOLECULAR PROFILING OF A SQUAMOUS CELL CARCINOMA OF THE LUNG TREATED WITH ANTI-TKI INHIBITORS. CASE REPORT

G. De Maglio[1], A. Follador[2], F. Cortiula[2], M.G. Cangi[3], I. Francaviglia[3], S. Bonura[4], A. De Pellegrin[1], C. Doglioni[3], G. Fasola[2], S. Pizzolitto[1]

*[1] Pathology Department, University Hospital of Udine, P.le S.M. Misericordia, Udine, Italy; [2] Department of Medical Oncology, University Hospital of Udine, P.le S.M. Misericordia, Udine, Italy; [3] Unit of Pathology, IRCCS San Raffaele Scientific Institute, Milano, Italy; [4] Medical Oncology Department, ASL5 Bassa Friulana, Gorizia, Italy*

**Background and aims**. Sensitizing mutations of EGFR (Epidermal Growth Factor Receptor) are uncommon in squamous cell lung cancer (SCC), occurring in about 3-4% of patients. Therefore, EGFR testing is not routinely recommended, unless in never or former light smokers. TKIs (Tirosin Kinase Inhibitors) are effective treatments in SCC harboring activating mutations of EGFR gene and successively osimertinib is an active therapeutic strategy when p.(Thr790Met) mutation of EGFR occurs as a resistance mechanism. Herein we report the case of a patient affected by metastatic SCC harboring a complex inframe deletion in exon 19 of EGFR, treated with second-line erlotinib and third-line osimertinib.
**Methods.** Molecular characterization was performed by a Mass-ARRAY system (Agena bioscience) with Myriapod Lung status kit (diatech pharmacogenetics) and Real time PCR with Easy® EGFR kit (diatech pharmacogenetics). ALK and ROS1 rearrangements were investigated by Fluorescence in situ hybridization (FISH) with ALK Break Apart FISH Probe kit (Abbott) and ZytoLight® SPEC ROS1 Dual color Break Apart Probe (ZytoVision) probes respectively. PD-L1 status was assessed by immunohistochemistry staining with Ventana PD-L1 (SP263) assay on a BenchMark/VENTANA Ultra platform (Ventana), according with manufacturer's instructions. NGS analysis was performed with Oncomine solid tumor DNA kit (Thermo scientific) on Ion torrent S5 equipment and bioinformatic analysis on Torrent Suite Software 5.2, (Thermo Scientific).
**Results.** A 54-year-old male, former light smoker (3 packs per year) presented with stage IV SCC. Immunohistochemistry on transbronchial biopsy showed p63 positive, thyroid transcription factor 1 (TTF-1) negative immunophenotype.
**Molecular** analysis for EGFR was planned in consideration of light smoking history, but because of the rapid drop in clinical conditions, a first line platinum-based chemotherapy was started. Molecular characterization highlighted deletion c.2237_2255>T in exon 19 of EGFR. Any other driver mutation or rearrangement in ALK/ROS1 were detected and PD-L1 expression was negative. After two cycles of chemotherapy, disease progression occurred. Therapy with erlotinib at full dose was started. No significant side effects were reported. After six months CT scan showed progression of the lung localization. A liquid biopsy was done with the purpose of investigating p.(Thr790Met) mutation. Previously described EGFR deletion was confirmed and a new p.(Thr790Met) mutation was detected. Thus osimertinib was started, within the ASTRIS clinical trial. After five cycles, a new progression of the lung

lesion was experienced and Nivolumab was started. Concomitantly, a new bronchial biopsy was performed, confirming squamous histotype and presence of EGFR deletion. Point mutation p.(Thr790Met) was not detectable anymore. After two administrations of Nivolumab the patient underwent a brain CT scan for sudden onset of buccal rhymes deviation. A new brain lesion was detected. Based on both patient's characteristics and treatment history, a re-challenge with Erlotinib was attempted, but progression occurred after two months and Docetaxel was started. After three cycles, a new progression occurred and the treatment was therefore stopped. Skin metastasis was sampled for molecular reassessment. EGFR deletion was still present, p.(Thr790Met) mutation was not detectable anymore. NGS analysis for single nucleotide variants and short indels of eleven genes (ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, and TP53) confirmed EGFR deletion c.2237_2255>T p.E746_S752>V (AF 56%), absence of T790M along with EGFR p.G724S, c.2170G>A (AF 25,38%), TP53 p.P152L, c.455C>T (AF 69,83%), CTNNB1 p.A13T, c.37G>A (AF 0.57%), FBXW7 p.R278* c.832C>T (AF 0.69%), PTEN p.Q171* c.511C>T (AF 0.75%). No clinical trials being available targeting mutation detected, supportive care was implemented. Patient died after six months.

**Conclusions.** Programmed death-1 checkpoint inhibitors are the standard of care in SCC in second line of therapy or in first line depending on PD-L1 level of expression. A meta-analysis of the randomized trials comparing immunotherapy with chemotherapy, showed no improvement in survival for patients with EGFR activating mutations. The use of TKIs in SCC patients unselected for EGFR mutational status has shown controversial results. We reported the case of a SCC of the lung harboring sensitizing EGFR mutation, treated with targeted anti-TKIs and then osimertinib. Molecular driver overcame histologic diagnosis in treatment strategy. Furthermore, we confirm the previously described possible emerging role of EGFR exon 18 p.(Gly724Ser) mutation, as a resistance mechanism to osimertinib.

## APPLICATION OF SIRE® NEXT-GENERATION SEQUENCING PANEL ON NSCLC ROUTINE SAMPLES

F. Pepe, C. De Luca, R. Smeraglio, P. Pisapia, R. Sgariglia, M. Nacchio, M. Russo, N. Serra, G. Gragnano, E. Vigliar, C. Bellevicine, U. Malapelle, G.Troncone

*Department of Public Health, University of Naples Federico II, Naples, Italy*

**Aim.** As a general rule the adoption of small gene panels require a low quantity DNA input; this makes NGS analysis feasible even on small tissue samples [1]. In particular, in most cases, Non Small Cell Lung Cancer is diagnosed in an advanced disease stage and for this reason only minimal invasive procedure are possible to obtain specimens for molecular purpose [2 3]. Following the previous experience of development for liquid biopsies of the SiRe® Next Generation Sequencing (NGS) panel, that covers 568 clinical relevant mutations in EGFR, KRAS, NRAS, BRAF, cKIT and PDGFRa genes [4], and the experience on liquid biopsy in a setting of lung cancer patients in a basal setting [5]. In particular, in this study we adopted this narrow NGS panel on tissue samples of lung cancer.

**Material and methods.** N = 322 specimens were prospectively tested and technical parameters were analyzed on both cytological and histological samples. For N = 75 samples, the EGFR SiRe® results were compared to those obtained by the CE – IVD EGFR assay on IdyllaTM platform. Moreover, the clinical outcomes of N = 11 patients, harboring a mutation in EGFR gene discovered by SiRe® panel, treated with EGFR tyrosin kinase inhibitors (TKIs) were also evaluated.

**Results.** Only N = 28 (8.7%) samples failed to produce a library. On the N = 294 remaining adequate samples, a total of 168 somatic mutations were identified. In 74/75 (99%), the EGFR SiRe® results were correctly confirmed by IdyllaTM. In general, SiRe® analytical parameters were excellent. However, only in some instances histological and cytological specimens differed in relation to average reads for sample (p = 0.0379), mean number of mapped reads (p = 0.0435), median read length (p = 0.0009) and average reads for amplicon ( p = 0.0236). On the N = 11 patients with the analysis of the clinical outcome, N = 9 (82%) showed a partial response with a median PFS of 340 days.

**Conclusions.** The narrow SiRe® gene panel is a clinically feasible to widespread the adoption of NGS in predictive molecular pathology laboratories.

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# PATOLOGIA NEFROLOGICA

## MALDI-MSI APPROACH TO RENAL BIOPSIES OF PATIENTS WITH FABRY NEPHROPATHY

V. L'Imperio[1], A. Smith[2], V. Scollo[3], F. Rossi[3], R.A. Sinico[3], M. Nebuloni[4 5], F. Pieruzzi[3], F. Magni[2], F. Pagni[1 4]

*[1] Dept of Medicine and Surgery, Pathology, UNIMIB, San Gerardo Hospital, Italy; [2] Dept of Medicine and Surgery, Proteomics and Metabolomics Unit, UNIMIB, Monza, Italy; [3] Dept of Medicine and Surgery, Nephrology Unit, UNIMIB, Monza, Italy; [4] Research Center for renal Immunopathology, University of Milan, Milan, Italy; [5] Dept of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy*

**Introduction and aims.** Fabry disease (FD) is a X-linked hereditary condition due to the mutation of the enzyme alpha-galactosidase A with consequent intracellular accumulation of its substrate (Gb3). Fabry nephropathy (FN, Fig. 3) can progress to end-stage renal disease (ESRD) if not adequately recognized and treated. Different mutations can determine different phenotypes (e.g. classic and atypical). Moreover, differences exist among males and females in terms of severity and time of onset of the disease, probably due to the Lyonization phenomenon. MALDI-MSI is a proteomic technique that analyzes in situ FFPE renal biopsies.

**Methods.** FFPE renal biopsies from 14 patients with FN (6

**Figure 1.** Characteristics of the cohort and mutation determining the disease. LO, late-onset; CL, classic; IND, undetermined; eGFR, estimated glomerular filtration rate.

| ID | GENDER | AGE | MUTATION | PHENOTYPE | PROTEINURIA (mg/24h) | CREATININE (mg/dl) | eGFR (EPI-CKD) ml/min |
|---|---|---|---|---|---|---|---|
| 1 | F | 34 | p.I91T | LO | 66 | 0.5 | 120 |
| 2 | F | 36 | p.R 301X | CL | 490 | 0.8 | 109 |
| 3 | M | 19 | p.Q2X | CL | 875 | 0.8 | 90 |
| 4 | M | 39 | c.317_327 del 11 | CL | 3240 | 1.3 | 51 |
| 5 | F | 52 | C.124-125 del AT | CL | 176 | 0.6 | 90 |
| 6 | F | 50 | p.G261c | IND | 360 | 0.8 | 73 |
| 7 | M | 46 | p.R356W | LO | 2000 | 0.8 | 100 |
| 8 | F | 50 | p.R356W | LO | 80 | 0.9 | 79 |
| 9 | F | 43 | p.R356W | LO | 200 | 0.8 | 95 |
| 10 | M | 27 | p.R356W | LO | 1000 | 4.5 | 10 |
| 11 | M | 32 | p.R356W | LO | 250 | 0.9 | 98 |
| 12 | F | 60 | c.667T>G | CL | 276 | 2.1 | 58 |
| 13 | M | 46 | p.R356W | LO | 1700 | 2 | 60 |
| 14 | F | 33 | p.R356W | LO | 152 | 0.7 | 120 |

**Figure 2.** Histological features of patients with comparison of male vs female and classic vs atypical groups. *Relevant difference.

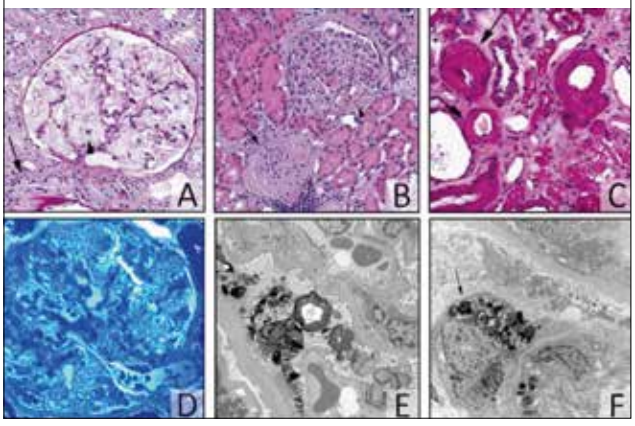
| Histological features | All (n=14) | | | Male (n=6) | | | Female (n=8) | | | p value | Classic (n=5) | | | Atypical (n=9) | | | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glomeruli, number in biopsy (LM) | 13 | ± | 6,98 | 12,38 | ± | 6,66 | 12 | ± | 7,6 | 1,00 | 13,8 | ± | 7,95 | 11 | ± | 6,63 | 0,55 |
| Segmental sclerosis (mild + severe) (%) | 3% | ± | 0,05 | 4% | ± | 0,07 | 1% | ± | 0,0 | 0,23 | 4% | ± | 0,06 | 2% | ± | 0,05 | 0,38 |
| Global sclerosis (%) | 5% | ± | 0,02 | 11% | ± | 0,20 | 1% | ± | 0,0 | 0,18 | 4% | ± | 0,06 | 6% | ± | 0,18 | 0,75 |
| Glomeruli without sclerosis (%) | 78% | ± | 0,29 | 73% | ± | 0,22 | 82% | ± | 0,3 | 0,55 | 78% | ± | 0,82 | 78% | ± | 0,69 | 0,99 |
| Interstitial fibrosis (%) | 8% | ± | 0,10 | 7% | ± | 0,10 | 9% | ± | 0,1 | 0,71 | 6% | ± | 0,11 | 6% | ± | 0,06 | 0,96 |
| Arterial sclerosis (0-3) | 0,9 | ± | 0,99 | 1,05 | ± | 0,07 | 0,82 | ± | 0,0 | 0,01* | 0,75 | ± | 0,43 | 0,96 | ± | 0,70 | 0,57 |
| Podocyte vacuoles (LM; 0-3) | 1,65 | ± | 0,61 | 2,11 | ± | 0,58 | 1,31 | ± | 0,6 | 0,20 | 1,57 | ± | 0,82 | 1,79 | ± | 0,69 | 0,59 |
| Podocyte inclusions (EM; 0-4) | 3,68 | ± | 0,47 | 3,40 | ± | 0,85 | 3,81 | ± | 0,24 | 0,37 | 3,8 | ± | 0,57 | 3,88 | ± | 1,80 | 0,53 |

males and 7 females) with different mutations of the galactosidase locus (5 classic, 8 atypical and 1 undetermined) were firstly analyzed on light and electron microscopy to assess the severity score (Fogo 2009 NDT) (Figs. 1, 2). For every patient, a 4-µm thick section from the corresponding FFPE block was cut and mounted onto a ITO slide. MALDI-MSI analysis was performed in reflectron positive mode in the mass range of m/z 750 to 2500 with the UltrafleXtreme (Bruker Daltonik GmbH) MALDI-TOF/TOF MS. Images were acquired with a laser diameter and raster of 50 µm, data elaboration was performed as previously described (Smith BBA 2016).

**Results.** Proteomics highlighted the absence of significant differences of spectra between males and females (Fig. 4A). On the other hand, differences in terms of protein profiles were found comparing classic and atypical variants (Fig. 4B), allowing the identification of a putative peptide (1325.87 m/z), corresponding to Histone H4 protein, which demonstrated a significantly different expression between the two groups (AUC≥0,8). Finally, proteomic analysis of the case with undetermined mutation demonstrated similarities with the classical average spectrum as depicted in Fig. 4C.

**Conclusions.** The present study demonstrated the feasible application of MALDI-MSI in the analysis of FN FFPE renal biopsies. The comparison of classic and atypical variants showed differences in terms of protein expression, allowing the identification of a putative diagnostic biomarker (Histone H4). Finally, proteomics can be successfully employed in cases with undetermined mutations with classification purposes.Due to the rarity of this condition, one of the limitation of the present study is represented by the few cases analyzed. For this reason, a future multicentric enrollment of further specimens is needed to confirm these findings and introduce them in the routine practice.

**Figure 3:** Histopathological and ultrastructural features of FN. In A vacuolization of podocytes (arrowhead) and tubular cells (black arrow). In B segmental vacuolization of podocytes (white arrow) with segmental (black arrowhead) and global glomerulosclerosis (black arrow). In C arteriolar hyalinosis (black arrows. In D a semithin section stained with toluidine blue shows lamellar inclusions, better appreciated in EM (E) also involving vascular muscle cells (vm) (black arrow, F).



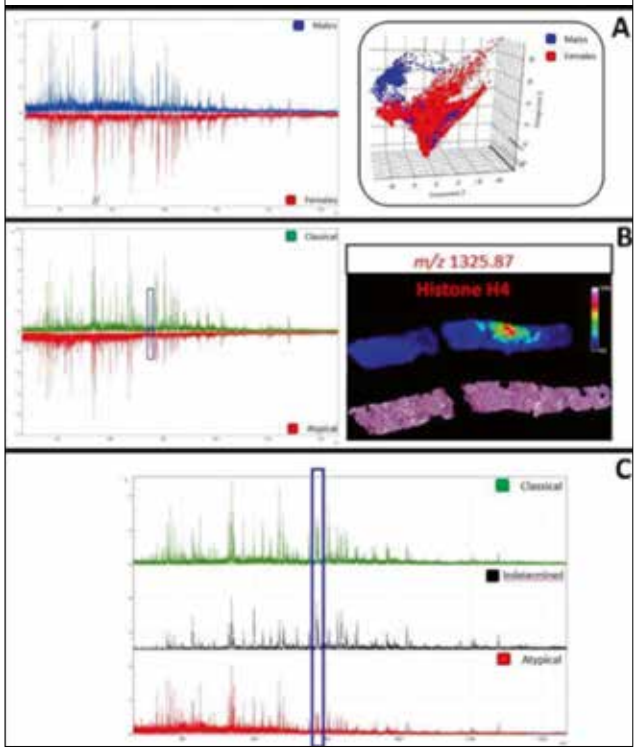

## RENAL MANIFESTATIONS REVEALED UNDIAGNOSED PRIMARY SJOGREN SYNDROME. CLINICAL-PATHOLOGICAL CORRELATION OF TWO CASES

G. Cenacchi[1], C. Zanella[2], A. Frigo[3], G. Cavalcanti[4], M. Greco[4], V. Papa[1], R. Colombari[2], A. Remo[2]

*[1] University of Bologna, Department of Pathology Bologna; [2] Pathology Unit ULSS9 "Scaligera", Verona; [3] Internal Medicine, ULSS9 "Scaligera", Verona; [4] Nephrology Unit ULSS9 "Scaligera", Verona*

**Background.** Classic clinical manifestations of Syogren syndrome (SS) include sicca, defined as dry eyes and mouth, which occurs in more than 90% of the patients. Extra-glandular manifestations of SS are variable and can involve most organ systems [1]. The prevalence of renal involvement in SS has been

**Figure 4.** In the top part (A) on the left males vs female spectra comparison, on the right PCA, no differences were noted. In the middle (B), on the left classic vs atypical variant spectra comparison showing differently expressed peaks, corresponding to statistically significant signals with an AUC value >0.8, one up-regulated in classical cases and corresponding to the peptide with a m/z value of 1325.87 (Histone H4 protein). This peptide seemed to be localized in the glomerular region of the biopsy. Finally, in the bottom part (C), the comparison of the average spectra of classical and atypical phenotypes with the case with undetermined mutation, demonstrating similarities with the classical group.




reported to range approximately from 10% to 30% [2]. Renal disease in SS is due to 2 distinct pathophysiological processes: (a) Epithelial disease with significant lymphocytic infiltration, resulting in different conditions like tubulointerstitial nephritis (TIN), electrolyte disturbances like hypokalemia, distal renal tubular acidosis, proximal renal tubular acidosis, Fanconi syndrome, diabetes insipidus, Gitelman syndrome, nephrolithiasis and nephrocalcinosis, and (b) Non-epithelial disease that can lead to glomerulopathy as a result of an immune complex- mediated process [3].

**Patients.** Case report 1. A 63-years-old Caucasian woman was admitted in Emergency Department because of signs of heart failure, severe dyspnoea and anasarca. Symptoms had been worsening over the last week. She presented also severe microcytic anaemia (Hb 4.8 g/dL). She was a heavy tobacco smoker (1 pack/die) since an early age and was irregularly following treatment for hypertension. She was admitted in hospital eight months before because of heart failure and pneumoniae. She presented anemia, mild renal failure and atrial fibrillation. She was submitted to a thoracic computed tomography (TC) that revealed signs of non specific interstitial pneumonia and pulmonary hypertension. She refused to pursue investigations to rule out underlying aetiologies and she was discharged. She performed a gastroscopy and a colonoscopy both negative. During the new admission, she was evaluated for heart failure attributed to pulmonary hypertension. The patient was treated with blood transfusions, diuretics and antibiotics for urinary tract infection, but she did not improve with conventional therapeutic measures. The outcome was unfavourable with development of acute renal failure. A basic metabolic panel revealed significant proteinuria, microscopic haematuria and reduced renal excretory function with hyperkalemia. Examinations showed severe nephrotic syndrome and pulmonary hypertension. The Patient was worked up for connective tissue disease and was found to have increased ANA and Sjögren antibody A and B were both found to be positive. A kidney biopsy revealed tubulointerstitial nephritis (TIN) and glomerulosclerosis with infiltration of lymphocytes and rare neutrophils. This infiltration focally extended into intact cortical parenchyma. Electron microscopy confirmed inflammatory infiltrate in the interstitial compartment together with unspecific glomerular alterations such as occluded capillary lumina by degenerated endothelial cells; no mesangial or capillary loop deposits are seen. The overall features were compatible with Primary Sjögren Syndrome (PSS). For PSS treatment, she started steroids and diuretics, but few weeks after she needed dialysis treatment.

Case report 2. A 69-years old Caucasian woman was admitted in Hospital because of generalized weakness, fever and lower limbs hypostenia. Past medical history was silent. On admission she was dehydrated, afebrile. Blood pressure was 100/60 mmHg, pulse 86/min, respiratory rate 25/min. She refered dry oral mucosa in the last year and lower limbs hypostenia, oliguria and constipation for about a week. She presented Raynaud phenomenon. Laboratory data showed serum creatinine 2.74 mg/dL, d- dimero 5310 ug/L (<700 ug/L). She was hydrated without improvement. Abdominal ultrasound showed normal-size kidney and no evidence of urinary obstruction. A TAC of abdomen, with subsequent dialysis session, revealed thrombosis of mesenteric superior vein. The woman started dialysis treatment and anticoagulation therapy. Serum immunoglobulines and serum C4 were low. ANA were increased (1/640, speckled pattern) and Anti-SSA and anti-SSB were both positive. A diagnosis of primary Sjögren's syndrome was made. Elettromiography showed infiammatory polineuropathy. The renal biopsy revealed acute interstitial nephritis, showing a focal infiltrate of lymphocytes. Ultrastructural analysis evidenced glomerular alterations characterized by mild expansion of the subendothelial space and tubular epithelium degenerative aspects. She started steroid therapy and diuretics with rapid improvement of renal function.

**Conclusions.** While the retrospective studies found a rate of renal involvement in SS of 4.3-6.5 %, the prospective studies observed a much higher rate of 28-42 % [2]. In literature are rare the cases in which the renal manifestations revealed an undiagnosed Syogren syndrome as in our cases. The rarer glomerular lesions, typically the characteristic membranoproliferative glomerulonephritis (MPGN), occur due to immune complex deposition following B-cell expansion. The more typical tubulointerstitial nephritis can manifest in a number of ways, including with a mild to moderate reduction in the glomerular filtration rate, although progression to end stage renal failure with SS TIN is reported.2,3 Many authors would advocate renal biopsy in all patients with SS and tubular defects to confirm the diagnosis of TIN, and to distinguish from other potential causes of localised tubular defects (eg. the presence of light chain). Our experience suggest to include in differential diagnosis the Sjogren syndrome also in patients without an apparent clinical diagnosis. However, there are no proven effective systemic immunosuppressive treatments for pSS; the few randomized trials have been inconclusive or contradictory. In our serie the steroid therapy results efficacy in one case (case 2) while in the other (case 1) was not possible to make clear a valuation in considerations of the general conditions.

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# PATOLOGIA APPARATO DIGERENTE, PACREAS E FEGATO

## EPSTEIN-BARR VIRUS (EBV) INFECTION IN THE GASTRIC CARCINOGENETIC CASCADE

S. Brignola[1], A. Bressan[1], V. Angerilli[1], C.A. Dal Pozzo[1], P. Mattiolo[1], I. Coati[1], G.N. Fanelli[1], G. Munari[1], E.S. d'Amore[2], A. Vanoli[3], F. Farinati[4], S. Lonardi[5], F. Loupakis[5], A. Gallo Lopez[6], W.J. Oblitas[7], M. Fassan[1], M. Rugge[1]

*[1] Department of Medicine (DIMED), Surgical Pathology Unit, University of Padua, Padua, Italy; [2] Department of Pathology, San Bortolo Hospital, Vicenza, Italy; [3] Department of Molecular Medicine, University of Pavia and Pathology Unit, IRCCS San Matteo Hospital, Pavia, Italy; [4] Department of Surgical Oncology and Gastroenterology (DiSCOG), University of Padua, Padua, PD, Italy; [5] Oncology Unit, Istituto Oncologico Veneto, IOV-IRCCS, Padua, Italy; [6] ARCPE, Applied Research Cancer Center Perú, Lima, Perú; [7] Centro Médico Arequipa Puente Grau, Arequipa, Perú*

**Background.** Recent comprehensive molecular profiling studies identified an Epstein Barr virus (EBV) positive gastric cancer sub-type. These tumors are characterized by a significant lymphocytic infiltration and activation of the PD1-PD-L1 axis. The prevalence of this peculiar histotype is still controversial and most of the available information is deriving from Asiatic populations. Moreover, the EBV pathogenetic role in the gastric mucosa has not been elucidated, so far.

**Materials and Methods.** A large phenotypical and molecularly characterized series of 594 gastric and gastroesophageal junction cancers were investigated for EBV by EBER in situ hybridization. Positive cases were re-evaluated to assess the main histopathological features of the tumors. In these EBV-positive cases, normal gastric mucosa, atrophic gastritis and dysplastic samples (when present) were further assessed for EBV presence.

**Results.** EBV was identified in 23 cases (3.9%). Among these tumors, the male/female ratio was 17/6 and the EBV-positive status was associated to an increased prevalence of diffuse type tumors (52%) and infiltrative pattern of growth (61%). None of the normal gastric mucosa (n=23), atrophic gastritis (n=15) or dysplastic (n=5) samples showed any EBER epithelial positivity.

**Conclusions.** EBV-associated gastric cancer is a relatively rare entity in the Caucasian population. EBV is present only in the late phases of the gastric mucosa transformation process.

## MIR-224 IS SIGNIFICANTLY UP-REGULATED AND TARGETS CASPASE-3 AND CASPASE-7 DURING COLORECTAL CARCINOGENESIS

S. Brignola[1], V. Angerilli[1], A. Bressan[1], C.A. Dal Pozzo[1], P. Mattiolo[1], R. Cui[2 3], P. Gasparini[2], C. Mescoli[1], V. Guzzardo[1], R. Salmaso[1], C. Vicentini[4], G. Munari[1], F. Loupakis[5], S. Lonardi[5], C. Braconi[6 7], R. Gafà[8], G. Lanza[8], W.L. Frankel[9], N. Valeri[7 10], M. Rugge[1], M. Fassan[1]

*[1] Department of Medicine (DIMED), Surgical Pathology Unit, University of Padua, Padua, Italy; [2] Human Cancer Genetics Program, The Ohio State University Comprehensive Cancer Center, Columbus, OH; [3] School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, Zhejiang, China; [4] ARC-NET Research Center, University of Verona, Verona, Italy; [5] Oncology Unit, Istituto Oncologico Veneto, IOV-IRCCS, Padua, Italy; [6] Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, London, UK; [7] Department of Medicine, The Royal Marsden NHS Trust, London, UK; [8] Department of Pathology, University of Ferrara, Ferrara, Italy; [9] Department of Pathology, The Ohio State University Comprehensive Cancer Center, Columbus, OH; [10] Molecular Pathology Division, Institute of Cancer Research, London and Sutton, UK*

**Background.** Colorectal cancer molecular sub-typing significantly affects the therapeutic decision process. However, limited reliable diagnostic and predictive biomarkers have been introduced into clinical practice, so far. miR-224 has recently emerged as a driver oncomiR in sporadic colorectal carcinogenesis, but its pathogenetic role is still controversial.

**Materials and Methods.** A large phenotypical and molecularly characterized series of pre-invasive and invasive colorectal lesions were investigated for miR-224 expression by qRT-PCR and in situ hybridization. The caspase-3 and caspase-7 status was also assessed and correlated to miR-224 dysregulation.

**Results.** miR-224 was significantly upregulated during the adenoma-carcinoma sequence and in the context of inflammatory bowel disease (IBD) dysplastic lesions, whereas its expression was significantly down-regulated among BRAF-mutated tumors and in the presence of a DNA mismatch repair deficiency. miR-224 targets caspase-3 and caspase-7 in colorectal cancer, and this inverse relation was already evident from the earliest phases of transformation in intestinal mucosa.

**Conclusions.** miR-224 is a driver oncomiR in sporadic and IBD-related colorectal carcinogenesis. The miR-224/caspases axis may represent an interesting field of study for innovative biomarkers/therapeutics for BRAF-mutated/DNA mismatch repair-deficient tumors.

## MULTIPLE PRIMARY MALIGNANCIES: CASE REPORT OF SYNCHRONOUS OCCURRENCE OF EXTRA-HEPATIC CHOLANGIOCARCINOMA AND NON- FUNCTIONING NEUROENDOCRINE CARCINOMA OF THE PANCREAS HEAD

M.R. Campanino[1], A. Travaglino[1], E. Guadagno[1], A.Corvino[1 2], F. Corvino[3], F.P. D'Armiento[1], D'Armiento[1]

*[1] Department of Advanced Biomedical Sciences, University of Naples "Federico II", Italy; [2] University of Naples "Parthenope", Naples, Italy; [3] Department of Vascular and Interventional Radiology, AORN "A. Cardarelli", Naples, Italy*

**Introduction.** Multiple Primary Malignancies (MPM) are defined as the occurrence of two or more primary malignant tumors arising in the same patient. The incidence of these lesions is being increasingly reported due to the wide use of diagnostic modalities and different associations of tumor lesions have been described [1 2]. We report the case of a patient with a new association of malignancies: in fact, an extra-hepatic cholangiocarci-

noma was found simultaneously with a small non-functioning neuroendocrine pancreatic tumor. To our knowledge, simultaneous cholangiocarcinomas and neuroendocrine pancreatic tumors in the same patient have not yet been reported in literature.
**Case report.** A 55-year-old male with a history of biliary stenosis with jaundice and significant increase in cholestasis laboratory indices undergone ERCP with biliary stenting was admitted to the diagnostic department of our institution to evaluate for the nature of the biliary stenosis. Initially, the patient underwent abdominal ultrasound which demonstrated intrahepatic bile ducts dilatation, especially in the left liver. The extrahepatic bile ducts were not visible; the liver echopattern appeared normal. Successively, MR cholangiography sequences showed dilatation of the biliary tree including the intrahepatic ducts, primarily of the left hepatic lobe, and the common hepatic duct. Furthermore, MR images showed a lengthy stricture with abrupt and asymmetric narrowing in the middle and lower third segment of the common bile duct. A solid circumferential wall-thickening of the common bile duct with a firm component projecting into the duct lumen was also detected at same level of the stricture. Furthermore, MR views demonstrated mild enlargement of the pancreas associated with a non-homogeneous signal intensity of the pancreatic tissue due to the presence of a hypointense nodule. Although no definite demonstration of malignancy was obtained, on the basis of MR findings which were highly suggestive of a malignant stenosis, the patient underwent surgical treatment: a Whipple's pancreatico-duodenectomy with end-to-side gastrojejunostomy, T-L hepaticojejunostomy and T-L pancreaticojejunostomy were carried out. The resected specimens were submitted to our pathology department for histological evaluation: they included the distal stomach, the duodenum, part of the proximal jejunum, part of the pancreas (head, neck and uncinate process) and the distal biliary tree. Histological examination revealed well-differentiated distal common bile duct adenocarcinoma, which was confined to the muscular wall without infiltrating the pancreatic head and other surrounding tissues; the margins were tumor free and there was no vascular invasion; all loco-regional lymph nodes were negative for malignancy. According to the TNM classification system, the tumor was staged as pT1-N0-M0, stage 1A. In addition, a macroscopically solid well-defined lesion measuring 0.4 cm was found within the pancreatic head; the surgical margins were clear. Immunocytochemistry showed that the tumor cells were positive for chromogranin A, synaptophysin and neuron-specific enolase; moreover, the tumor tissue showed a negative reaction to vimentin. Ki67 labeling index was around 2%. No necrosis, nor mitotic figures were evident. The final pathology report classified the tumor as a non-functioning well-differentiated neuroendocrine tumorof the pancreas (pNET G1), according to the actual Classification System of Neuroendocrine tumors of the pancreas [3].
**Conclusions.** The first report regarding multiple primary malignancies was in 1889 by Billroth who described a patient with a spinocellularepithelioma of the right ear and a gastric carcinoma [4]. Since that time, multiple primary malignancies have been the object of medical research [2].The occurrence of a second tumor in patients with pancreatic cancer is described [5-8]; however, only a single case of simultaneous cholangiocarcinoma was reported [8]. Conversely, the most frequently associated tumors were gastric and colorectal cancers, followed by pulmonary neoplasms. In our report, also, the tumor association that we observed is different since the pancreatic lesion consisted of a different histological type represented by neuroendocrine tissue. In conclusion, an extra-hepatic cholangiocarcinoma and a neuroendocrine pancreatic tumor may occur simultaneously: this association should thus be considered in multiple primary malignancies.

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## IMPACT OF FORMALIN FIXATION ON THE RISK ASSESSMENT OF GASTROINTESTINAL STROMAL TUMORS ACCORDING TO THE AFIP CRITERIA


T. Musarra, V. Fiorentino, F. Rosa, A. Biondi, F. Castri, M.C. Giustiniani, R. Persiani, S. Alfieri, R. Ricci
*Departments of 1 Pathology and 2 Surgery, Catholic University, Foundation "Agostino Gemelli" School of Medicine, Largo F.Vito 1, 00168, Rome, Italy*


**Aims.** Gastrointestinal stromal tumors (GISTs) form a continuum in terms of biologic potential [1]. As a consequence, the definition of a reliable prognostication system on an individual basis has been a problematic process. Currently, the most powerful and widely accepted descriptors for determining the biologic potential of GISTs are size, mitotic activity and location [1]. These parameters are variously combined in different risk classifications, all of which with a well-established relationship with tumor behavior [2]. Risk assessment systems employing the abovementioned parameters as continuous variables probably better reflect the continuous spectrum of GIST biological aggressiveness, with prognostic contour maps being likely the most accurate method for estimating individualized outcomes [3]. Despite this, prognostication systems using biological descriptors as categorical variables are commonly preferred in daily practice, due to their relative simplicity. In particular, the risk assessment based on AFIP criteria and tumor rupture in vivo is the most widely used method for assessing the biological potential of GISTs [1 4]. An accurate definition of risk descriptors is therefore fundamental in a GIST pathological report, especially when detected data approach the threshold values separating risk classes. Formalin fixation is known to determine variable amounts of shrinkage in biological specimens, depending on tissue type [5]. This implies that whenever a lesion is measured in a tissue specimen, the status of the specimen relative to fixation should be specified. This is particularly true when tumor measurement is part of a pathological classification, as both the accurate biological validation and the reliable application in daily practice of the latter would be negatively influenced by a lack of clarity on the point. However, neither published

GIST risk classification systems, nor the papers which they are based on, specify whether the considered GIST specimens had been formalin-fixed prior to size measurement or not [1 6-9]. Only Fletcher et al. state that GIST size "may vary somewhat between prefixation and postfixation" [10]. Given these premises, we investigated the impact of formalin fixation on size in a series of 18 consecutive GISTs resected at our institution.

**Materials and Methods**. GISTs were half cut up to their maximum diameter; the latter was measured with a metallic, rigid ruler adhering to the bottom of the resulting incisura. The same measurement was performed after 24 and 48 hours of formalin fixation. Statistical analysis was performed using Statistica software version 12 (StatSoft Inc., Tulsa, OK, USA). Wilcoxon matched pairs test was applied for differences between the fixed and unfixed specimen groups. A p value less than 0.05 was considered statistically significant.

**Results.** GIST size did not vary between 24 or 48 hours-fixed specimens. However, the mm tumor size in formalin-fixed samples (mean 39.4, SD 34.1, median 28.5, range 13-145) was significantly lower than in unfixed ones (mean 42.2, SD 36.1, median 30.5, range 13-153) (Wilcoxon matched pairs test, p <0.01). This result was confirmed also after excluding the two GISTs resected following imatinib neoadjuvant therapy, which showed a remarkable size decrease (from 117 mm and 39 mm to 109 mm and 31 mm, respectively) likely related to their relevant mixedematous component. In fact, in the imatinib-naïve GIST population, the size of formalin-fixed tumors (mean 35.6, SD 31.3, median 27.0, range 13-145) again was significantly smaller than that recorded prior to formalin fixation (mean 37.8, SD 32.9, median 29.0, range 13-153) (Wilcoxon matched pairs test, p <0.01). Additionally, the risk class classification according to AFIP criteria (1) did not vary in any of the investigated tumor.

**Conclusions.** The clinical impact of the observed tumor size artifact due to formalin fixation is presumably low. In fact, provided the size of cases considered for establishing GIST risk were consistently measured with respect to fixation, in case of methodological inconsistency, our results suggest that at worst only a small fraction of GISTs would shift between close risk class extremes whatever the direction (which would depend on the fixation state of the specimen at the moment of size determination). To get an idea of the possible impact of formalin fixation on GIST risk according to the AFIP criteria (1), we applied an increase of 6% to tumor size, as detected after fixation (i.e. the average value by which the size determination on unfixed GISTs exceeded those after formalin fixation based on the herein investigated sample, excluding the post-NAD cases) to a series of 293 consecutive naïve GISTs surgically treated at our Institution: under these circumstances, 19 cases (6,5%) featured a risk class shift, obviously with a risk increase in the "formalin unfixed setting". Although these tumors changing prognostic group raise the issue of an unambiguous validation of GIST prognostication according to the specimen fixation state, not specifying the latter probably does not add significant imprecision with respect to those intrinsically present in risk assessment systems employing categorical variables.

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## THE VALUE OF HISTOLOGY, TUMOR INFILTRATING LYMPHOCYTES, AND MISMATCH REPAIR STATUS AS RISK FACTORS OF NODAL METASTASIS IN SCREENING DETECTED AND ENDOSCOPICALLY REMOVED PT1 COLORECTAL CANCER


L. Nicolè[1], R. Cappellesso[1], F. Zanco[1], A. Fassina[1], L. Ventura[2], M. Lo Mele[1], E. Rosa-Rizzotto[3], E. Guido[3], F. De Lazzari[3], P. Pilati[4], M. Tonello[4 5], M. Fassan[1], M. Rugge[1]

*[1] Department of Medicine (DIMED), Surgical Pathology Unit, University of Padua, Padua, Italy; [2] Department of Statistics, University of Padua, Padua, Italy; [3] Gastroenterology Unit, S. Antonio Hospital, Padua, Italy; [4] Unit of Surgical Oncology of the Esophagus and Digestive Tract, Veneto Institute of Oncology, I.R.C.S.S, Padua, Italy; [5] Department of Surgery, Oncology and Gastroenterology (DISCOG), University of Padua, Padua, Italy*


**Objective.** The number of patients with pT1 CRCs resected during colonoscopy is increasing due to the screening programs. Such tumors are potentially metastatic, but only 15% of patients have nodal involvement. Histologic criteria currently used for the selection of patients needing bowel resection are imprecise and the rate of overtreatment is high. TILs and MMR status impact on CRC prognostic but have not yet been tested as risk factors of nodal metastasis. To aid in the identification of patients requiring completion surgery, the value of histologic variables, tumor infiltrating lymphocytes (TILs), and mismatch repair (MMR) status as risk factors of nodal metastasis was investigated in screening detected and endoscopically removed colorectal cancers (CRCs) invading the submucosa (pT1).

**Materials and methods.** Histologic variables, CD3+ and CD8+ TILs, and MMR status were assessed in 102 endoscopically removed pT1 CRC. Univariate and multivariate analyses were used to evaluate the correlation with nodal metastasis.

**Results.** Positive resection margin, evidence of vascular invasion, presence of tumor budding, wide area of submucosal invasion, and high number of CD3+ TILs were associated with nodal involvement in univariate analyses. Vascular invasion and incomplete resection were statistically independent factors by multivariate analysis.

**Conclusions.** Completion surgery should be mandatory only for patients with pT1 CRC with vascular invasion or with tumor cells reaching the margin. In all other cases, the treatment choice should be entrusted to the evaluation of the risk-benefit ratio of each patient considering the rarity of nodal metastasis.

# NEUROPATOLOGIA

## LEF-1 EXPRESSION IN A SERIES OF 56 MEDULLOBLASTOMAS

A. Fioravanzo, C. Ghimenton, A. Eccher, A. Sorio, M. Brunelli, A. Scarpa

*Section of Pathology, Department of Diagnostics and Public Health, and Azienda Ospedaliera Universitaria of Verona, Italy*

**Aims.** The aim of our study was the evaluation of the expression of LEF-1 (lymphoid enhancer-binding factor) in a series of medulloblastomas, its relationship with the histopathological features and with the cytoplasmic-membranous or nuclear β-catenin expression, considering the partnership of the two factors in the WNT/wingless signalling pathway.

**Material and Methods.** We extracted all cases of medulloblastomas, registered with the SNOMED international identification code, from the files of the Unità Operativa Complessa (UOC) of Pathology of the University of Verona from 2000 to the first semester of 2018. We recovered 56 paraffin-embedded blocks to obtain sections and investigate the expression of both β-catenin and LEF-1 using Leica Bond-Max (Leica Biosystems). Slides were subjected to antigen retrieval by steaming (20 minutes at 80°C) with the Bond Epitope Retrieval Solution 2 (Leica Biosystems), incubated at room temperature for 15 minutes with an antibody directed towards β-catenin (clone 15B8, at a diluition of 1:200, SIGMA) and with an antibody directed towards LEF-1 (clone EPR2029Y, at a diluition of 1:200, Abcam). Antibodies were detected using the Bond Polymer Refine Detection (Leica Biosystems) with diaminobenzidine serving as chromogen. The results of β-catenin immunohistochemistry were interpreted on the percentage of tumour cells with β-catenin nuclear accumulation: if it was lower than 50% we categorized the immunoreactivity as restricted to the plasma membrane and cytoplasm; if it was higher than 50% we considered it as a nuclear positivity and thus as a WNT-activated medulloblastoma. The expression of LEF-1 was scored with the percentage of nuclei stained by the immunoreaction.

**Results.** Our cohort of medulloblastomas comprised 35 classic, 15 desmoplastic/nodular type, 6 large cell/anaplastic and no one with extensive nodularity. In 29 classic medulloblastomas there was a cytoplasmic-membranous expression of β-catenin and a staining of LEF-1 variable from null (26 cases) to 20-25% (3 cases). These tumors were classified, due to β-catenin immunoreactivity, as non-WNT. In 4 classic medulloblastomas we found a nuclear accumulation of β-catenin and a strong (80-90% of nuclei) positivity of LEF-1. Thus they were considered as WNT-activated. There was no correspondence, in terms of nuclear staining, between β-catenin and LEF-1 in 2 classic medulloblastomas with a cytoplasmic-membranous β-catenin and a diffuse positivity of LEF-1. Among desmoplastic/nodular cases we found always a cytoplasmic-membranous β-catenin and in 5 of them an expression of LEF-1 variable from 5 to 70%, especially around the pale nodular areas. In large cell/anaplastic medulloblastomas β-catenin was restricted to the plasma membrane and cytoplasm, whereas LEF-1 was in 5 cases negative and in 1 case with a weak 10% positivity.

Figure 1. Examples of a classic medulloblastoma non-WNT (A), a classic medulloblastoma WNT-activated (B) and a nodular medulloblastoma with membranous β-catenin and LEF-1 positivity (C).

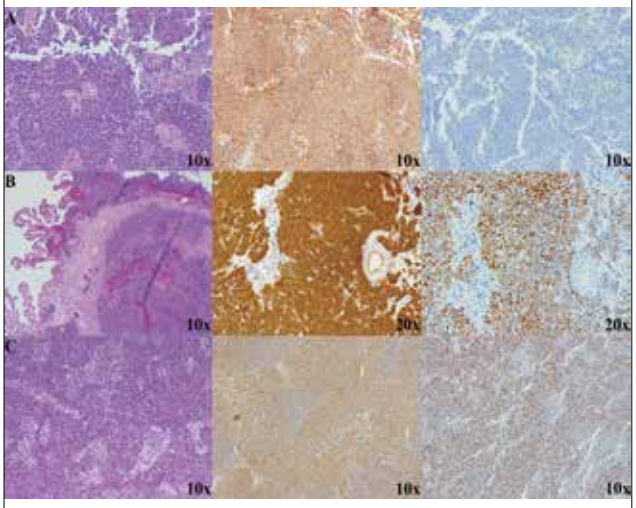
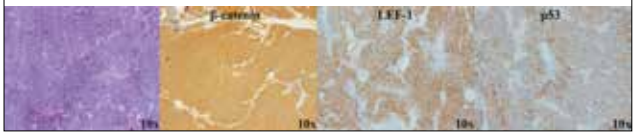



Figure 2. An example of classic medulloblastoma in a Li-Fraumeni syndrome with cytoplasmic-membranous β-catenin, a strong positivity for LEF-1 and an overexpressed p53.



**Discussion.** The transcription factor LEF-1 and β-catenin form a complex that participate in the WNT/wingless signalling pathway. Because they have genetic targets in common, we wanted to prove that LEF-1 can be a feasible and reliable marker for WNT-activated medulloblastomas instead of β-catenin. In fact, the immunohistochemical evaluation of β-catenin nuclear accumulation can be difficult due to its concomitant cytoplasmic-membranous expression in medulloblastoma cells. Among our 31 classic medulloblastomas with a cytoplasmic-membranous β-catenin, the expression of LEF-1 was null or very low in 29 cases and diffuse (80-90%) in 2 cases. Instead among our 4 classic WNT- activated medulloblastomas, there was a perfect correspondence of strong nuclear positivity between the two markers. From these data we desumed that LEF-1 had an high negative predictive value: a negative expression of LEF-1 always matched with a non WNT-mutated medulloblastoma. On the other hand in the 6 classic medulloblastomas with a strong positivity for LEF-1, β-catenin was nuclear in 4 cases and cytoplasmic-membranous in the other 2. So LEF-1 had a sensitivity of 100%, but a lower positive predictive factor: a strong staining for LEF-1 matched with a diagnosis of WNT-activated medulloblastoma in 4 cases out of 6. In the 2 classic medulloblastomas with LEF-1 expression and cytoplasmic-membranous β-catenin, we observed a mutant p53 (more than 60% of nuclei stained) and one of the 2 patients had an ascertained Li-Fraumeni syndrome. Thus when the medulloblastoma was of classic type and had a wild-type p53, we could rely on LEF-1 for the detection of WNT-activated tumors. It could be interesting

to understand the reason why LEF-1 stains positively around pale nodular areas in desmoplastic/nodular medulloblastomas, acting independently from β-catenin.

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# PATOLOGIA PEDIATRICA

## ONCOCYTIC ADRENOCORTICAL TUMORS IN PEDIATRIC AGE: WHAT SCORE SYSTEM FOR MALIGNANCY?

L. Salvatorelli[1], G.M. Vecchio[1], G. Bartoloni[2], G. Broggi[1], G. Magro[1]

*[1] Department of Medical and Surgical Sciences and Advanced Technologies, G.F. Ingrassia, Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Anatomic Pathology Section, School of Medicine, University of Catania, Catania, Italy; [2] Pathology Department, University of Catania, Catania, Italy.*

**Background.** Rarely "oncocytic" adrenocortical tumors (ACTs) occur in pediatric patients. Due to their rarity, the classification is still based on the Lin-Weiss-Bisceglia score system [1 2], designed and applied for oncocytic adrenocortical tumors of adults. They can be classified into adenomas, ACTs with indeterminate malignancy (borderline tumors), or adrenocortical carcinomas. To the best of our knowledge only six cases of oncocytic ACTs have been reported in children so far, with five cases classified as benign (adrenocortical oncocytomas) and one case as "borderline tumor", while no case of oncocytic carcinoma has been described. Accordingly it is still to be established if the Lin- Weiss-Bisceglia score system is reliable to predict the biological behavior of the oncocytic ACTs in children.

**Aim.** i) To report the pathological features of two rare cases of oncocytic ACTs classified as "malignant adrenocortical carcinomas" in pediatric age; ii) to establish correlation between pathological features and clinical behavior.

**Materials and Methods.** We herein report on two rare cases of oncocytic ACTs, respectively in a 7- year-old boy with pseudo-precocious puberty and in a 4-month-old girl. The Lin–Weiss–Bisceglia scoring system used for the adult counterpart tumors was applied to classify both tumors [1 2]. The scoring system is based on the identification of major criteria (high mitotic rate: >5 mitoses per 50 high-power fields; any atypical mitosis; venous invasion) or minor criteria (large size and huge weight; necrosis; capsular invasion; sinusoidal invasion). If an oncocytic tumor shows at least one or more major criterion, it is classified as "malignant," while if a tumor exhibits one to four minor criteria, it should be included in the category of "uncertain malignant potential" tumors (borderline tumors). A tumor should be classified as "benign" if none of the above-mentioned major or minor criteria are identified.

**Results.** A 7-year-old boy presented a mass, weighed g 80 and measured cm 6x5x3,5, almost entirely surrounded by a capsule. The cut surface showed a solid tumor with elastic consistency and mahogany/brownish color. Histologically, tumor was composed almost entirely of cells with oncocytic features (i.e. large, granular, deeply eosinophilic cytoplasm), arranged in a solid growth pattern. A unique atypical mitosis, >5 mitoses per 50 high-power fields, as well as a focal sinusoidal invasion of tumor capsule were identified. In the second case the patient had a well capsulated mass with polylobate margins, weighed g 65 and measured cm 5x4x2,5. The cut surface showed elastic consistency and pink-grayish color and multiple necrotic areas. Histologically, tumor was predominantly composed of cells with oncocytic features (>90%) arranged in a solid growth pattern; neoplastic cells showed diffuse and severe nuclear pleomorphism. A high mitotic index (91 mitoses per 50 high-power fields), rare atypical mitosis, multiple foci of necrosis and focal sinusoidal invasion of tumor capsule were also observed (two major criteria plus two minor criteria according to Lin-Weiss-Bisceglia scoring system). According to the Lin-Weiss-Bisceglia scoring system [1 2] both tumors were classified as "oncocytic adrenocortical carcinomas" in that the first case had two major criteria (atypical mitosis; >5 mitoses per50 high-power field) plus one minor criterion (sinusoidal invasion), while the second case showed two major criteria (atypical mitoses; >5 mitoses per 50 high- power field) and two minor criteria (sinusoidal invasion; necrosis). Although both tumors were classified as histologically malignant, the patients are well with no evidence of local recurrence after a follow-up period of 48 and 8 months, respectively.

**Conclusions.** The rarity of pediatric oncocytic ACTs makes difficult the identification of pathological criteria useful for prognostic purposes [3]. We report on two cases of oncocytic adrenocrotical carcinomas without evidence of adverse events after a follow-up period of 48 and 8 months, respectively. Although we admit that follow-up is relatively short, our results raise the question of the reliability of the Lin– Weiss–Bisceglia score system in predicting clinical behavior in pediatric patients.

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# PATOLOGIA PLEUROPOLMONARE

## FULMINANT PULMONARY ASPERGILLOSIS IN TREATMENT FOR MULTIPLE SCLEROSIS: AUTOPSY AS A TOOL IN PHARMACOVIGILANCE

M.R. Campanino[1], M. Paternoster[2], C.V. Russo[3], F. Saccà[3], G. Mansueto[1 2]

*[1] Department of Advanced Biomedical Sciences, Pathology Section, University Federico II, Naples, Italy; [2] Department of Advanced Biomedical Sciences, Legal Medicine Section, University Federico II, Naples, Italy; [3] Departmet of Neurological Sciences, Reproductive and Odontostomatological Sciences, University Federico II, Naples, Italy*

**Aim.** Aspergillosis is a major cause of morbidity and mortality in immunosuppressed patients, particularly in those with hematologic disease. Aspergillus species are common molds, ubiquitous within the environment in a worldwide distribution, isolated form soil, decaying, vegetation, and organic debris.

Only a few are human pathogens, including A. fumigatus, the most common agent of invasive pulmonary aspergilli [1]. We report the first case of Invasive Pulmonary Aspergillosis (IPA) in a patient treated with Alemtuzumab for relapsing-remitting multiple sclerosis (SMRR) [2 3]. Alemtuzumab is a humanized monoclonal anti-CD52 antibody, a surface protein expressed on T- and B-cells, natural killer, monocytes, and dendritic cells. It is indicated for adult patients with SMRR with active disease defined clinically through magnetic resonance images (MRI). Several types of infections caused by Alemtuzumab are described in the literature: viral infections (Herpes Simplex and Zoster virus, Cytomegalovirus), tuberculosis, listeriosis mainly superficial fungal infections (mouth and vagina) [2]. No case of aspergilli in patients treated with Alemtuzumab has been described in the literature. Immunomodulation and immunosuppression are generally linked to an increased risk of infection. Aspergillus pneumonia is a potentially fatal consequence of these conditions and can be difficult to treat despite prompt diagnosis and adequate therapy [1].

**Material and Methods.** A 38-year-old Caucasian woman with an 11-year history of SMRR was treated, initially, with interferon beta-1b and Glatiramer Acetate, both suspended for breakthrough disease. Subsequently, she received Natalizumab, than replaced by Alemtuzumab for John Cunningham virus positivity and for two relapses during treatment. Two weeks later the new therapy, the patient experienced weakness, fever, cough, vomiting, abdominal pain and diarrhea. These symptoms were resistant to antibiotic therapy and they worsened over time. A few days later, the patient was admitted to unit of Neurology of "Federico II" Hospital of Naples-Italy with fever and dyspnea. She had leucopenia, high inflammatory indices and abnormal liver function tests. A high-resolution CT scan showed parenchymal thickening extended to almost the whole of the left lung, with cystic nodules and stenotic aspect of the main bronchus as from diffuse normal phlogistic involvement, in the right hemithorax the thickening of the pulmonary plot was associated with areas of "ground glass" as the initial inflammatory process (Fig. 1). An antibiotic therapy was performed but few hours after admission, she became severely hypoxic and she died. The autopsy showed an abnormal apical lobe of the left lung with a reddish-gray surface. The transition zone between the two lobes showed fibrous adhesions with whitish, compact and friable areas (Fig. 2a). The microscopic observation revealed widespread intra-alveolar fibrinous and necrotic material with rare elements of inflammation (Fig. 2b) hyphae with a characteristic "dichotomous" subdivision structure (Fig. 2c), perivasal and endovascular hyphae with septic emboli (Fig. 2d). This was compatible with a widespread necrotizing alveolar pneumonia that, together with the hyphae morphology and the endovascular arrangement, was diagnostic for Aspergillus pneumonia.

**Conclusions.** Immunosuppressive therapy is the main risk factor for opportunistic infections. The prognosis is severe with high mortality in some series, partly due to delayed initiation of specific treatment. Early diagnosis of IPA can be challenging and is frequently only post-mortem as direct microbiological diagnosis is impossible due to the rapid worsening of clinical conditions. Several types of infections are described in the literature that manifest themselves following immunosuppressive treatment. IPAs have never been reported in MS patients treated with Alemtuzumab, but are relatively frequent in hematological patients where Alemtuzumab is used at different doses, and patients have different integrity of the immune system [4].

IPA should be considered a fatal complication in immunocompromised patients with febrile illness and antifungal prophylaxis is required. The autopsy have an important role to collect useful data (e.g. fatal complications drug-related) for Pharmacovigilance's activity [5].

Figure 1. A high-resolution CT scan showed a complete involvement of the left lung and patchy pulmonary infiltrates in the right lung.

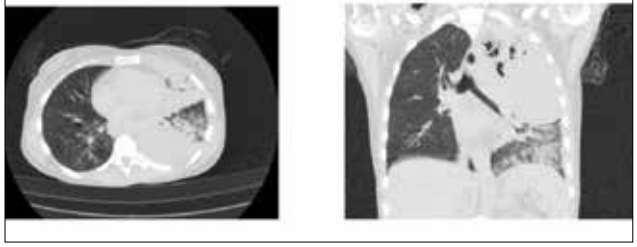
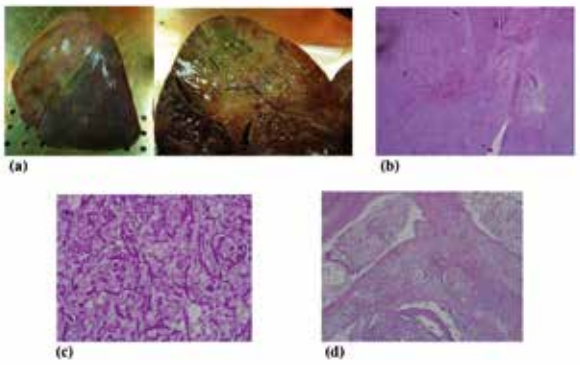


Figure 2. Macroscopic aspect left lung: (a) reddish-gray surface, with fibrous between lobes; the cut surface showed compact parenchyma, whitish and partly hemorrhagic; histology: (b) intra-alveolar fibrinous and necrotic material with rare elements of inflammation; (c) septate hyphae with characteristic dichotomous subdivision; (d) perivasal and endovascular hyphae.

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## PD-L1 EXPRESSION ON ROUTINE SAMPLES OF NON-SMALL CELL LUNG CANCER: A CENTRALIZED LABORATORY EXPERIENCE

E. Clery, A. Iaccarino, C.De Luca, G. Gragnan, C. Bellevicine, G. Troncone, E. Vigliar

*Dipartimento di Sanità Pubblica, Università degli studi di Napoli "Federico II", Naples 80131, Italy*

**Aims.** Non-small cell lung cancer (NSCLC) is one of the promising fields of application of checkpoint inhibitor therapy, targeting programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) proteins. Nowadays, several anti-

PD-L1 immunohistochemistry (IHC) clones, developed and validated on different staining platforms as part of clinical trials, each linked to a specific treatment, are available (22C3 for pembrolizumab, 28-8 for nivolumab, SP142 for atezolizumab and SP263 for durvalumab) [1-7]. In addition to the availability of 4 different clones and 2 different staining platforms, the usage of several cutoff levels (1, 5, 10, 25, and 50%) for positive staining and the possibility to implement laboratory developed test (LDT), has complicated matters further. Hence, a growing literature consisting of harmonization studies of PD-L1 testing has been produced and data are not always concordant with those generated by clinical trials. Since no routine series of IHC PD-L1 expression has been reported to date, the aim of this study was to report an over one year experience on routine IHC PD-L1 expression on NSCLC samples outsourced to the molecular laboratory at the cytopathology department of the University of Naples "Federico II", highlighting analogies and differences between our series and those reported in clinical trials, validation and harmonization studies.

**Materials and Methods.** From January 2017 to April 2018 we analyzed 211 consecutive requests for IHC PD-L1 evaluation The type of preparation, clinical informations, original diagnoses and sample sites were recorded for each sample. PD-L1 testing was performed by LDT, using 22C3 antibody concentrate on VENTANA BenchMark ULTRA platform (ultraView DAB Detection kit and Amplification kit). Trained pathologists, familiar with lung pathology and PD-L1 scoring, evaluated the sample adequacy (at least 100 viable cells); 3 cut-off levels of PD-L1 positive tumor cells (tumor proportion score, TPS) were used (<1%, 1-49%; >50%). Then, we compared our results with those reported in literature concerning the use of 22C3 antibody.

**Results.** The series included 147 men and 64 female, with an age range of 20-84 years (median age 65 yrs). Sample types include biopsies (n=112), surgical resections (n=51) and fine needle aspiration biopsies (FNAB) (n= 48). Samples preparations submitted for testing include paraffin blocks (n=129), unstained paraffin sections (n= 34), cell blocks (CB) (n= 45), direct smears (n=2) and liquid-based cytology vial (n= 1). Submitted material was obtained from primary lung tumors (n=95) or metastases (n=51); in 65 cases sample site was not reported. Overall, the sample received were diagnosed as adenocarcinoma (ADC) (n=104), squamous cell carcinoma (SQCC) (n=36), NSCLC not otherwise specified (n=28), carcinosarcoma (n=2), adenosquamous carcinoma (n=1) and small cell carcinoma (SCC) (n=1); in 39 cases diagnosis was not reported. Overall, 193 out of 211 samples (92%) met the criteria for adequacy. One hundred and twenty-one of 193 (62,7%) specimens did not express PD-L1 (TPS < 1%); 34 of 193 (17,6%) expressed PD-L1 with a TPS between 1% and 49% and 38 of 193 (19,7%) expressed PD-L1 with a TPS of >50%. The prevalence of TPS <1% in our series was similar to those reported in validation and harmonization studies; however, the difference between our results and clinical trials results was statistically significant ($p < 0.05$).

**Conclusions.** Our study shows the performance of a LDT that uses the 22C3 antibody concentrate on a routine series of IHC PD-L1 evaluation. In our series 62.7% of analyzed samples did not express PD-L1 (TPS <1%). Compering our data with those present in literature, this value is similar to those reported in validation and harmonization studies [1 5-7] but different from those reported in clinical trials [3 8], where the percentage of TPS<1% cases ranged from 33 to 39%. This evidence underlines the need to report further IHC PD-L1 routine series, to evaluate the prevalence of different TPS percentage and to investigate possible factors affecting the PD-L1 positive cases distribution.

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## GENOMIC CHANGES OF CHROMOSOMES 8P23.1 AND 1Q21: NOVEL MUTATIONS IN MALIGNANT MESOTHELIOMA


F. Fortarezza[1], A. Marzullo[1], A. Pennella[2], L. Vimercati[3], M. Gentile[4], D. Cavone[3], A. L Buonadonna[4], A. Scattone[5], G. Serio[1]

*[1] Department of Emergency and Organ Transplantation, Pathology Division, University of Bari, Italy; [2] Department of Surgery, Pathology Division, University of Foggia, Italy; [3] Medical Genetics Division, "Di Venere Hospital", Carbonara (Bari), Italy; [4] Department of Interdisciplinary Medicine, Occupational Health Division, University of Bari, Italy; [5] Pathology Division, IRCCS, National Cancer Institute "Giovanni Paolo II", Bari, Italy*


**Introduction.** Malignant mesothelioma (MM) is a lethal malignancy affecting the cells of the pleura or peritoneum surfaces, whose main known cause is occupational or environmental asbestos exposure. As in cases of exposure to other carcinogens, not all exposed individuals develop cancer even if they have inhaled high concentrations of asbestos. It has been shown that low concentrations of asbestos fibers can promote the development of mesothelioma, suggesting that a genetic predisposition (inherited or not) may play a role in mesothelioma development. There is no significant evidence that surgery, chemotherapy, immunotherapy, radiotherapy treatments can significantly influence survival. Although a number of studies have been conducted to evaluate genetic events associated with the development and progression of MM, the first molecular mechanism implicated in the initiation of genomic instability remains obscure, and the identification of predictive markers is crucial. In particular, the identification of the genes that are altered in this aggressive malignancy could be valuable, indicating potential therapeutic targets or prognostic indicators. To date, little it is yet known about the genetic events that trigger MM. Molecular changes consist of an altered expression and activation or inactivation of critical genes in oncogenesis, es-

pecially tumor suppressor genes at the 9p21(INK4) and 22q12 (NF2) loci. Also, BAP1 mutations have been identified in familial and sporadic mesotheliomas, so it could be hypothesized that germline BAP1 mutations may contribute to the susceptibility to asbestos-related mesothelioma through a mechanism involving gene-environment interaction. While most studies have been focused on pleural mesothelioma, peritoneal mesothelioma is an extremely rare condition and a clear history of asbestos exposure is not always well documented. Therefore, it is still unknown whether MMs from different sites share genomic alterations or undergo similar oncogenic transformations.

**Material and Methods.** Between 1990 and 2008, among the cases recorded in the Apulia Mesothelioma Register, we found 22 peritoneal mesothelioma cases, mostly arising in patients with occupational/environmental or domestic (cohabitant son/daughter or wife) asbestos exposure. Demographic data, including gender, age, age at first asbestos exposure, occupational history, domestic exposure based on the characteristics of the dwellings, namely the presence or use of asbestos-containing materials at home or by a cohabitant person, duration of the exposure (the difference between the start and the end data), and personal and family health history, were collected and archived from the questionnaire/interview employing National Mesothelioma Register Guidelines standard criteria. All patients lived in Apulia (southern Italy), in the city of Bari, where asbestos was actively produced at the Fibronit factory. The study was approved by the local Ethics Committee of the Policlinico-Hospital, Bari, Italy (accession number 5062, June 22 2016). All histological slides were classified according to WHO criteria [1]. Tumor tissues were fixed in 4% buffered formaldehyde and paraffin-embedded according to standard histopathologic methods. All diagnoses were supported by immunohistochemistry (IHC) according to current guidelines. High-resolution array-comparative genomic hybridization (a-CGH) was performed to identify genetic imbalances in a series of malignant peritoneal mesothelioma.

**Results.** At diagnosis patients ranged from 36 to 80 years old of age (mean 61.6 years), asbestos exposure was well documented in 18/22 cases and an occupational asbestos history was present in 10 of them (mean exposure 16.5 years). Patients (7 cases) with environmental asbestos exposure lived near the polluted sites and in one case the exposure was domestic. Fourteen tumors were epithelioid, five biphasic and three sarcomatoid types. The CGH-array analysis revealed multiple chromosomal imbalances. Deletions were less frequent than gains. Interestingly, deletion at 8p23.1 was observed in 12 cases, 10 of them with exposure to asbestos. Furthermore, another novel deletion at 1q21 was present in 11 cases and only one of them did not have a documented history of asbestos exposure. Often, 1q21 and 8p23.1 losses were present in the same patient (7 cases).

**Discussion.** The region at 8p23.1 contains the beta- defensin gene cluster (DEF). Defensins (α-defensin or ß-defensin) are produced in the respiratory, gastrointestinal, genitourinary tract, skin and blood cells. They are considered a first line of defense against invading pathogens (antimicrobial, chemotactic and regulatory functions). The fact that ß-defensins are expressed in most epithelial cells and are impaired in many inflammatory diseases vindicates the assumption that defensins are involved in the pathogenesis of inflammatory processes and also in many cancers (pancreas, lung, breast etc.). Asbestos causes DNA damage directly, by mechanically interfering with the segregation of chromosomes during mitosis, and indirectly by inducing mesothelial cells and macrophages to release mutagenic reactive oxygen and nitrogen species. Since malignant mesothelioma of the peritoneum has been observed in individuals with recurrent peritonitis (diverticulitis, Crohn's disease, Familial Mediterranean fever etc.) the chronic serosal inflammation might contribute to trigger mesothelioma. In a recent report, Sneddon et al. [2] report similar molecular characteristic in cell lines derived from tumour mesothelioma cells found in pleural effusion samples. Our results in tissue peritoneal samples suggest a common mechanism of asbestos-induced neoplastic transformation of mesothelioma in the pleura and peritoneum. The region at 1q21 contains ubiquitin E2 (UBEQ1), a gene that plays an important role in the conjugation of proteins and their degradation. Ubiquitin has been clarified to play a role not only as a protein marker but also to have other functions, such as directing the transport of proteins into and out of the cell. By connecting multiple ubiquitins together in short or long chains, or using different connections between the molecules, very different signals can be encoded. Numerous cellular processes are regulated by ubiquitination, mediated by growth factors, and potentially affect all aspects of a cell life. Consequently, there are also many signal transmission pathways that, in cases of alteration of this mechanism, may be seriously compromised, also giving rise to various types of cancer (ie breast and ovarian cancersin cases of BRCA1 mutations). Our results show a loss of function of the UBE2Q1 gene in 10 patients with long lasting asbestos exposure (occupational/environmental).

**Conclusions.** In conclusion, it could therefore be hypothesized that the loss of function of ubiquitination, as well as of the defensins, could play an important role in the initial development and subsequent progression of mesothelioma. These targets could therefore be considered as a focus for the development of new therapeutic approaches.

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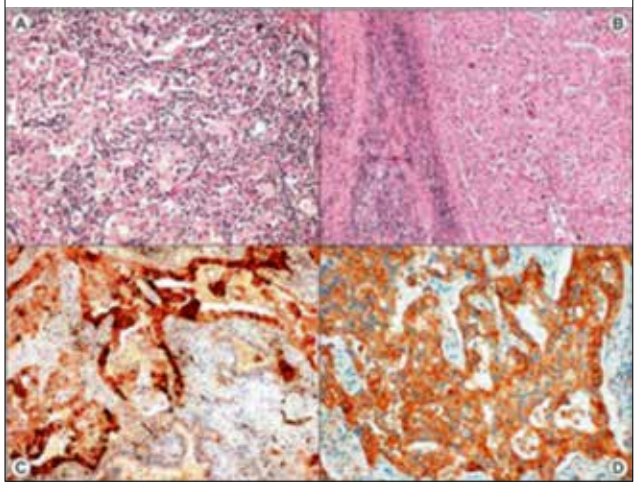
## ASSESSMENT OF TILS, IDO-1 AND PD-L1 IN RESECTED NON SMALL CELL LUNG CANCER: AN IMMUNOHISTOCHEMICAL STUDY WITH CLINICOPATHOLOGICAL AND PROGNOSTIC IMPLICATIONS


M. Mandarano[1], G. Bellezza[1], M.L. Belladonna[2], B.J. Van den Eynde[3], R. Chiari[4], J. Vannucci[5], I. Ferri[1], G. Mondanelli[2], V. Ludovini[4], F. Bianconi[6], R. Del Sordo[1], L. Cagini[5], E. Albini[2], G. Metro[4], Puma[5], A. Sidoni[1]

*[1] Department of Experimental Medicine, Section of Anatomic Pathology and Histology, Medical School, University of Perugia, Italy; [2] Department of Experimental Medicine, University of Perugia, Italy; [3] Ludwig Institute for Cancer Research, de Duve Institute, Université Catholique de Louvain, Belgium; [4] Department of Medical Oncology, Santa Maria della Misericordia Hospital, Perugia, Italy; [5] Department of Thoracic Surgery, University of Perugia Medical School, Perugia, Italy. 6Independent Researcher, Montefalco, Italy*


**Aims.** Several cancers, especially non small cell lung cancer (NSCLC), are able to escape the immunosurveillance of tumor infiltrating lymphocytes (TILs) [1-3]; among the molecules involved, the indoleamine 2,3-dioxygenase 1 (IDO-1) and the programmed cell death ligand-1 (PD-L1) play a crucial role [4 5]. These aspects are of great interest in the current immunotherapeutic era, although contrasting results about both their prognostic role and their function as potential biomarkers exist yet [2 3 5-8]. Therefore, the current study analyses the TILs, IDO-1

Figure 1. **A)** Intratumoral TILs (H&E 200X). **B)** Peritumoral TILs (H&E 200X). **C)** Heterogeneous stain for IDO-1 (100X). **D)** Intense immunostaining (3+) for PD-L1 (200X).



and PD-L1 interactions and their correlations with clinical-pathological parameters and prognosis in a NSCLC series.
**Materials and Methods.** 193 NSCLC surgical specimens collected between 2009 and 2015 in the Department of Experimental Medicine – Section of Anatomic Pathology and Histology, formalin – fixed, paraffin-embedded, were assessed for TILs intensity, TILs localization, IDO-1 (clone 4.16H1) and PD-L1 (clone E1L3N) immunohistochemical expressions (Fig. 1). This data was correlated with clinical-pathological parameters, disease free and overall survivals.
**Results.** IDO-1 and PD-L1 high expressions were related with adenocarcinoma solid pattern (respectively p=0.036 and p=0.026) and a high PD-L1 expression was correlated with squamous histotype (p=0.048). IDO-1 overexpression correlated with former smokers (p=0.041), higher adenocarcinoma stages (p=0.039) and with both higher TILs intensity (p=0.025) – in particular in squamous cell carcinomas subgroup (p=0.002) – and high PD-L1 expression (p=0.0003). A worse prognosis was associated with TILs peritumoral localization (p=0.029).
**Conclusions.** TILs localization affects NSCLC prognosis; the higher expression of IDO-1 and PD- L1 in poorly differentiated and more aggressive lung adenocarcinomas, as well as the correlation between high PD-L1 expression and squamous cell histotype, confirm the more efficient immunoescaping of these NSCLC subgroups. Further studies are advisable to better understand the interactions between these and other molecules in NSCLC microenvironment, in order to discover still unknown, but potentially useful, molecular pathways.

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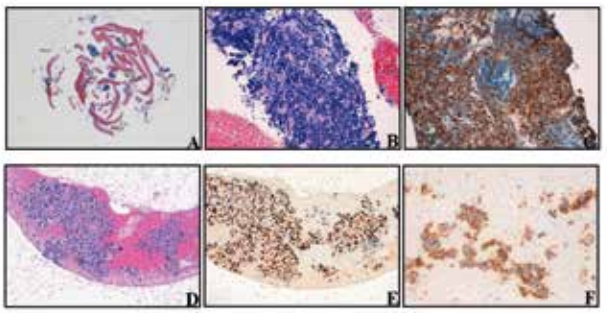
## IMPLEMENTATION OF THE AUTOMATED CELL BLOCK SYSTEM (CELLIENT TM ) FOR PROCESSING FINE NEEDLE ASPIRATION (FNA) MATERIAL IN THORACIC DISEASE : MAXIMUM OUTPUT WITH MINIMUM EFFORT


E. Prosperi[1], R. Colella[1], A. Fratto[1 2], M. Mandarano[1], S. Baglioni[3], G. Bellezza[1], Giovenali[2], A. Sidoni[1]

*[1] Department of Experimental Medicine, Section of Anatomic Pathology and Histology, Medical School, University of Perugia, Italy; [2] Diagnostic Cytology and Histology Unit, S. Maria della Misericordia Hospital, Perugia, Italy; [3] Pulmonary Division and Respiratory ICU, S. Maria della Misericordia Hospital, Perugia, Italy*


**Background.** Diagnosis of thoracic disease (and particularly staging of lung carcinoma) with the least invasive method, which allows however to acquire tissues in sufficient quantity even for all molecular investigations, is now of crucial importance for patients [1]. EBUS-TBNA (endobronchial ultrasound-guided transbronchial needle aspiration) and EUS-FNA (endoscopic ultrasound-guided fine needle aspiration) are minimally invasive techniques rapidly gaining ground in the non-surgical invasive diagnostic approach to thoracic diseases [2]. Fine needle aspiration procedures produces cytological material, which risks not being sufficient for all immunohistochemical and molecular investigations required today in lung carcinomas. Use of cell blocks (CBs) is becoming more and more an important diagnostic tool in cytopathology, to provide morphological details and to perform ancillary studies, such as immunohistochemistry (IHC) and molecular techniques [3], but is still not well standardized [4]. CellientTM Automated Cell Block System (Hologic Corporation, Marlborough, MA, USA) can produce standardized CBs in a short period of time (less than 1 hour), with a higher cellularity relative to traditional CB methods and excellent results also with IHC and molecular techniques [5 6].
**Objective.** Evaluate merits and defects of CellientTM system in routine diagnosis of FNA procedures.
**Material and Methods.** During the period February-July 2018, 78 samples of 46 consecutive patients underwent FNA of an thoracic lesion were analyzed in our Institution using the CellientTM Automated Cell Block System. Of the 46 cases, 14 were fixed in Cytolyt (30,4%) while 32 (69,6%) were formalin-fixed. In thirty-eight cases (82,6%) rapid on site evaluation (ROSE) was performed by a pathologist to ensure the adequacy of the sample. All the CBs produced have been sectioned to obtain a minimum of six sections, stained alternately with hematoxylin and eosin (H&E). Two expert pathologists, dedicated to thoracic and lung pathology, evaluated the material

**Figure 1. A)** Wide view of a CellientTM CB. **B)** Higher magnification of picture A, showing a small cell lung cancer. **C)** Immunohistochemistry for Ki67-Mib1. **D)** Lung adenocarcinoma. **E)** Immunohistochemistry for TTF1. **F)** Immunohistochemistry for PD-L1.




obtained with CellientTM Automated Cell Block System in terms of cellularity, cellular morphology and results of immunohistochemistry. In particular, in non-neoplastic lymph nodes, the material was considered adequate if there was a lymphocyte cellularity higher than 100 lymphocytes for HPF in more than one section [7]. Instead, in neoplastic lesions, cellularity has been classified as: poor (at least 10 cells), moderate (10-50), discrete (50-100) and high (>100).

**Results.** Forty-two patients underwent an EBUS-TBNA procedure ; two a TBNA and two an EUS- FNA (91,4%, 4,3% and 4,3% respectively). The clinical indications for these procedure were: diagnosis of a lung neoplasm in 32 cases (69,6%), stadiation of a known neoplasm in 9 cases (19.6%) and diagnosis of non-neoplastic pathology in 5 cases (10,8 %). The collected material allowed to formulate a diagnosis in all the samples, in particular 26 patients had a neoplastic disease, 4 a granulomatous lymphadenitis and 16 a reactive lymphoadenopathy (56,5%, 8,7% and 34,8% respectively). Cellularity has been evaluate as adequate in all the cases to support a diagnosis, in fact over 93% of cases showed high cellularity with the criteria shown before. Cell morphology was adequately conserved in all cases, regardless of the type of fixation. Immunohistochemistry was performed on 29 of 46 cases (63%) and, depending on the morphological suspicion, various antibody panels have been used (such as Cytokeratin 7, TTF1, p40,Synaptophysin, Chromogranin, Ki67-Mib1, ect.), without significant differences in antigenic expression in relation to the type of fixation used. For 9 patients diagnosed with squamous cell carcinoma or adenocarcinoma, immunohistochemistry for PD-L1 was also performed, which was positive (high expression) in 4 cases.

**Conclusions.** CellientTM Automated Cell Block System allows to obtain high quality CBs, in extremely short time, using a highly standardized procedure. CellientTM permits also to reduce the work of biomedical laboratory technicians, in terms of preparation of CBs and of number of sections to be set up to obtain sufficient material, providing an excellent diagnostic yield. In the era of precision medicine, the management of the diagnostic "material" for the vast majority of cases is critical and decisive, so it is crucial to handle it with techniques that permit to maximize both diagnostic and prognostic power of the samples.

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# CITOPATOLOGIA

## MALIGNANT PLEURAL MESOTHELIOMA WITH "SIGNET-RING" CELLS: A DIAGNOSTIC CHALLENGE AND AN UNUSUAL CASE REPORT

D. Bonazza[1], A. Mangogna[1 2], B. Belmonte[3], A. Romano[1], M. Cortale[1], R. Bulla[2] and F. Zanconati[1]

*[1] Department of Medical, Surgical and Health Science, University of Trieste, Trieste, Italy; [2] Department of Life Sciences, University of Trieste, Trieste, Italy; [3] Tumor Immunology Unit, Department of Health Sciences, Human Pathology Section, University of Palermo, Palermo, Sicily, Italy*

**Background.** Malignant pleural mesothelioma (MPM) remains one rare cancer of the pleural surface, typically associated with exposure to asbestos. It has a breathtakingly rapid natural history with a median survival of 6 to 8 months when untreated and a significant economic and social impact. MPM is a disease with limited therapeutic options and its management is still controversial. Diagnosis is usually made by thoracoscopy, which allows multiple biopsies with histological subtyping and is indicated for staging purposes in surgical candidates [1]. Signet-ring cell MPM is an uncommon histological subtype of mesothelioma that exhibit signet-ring features and that can be misdiagnosed as signet-ring cell adenocarcinoma [2]. Exclusion of pleural metastasis of signet-ring cell adenocarcinoma is the critical point for differential diagnosis.

Here we report a case of pleural effusion diagnosed as MPM with neoplastic cells that exhibit prominent signet-ring-like features, which was identified by pleural effusion cell block immunohistochemistry (IHC) and also confirmed by IHC of histological sections from pleural biopsy specimen.

**Results.** A 71-year-old Caucasian male was admitted to our hospital with dyspnea, fatigue and weight loss. His past medical history was unremarkable, and he has not recently used drugs. He does not have family history of note and was not on regular medication. There was a history of exposure to asbestos during the occupation. After clinical examination, computed tomography of the chest showed left sided massive pleural effusion and likely pleural plaques. A thoracentesis was performed and serohemorrhagic fluid was determined. Exfoliative cytological examination of pleural effusion showed reactive mesothelial cells and some clear cells discretely showing intracytoplasmic vacuoles and eccentric atypical nuclei reminiscent of ''signet-ring'' cells. In view of signet-ring cells, diagnosis of likely metastatic adenocarcinoma with background mesothelial cells was done. Screening for a primary tumor of other parts of the body was negative. Pleural effusion cell block IHC was CEA, TTF-1, CK20, CDX2 negative and CK5/6, CK7, EMA (membranous), WT-1 (nuclear), calretinin positive suggesting a MPM diagnosis. Finally, also immunohistochemical analysis of pleural biopsy confirmed the diagnosis of MPM. The patient was considered operable for his good performance status. It was performed a pleurectomy/decortication surgery with atypical lung resection of the left upper lobe. Histopathological examination of surgical specimen detected a biphasic mesothelioma with signet-ring-like features.

**Discussion.** Mesotheliomas have been classified into four major histologic subtypes: epithelioid, sarcomatoid, mixed epithelioid and sarcomatoid (biphasic), and desmoplastic, the most common of which is epithelioid [3]. The signet-ring configuration seen in adenocarcinomas has traditionally been associated with round shapes and eccentric nuclei and with the accumulation of large amounts of intracytoplasmic mucin [4]. Signet-ring cell carcinomas can arise in a wide variety of organs, including lung, stomach, colon, breast, urinary bladder, pancreas, salivary glands, prostate, as well in stromal tumors of the ovary and testis [5-9].

**Conclusions.** This case illustrates that pathologists should know that mesotheliomas can also present prominent signet-ring-like features and that immunohistochemistry of histological sections from a cell block combined with the immunohistochemical studies may be helpful to determine the type of malignancy.

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## IMMUNOHISTOCHEMICAL DETECTION OF CD56, CK19, GALECTIN 3, HBME1 AND BRAF V600E IN THYROID FOLLICULAR PROLIFERATIONS ON CELL BLOCK: PRELIMINARY STUDY

G. Gazzano, S. Palazzo, L. Ermellino, L. Alasio, , R. Consonni, A. Grindati, M. Donisi, M. Biancardi, T. Zacchi, S. Sernagiotto, S. Gatti, R. Giardini

*Istituto Auxologico Italia no, IRCCS, Ospedale Capitanio*

**Objective.** The present study was aimed to evaluate the diagnostic reliability of a IHC panel (CD56, CK19, Galectin 3, HBME1 and BRAF) on conventional cell block for the investigation of thyroid follicular proliferation.

**Material and Methods.** We selected 36 patients from 1100 thyroid FNA cases collected during last year. All selected cases, defined as follicular proliferations in according with guidelines of The Bethesda System for Reporting Thyroid Cytopathology, 2008; Consensus Statement AIT, SIE, SIAPEC-IAP for Class and Reporting of Thyroid Cytology, 2014 and subsequently undergone to thyroidectomy. Comparative analysis has been performed between cytological and histological slides. Cell blocks of all cases have been quantitatively evaluated and 19 of them were excluded because poor cellularity. The remaining cases (8 TIR3b; 2 TIR4 and 7 TIR5) were stained with CD56 (Cell Marque,MRQ-42), Mesothelial cell(Cell Marque, HBME-1), CK19 (Cell Marque, A53- B/A2.26) and Galectin-3 (Cell Marque, 9C4) has been done by automatic device (BENCHMARK XT) on cell block before surgery.

**Figure 1.** Galectin-3.

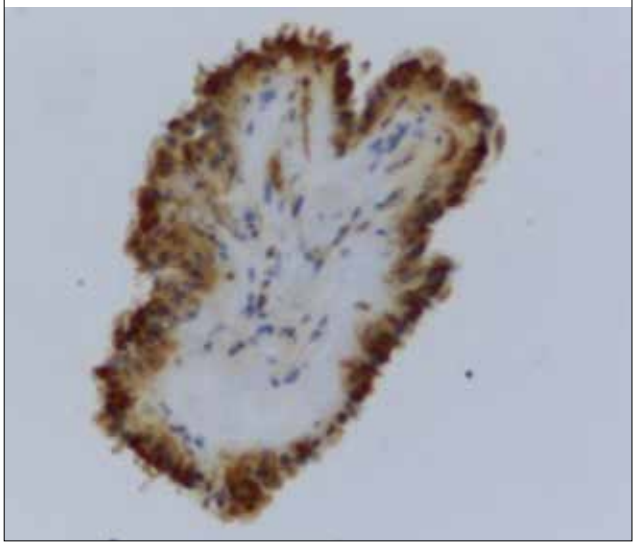


**Figure 2.** CK19.

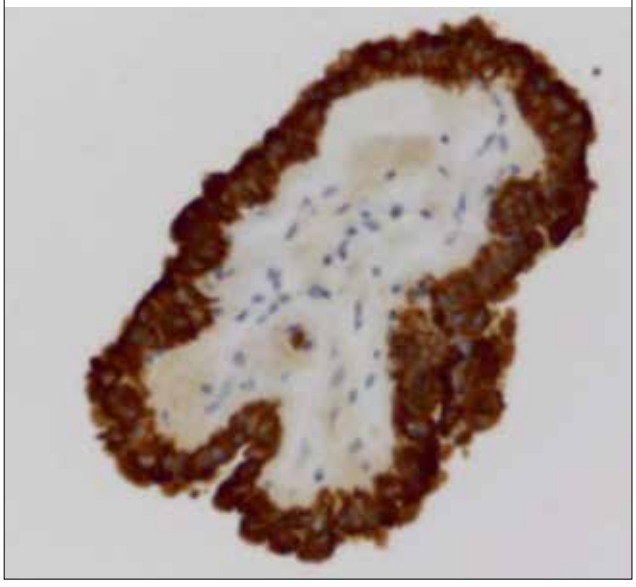


**Figure 3.** HBME-1.

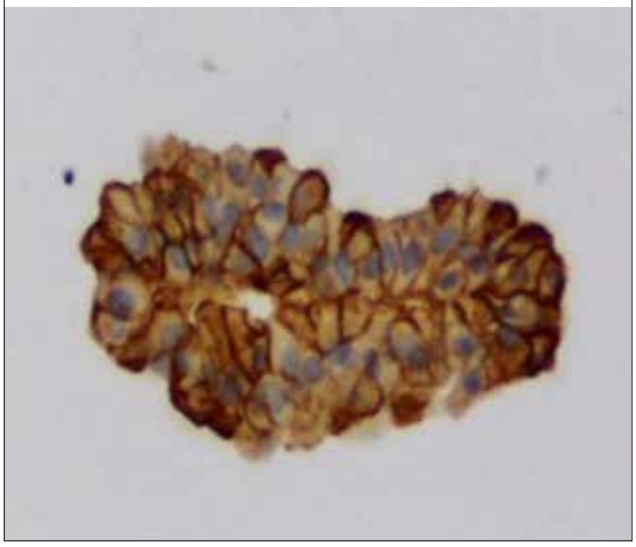


**Figure 4:** CD56.

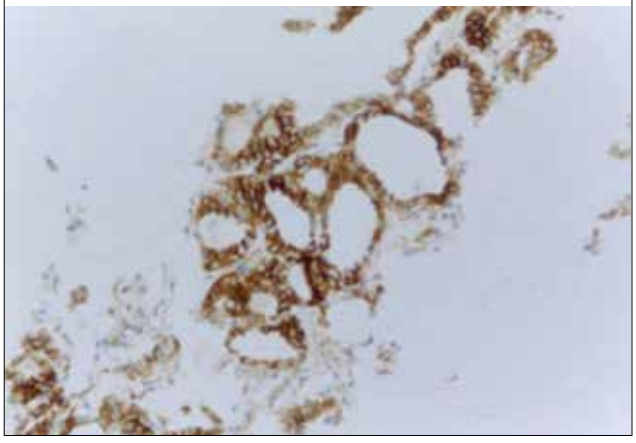
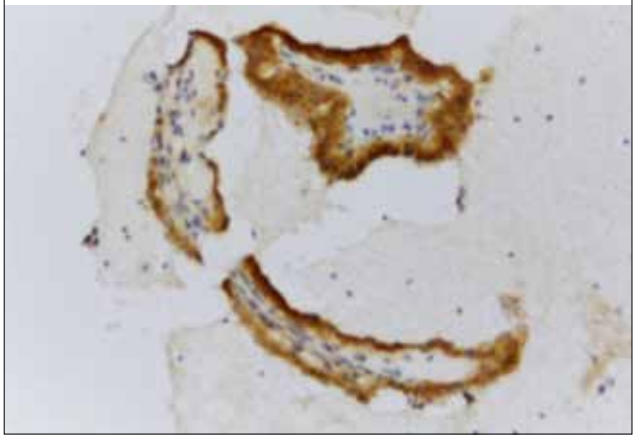


**Figure 5.** BRAFV600E.



Immunohistochemistry with BRAF V600E (ROCHE, VE1) has been performed both on cell block and on embedded surfical specimens of all malignant lesions.
Membranous, cytoplasmic and nuclear immunoreactivity was evaluated for each staining.

**Results.** In TIR3 B group the histological examination revealed benign lesions in 7 and malignant in 1; malignant lesions of TIR4 and TIR5 groups have been confirmed. Malignant tumors were represented from 8 papillary carcinomas, 1 medullary carcinoma and 1 anaplastic. Galectin 3 showed immunoreactivity (Fig. 1) in all papillary carcinoma and anaplastic tumor (90%); no staining in medullary carcinoma and in any benign lesions (100%). Cytocheratin 19 wasexpressed (Fig. 2) in all malignant tumours (100%); among benign lesions strong staining was detected in 1 (14%) and focal in 2 cases (28%) ,no staining in the remaining 4 cases (58%). HBME1 showed staining (Fig. 3) in 9 malignant tumours (90%) and no staining in medullary carcinoma; in benign lesions it showed staining in 2 cases (28%) and no staining in the remaining cases(72%). CD56 was negative (Fig. 4) in 8 malignant tumours (80%); only 2 cases showed focal staining (papillary carcinoma and medullary carcinoma). Immunoreactivity for BRAF V600E has been detected (Fig. 5)

in 7 papillary carcinoma ; no staining in 1 papillary carcinoma and in medullary carcinoma.

**Conclusions.** Our preliminary results suggest that an immunohistochemical panel consisting of Galectina 3, CK19, HBME1 and CD56 may improve the cytology diagnostic reliability in management of thyroid cancer. Furthemore IHC is a suitable method to screen BRAF V600E mutation in cell block.

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## FINE NEEDLE ASPIRATION OF ACCESSORY SPLEEN, A POTENTIAL MIMIC OF MALIGNANCY: REPORT OF TWO CASES

E. Kuhn[1], S. Lega[1], F. Poli[1], A. Lisotti[2], P. Fusaroli[2], M. Puccetti[1]

*[1] Pathology Unit, Azienda Unità Sanitaria Locale (AUSL) di Imola, Imola (BO), Italy; [2] Gastroenterology, Azienda Unità Sanitaria Locale (AUSL) di Imola, Imola (BO), Italy*

**Objectives.** The use of ultrasound-guided fine needle aspiration has become a routine diagnostic method widely used. This minimally invasive method allows obtaining a reliable cytologic diagnosis with reduced morbidity and mortality. However, the small dimension of the sample and lack of tissue architecture often make more difficult the interpretation of otherwise easily diagnosed entities. We present two cases of accessory spleen diagnosed on FNA aspiration, sent with the clinical suspicion of malignancy.

**Material and Methods.** Fine needle aspiration was performed on two abdominal nodules. In both cases, all aspirated material was processed by cell block technique. Immunohistochemical staining was performed for CD3, CD8, CD15, CD20, CD30, CD31, CD34, CD45, CD68, CD117, CD138, D2-40, DOG1, pankeratin (AE1/AE3), and S100.

**Results.** The first was a 3 cm oval hypoechoic nodule located in the gastric wall, specifically in the tunica muscolaris of a 47-year-old woman, suspected to be a gastrointestinal stromal tumor. The second was a 2.2 cm homogenously solid nodule in the tail of the pancreas of a 29-year-old woman, doubtful for neuroendocrine tumor. At cytological examination, both samples presented a heterogeneous hematopoietic cell population composed of small lymphocytes, eosinophils, histiocytes, neutrophils and plasma cells. Moreover, a background of a regular meshwork of channels with flattened wall and dense fibrous bands could also be noted. Immunohistochemically, CD3 and CD20 highlighted the mixed lymphocytes population and the presence of occasional germ centers. Some lymphocytes and most granulocytes were positive for CD15. CD138 marked isolated plasma cells and some small aggregates. CD68 revealed labyrinth-arranged macrophages. CD34 stained small vessels, while CD31 demonstrated a diffuse granulated positivity with intense scattered endothelial cells. D2-40, S100, pankeratin, CD117, DOG1 and CD30 were completely negative. Finally, CD8 stained intensely a meshwork of large vessel endothelium, demonstrating the splenic sinus architecture of the redpulp.

**Conclusions.** The accessory spleen is a quite common find, easily diagnosed on surgical resection, but challenging in cytologic samples, particularly when it has an unusual clinical presentation. Based on the location, it may clinically simulate different malignant neoplasias or lymph node metastasis, whereas morphologically it enters in differential diagnosis with lymphoproliferative diseases. The main morphologic diagnostic clue is the presence of well-organized heterogeneous hematopoietic cells. It is important to know that CD8 immunohistochemistry stains the venous sinusoids characteristic of the spleen and confirms the diagnosis of a suspected accessory spleen.

## REVIEW OF CLINICAL USEFULNESS OF THE CEREBROSPINAL FLUID CYTOLOGY

Nastassia Nardini[1 3], Saima Khan[2], Michael Sheaff[2], Ashirwad Merve[1]

*[1] Department of Neuropathology, National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS Foundation Trust, London WC1N 3BG; [2] Department of Cellular Pathology, The Royal London Hospital, Barts Health NHS Trust, London E1 2ES; [3] Institute of Pathology, S. M. Misericordia Hospital, Udine*

**Aim of the study.** Several cerebrospinal fluid samples (CSF) samples are received routinely to neuropathology departments contributing to significant workload. There is seldom any significant microscopic finding with bulk of it being negative. Here we review the usefulness of CSF cytology and put forth facts and opinions of whether CSF cytological examination has any continued clinical value. Active use of CSF cytology in diagnosing and monitoring CNS neoplasia, inflammatory and infectious diseases is discussed. Also discussed is the latest development in way of molecular testing and single cell detection.

**Methods.** We performed a retrospective survey in two large teaching hospitals – National Hospital for Neurology and Neurosurgery (NHNN), a tertiary specialist centre for neurological diseases and The Royal London Hospital (RLH), comprising one of the largest cellular pathology departments in the UK. The cytology reports of over 100 cases examined over approximately 3 months duration in the latter half of 2017 were reviewed in each centre and the results were correlated with the clinical outcomes to derive clinical usefulness of the CSF cytology examination.

**Results.** NHNN results: A total of 110 patient's CSF cytology reports were analysed between August to October 2017. Of them only 4 cases (3.6%) which showed non-specific increase in inflammatory cells actually had clinical diagnosis of an inflammatory disease. 83% despite clinical symptoms had a negative CSF with no significant findings (either acellular or low cellularity). 7 patients who went on to have brain biopsy where a pathology was detected, only 1 patient had increased inflammatory cells and the other 6 patient's CSF was reported as negative. RLH results. A total of 103 patient's CSF cytol-

ogy reports were analysed between August to October 2017. Of them, 86 (83,5%) were acellular or hypocellular with no positive findings. Only 10 cases (9.7%) had positive findings such as the presence of significant amounts of inflammatory cells (8/103), or presence of neoplastic cells (2/103).

**Literature Review and Discussion.** The CSF is of diagnostic aid in the evaluation of inflammatory conditions, infectious or non- infectious, involving the brain, spinal cord, and meninges as well as CT-negative subarachnoidal haemorrhage and in leptomeningeal metastases. The diagnostic sensitivity and specificity will increase combining a set of CSF variables referred to as routines parameters: determination of protein, albumin, immunoglobulin, glucose, lactate and cellular changes, as well as specific antigen and antibodies testing for infectious agents. Several methods, other than the cytological examination, are available for the identification of the diseases in the CSF.

Adapted lens-free microscopy is emerging as an operator-independent technique for the rapid numeration of leukocytes and erythrocytes in cerebrospinal fluid. In particular, this technique is well suited to the rapid diagnosis of meningitis at point-of-care laboratories [1].

Flow cytometry of CSF is increasingly being considered as the method of choice in patients suspected of leptomeningeal localization of hematological malignancies. Additionally, in several neuroinflammatory diseases such as multiple sclerosis and paraneoplastic neurological syndromes, flow cytometry is commonly performed to obtain insight into the immunopathogenesis of these diseases [2].

Cell-free DNA (cfDNA) shed by cancer cells has been shown to be a rich source of putative tumor- specific biomarkers. Because cfDNA from brain and spinal cord tumors cannot usually be detected in the blood, Momtaz et al. investigated if mutated BRAF V600 cfDNA could be quantified in the cerebrospinal fluid (CSF) of patients with central nervous system metastases of melanoma. Conventional cytology was negative in all the patients except in two patients with markedly elevated levels of tumor-derived cfDNA. In addition, CSF tumor-derived cfDNA levels reflected response to treatment or progressive disease. They demonstrate that CSF tumor-derived cfDNA has the potential to serve as a diagnostic tool that complements MRI and may be more sensitive than conventional cytology [3].

In another similar study, Wang et al. investigated whether the CSF that bathes the CNS is enriched for tumor DNA. They analyzed 35 primary CNS malignancies and found at least one mutation in each tumor using targeted or genome-wide sequencing. Using these patient-specific mutations as biomarkers, they identified detectable levels of cfDNA in 74% of cases. All medulloblastomas, ependymomas, and high-grade gliomas that abutted a CSF space were detectable, whereas no cfDNA was detected in patients whose tumors were not directly adjacent to a CSF reservoir. These results suggest that CSF-tDNA could be useful for the management of patients with primary tumors of the brain or spinal cord (4).

**Summary/Conclusions.** Here we describe recent literature highlighting new technologies such as flow cytometry, single cell and DNA analysis for identification of inflammatory, neoplastic and neurodegenerative diseases. This may give an impression that cytological examination is of poor diagnostic value. However our survey results show equivocal results, with clinical usefullness detected in 3.6 – 9.7%. Moreover negative cytology may be of prarmount importance for clinicians. As CSF examination is performed as a part of routine work up of several neurological assessment, it is likely that they will continue to need our input in cytological examination of CSF. It prompts a larger study to understand the cost-effectiveness and possible impletmentation of protocol for CSF cytology on broader platform.

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## ANAL PAP SMEAR IN A HIGH RISK POPULATION: THE L. SACCO HOSPITAL EXPERIENCE


M. Nebuloni[1], D. Cominetti[1], L. Carsana[1], S.Rosatto[1], M. Maresca[1], L. Lorella[1], N. Zanchetta[2], MR. Gismondo[2], P. Zerbi[1]

*[1] Pathology Unit, Department of Clinical Sciences, L. Sacco Hospital, Università degli Studi di Milano, Italy; [2] UOC Microbiologia Clinica, Virologia e Diagnostica delle Bioemergenze, L. Sacco University Hospital, Milan, Italy*


**Introduction.** Persistent Human papillomavirus (HPV) infection can induce a broad spectrum of mucocutaneous alterations, causing also squamous cell carcinoma. Anal intraepithelial lesion (AIN) is one of the most common morbidity in case of HPV infection, especially in some population groups, considered at higher risk of transmissible sexual diseases. Furthermore, Human Immunodeficiendcy Virus (HIV) is strongly associated with a higher risk of chronic HPV infection, making the HIV-HPV coinfected patients at higher risk of developing AIN. In this context, it is imperative to strictly screen patients at high risk of developing AIN, in order to reduce its morbidity and mortality.

**Material and Methods.** We retrospectively analyzed the cytological reports of anal pap smear (n=113), screened in a period of 6 months, sent to the Pathology Unit of the L. Sacco Hospital, Milan. Pathologists were blinded to the HPV or HIV status of the patients at the moment of the microscopic evaluation. Subsequent anal biopsies were graded as low (AIN1) or high (AIN2 and AIN3) grade. Anal pap smear reported as Atypical Squamous Cells of Undetermined Significance or worse were classified as abnormal anal cytology. HPV and HIV status, the cytological grade, condyloma presence, previous anal cytology, histological and eventual cervical cytology were also evaluated. HPV-typing (High Risk HPV vs Low Risk HPV) was performed at the Microbiology Unit of the same Hospital.

**Results.** In High Risk HPV (HPV-HR) infected patients, a higher percentage (48%) of squamous lesions was identified by cytology, especially in HIV- HPV-HR coinfected patients. In particular, LSIL showed a higher incidence among HIV-HPV-HR coinfected patients, respect to HPV-HR positive, HIV negative, patients (52 vs 36%, respectively). No high grade lesion was identified in HPV-HR negative samples. Histological examination following a positive cytology identified n=21 AIN, more often after a LSIL cytological diagnosis. All histology confirmed AIN had an abnormal cytology, but one HIV patient with AIN2 had a HPV-HR test negative. Among this high risk population (69% HIV infected), all but one AIN patients were

HIV positive. Female represented the 19% of the study population; 45% of them had a concomitant abnormal pap smear.
**Conclusions.** Screening tests for anal lesions is of utmost importance in a context of a high risk population. HIV and HPV-HR positive patients are more prone to develop an AIN. Concomitant cervical pap smears seems mandatory in the high risk female population.

## THE RARE ENTITY OF ADENOSQUAMOUS CELL CARCINOMA OF THE PANCREAS: REPORT OF A CASE WITH CYTOLOGICAL DIAGNOSIS

M. Nebuloni[1 2], D. Taietti[2], L. Carsana[1], E. Molteni[1], F. Falappa[1], E. Volontè[1], C. Marras[1], L. Terni[2], Zerbi[1 2]

*[1] 2Pathology Unit, L. Sacco Hospital, Department of Biomedical and Clinical Sciences; [2] Università degli Studi di Milano, Italy*

**Introduction.** Adenosquamous carcinoma of the pancreas (ASCP) is rare variant of pancreatic carcinoma, accounting for 0.38% to 10% of all the exocrine malignancies. The current guidelines to diagnose adenosquamous pancreatic cancer arbitrarily stable that the presence of squamous component in the pancreas tumour must to be of at least 30%. In the past, it has been variously referred to as adenoacanthoma, mixed squamous and adenocarcinoma, and mucoepidermoid carcinoma. Patients with adenosquamous carcinoma typically present with symptoms similar to adenocarcinoma of the pancreas, including abdominal pain, weight loss, anorexia, and jaundice. Computer tomography imaging of ASCP lesions commonly show the presence of central necrosis within the tumour mass, which is rarely seen in pancreatic ductal adenocarcinoma or in endocrine tumours of the pancreas.
The overall survival of ASCP is poor, even worse than the classical adenocarcinoma.
**Case presentation.** We report a case of 77 year old woman presenting abdominal pain with dorsal irradiation, firstly interpreted as osteo-articular disorder. The duration of symptoms prior to appearance was 5 months. She came to the L.Sacco Hospital, Milan, Italy, after developing scleral- cutaneous jaundice. Abdominal ultrasound showed a hypoechogen cefalopancreatic mass of 26 mm and computerized tomography scan highlighted a mass in the head of the pancreas measuring 4 cm in size. Clinical staging by CT excluded secondary malignant growths. The cefalopancreatic mass, partially colliquated, was 52 mm in dimension, invading the contiguous portion of the duodenum with minimal ectasia of Wirsung duct and dilatation of the gallbladder and the intra/extrahepatic biliary tree. There wasn't extension to the superior mesenteric artery, celiac axis and portal vein.
Laboratory studies showed elevated bilirubin (total 12,52 mg/dL, direct 9,52 mg/dL), Ca 19-9 of 65 U/mL, CEA of 3,5 ng/mL, NSE 12 ng/mL and CgA of 206 ng/mL.
Endoscopic ultrasonography revealed a 43x30 mm mass of the head of the pancreas, with faded borders and no extension to mesenteric vessels, with Wirsung duct and principal biliary tract dilatation. Fine needle aspiration executed during the exam exited in a poor differentiated carcinoma with abundant necrosis and dominated by squamocellular differentiation.
The decision for surgical intervention was taken and a pylorus preserving pancreaticoduodenectomy was performed. The patient had a postoperative course without relevant complications and was discharged on the 24th day.
At macroscopic examination, the lesion was described as an infiltrative white and firm mass of fibrous consistency. Taking into account the cytological preoperative diagnosis, an extensive sampling of the lesion was performed in order to correctly document the histotype of the carcinoma. Histological evaluation of the pancreatic tumour showed an infiltrative carcinoma with involvement of peripancreatic lymph nodes and all the thickness of the duodenum wall. The tumour exhibited a biphasic malignant growth with a predominantly squamous component, identified as poorly differentiated squamous cell carcinoma associated with focal and limited areas of glandular differentiation. Squamous differentiation was characterized by irregular and infiltrative nests of polygonal cells with distinct cellular borders, intercellular bridges, eosinophilic cytoplasm and varying degrees of keratinization. The adenocarcinoma component contained glandular structures with focal intracellular or extracellular mucin. Necrosis within tumour and desmoplastic stromal reaction were present.
Immunohistochemistry showed the squamous component positive to Citokeratin 5/6 and p63, while the glandular part mainly positive to Citokeratin 7. Alcian Blu-PAS staining marked focal intracellular or extracellular mucin in the adenocarcinoma areas. The whole neoplasia was negative for CK 20, chromogranin and synaptophysin.
**Conclusion.** Adenosquamous carcinoma of the pancreas is a rare aggressive subtype of pancreatic ductal adenocarcinoma. Even if the role of endoscopic US-guided FNA biopsy is still controversial, it is undoubted its role in defining an extremely rare entity, and thus modulating the surgical and therapeutic approach.

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## VALIDATION OF THE PARIS SYSTEM TO IDENTIFY ATYPICAL CELLS AS DIAGNOSTIC CATEGOR Y IN URINARY CYTOLOGY: AN EXPERIENCE FROM ONE SOUTHERN ITALY PATHOLOGY INSTITUTION

A. Ieni, C. Pizzimenti, A.M. Bonanno, R.A. Caruso and G.Tuccari

*Department of Human Pathology of Adult and Evolutive Age "Gaetano Barresi", Section of Pathological Anatomy and Cytopathology, University of Messina, Messina, Italy*

**Objective.** In 2013, during the International Congress of Cytology in Paris, members of the American Society of Cytopathology and the International Academy of Cytology formed a committee, which published their guidelines, known as the Paris System (TPS) for Reporting Urinary Cytology. Emphasizing the prominent role of urinary cytology in the detection of high grade lesions, the system applied a series of morphological criteria for the identification of atypia and mainly for the separation atypical from reactive and neoplastic cellular features. The aim of this analysis was to apply this new reporting system in urinary cytology in order to verify its reliability and reproducibility in the field of atypia.

**Materials** & Methods. We retrieved from our database 410 urinary samples from January 2014 to January 2018. All voided urine samples corresponded to patients with symptoms like haematuria or pain (145 samples, 36,27%) or patients with a history of urothelial neoplasm (285 samples, 63,73%). All urine specimens have been processed by conventional and liquid-based (Thinprep®; Hologic, USA) technique.

**Results.** Of 410 urine samples, 248 (60.50%) were negative for malignancy, 106 were malignant (25,85%) and 56 were atypical (13.65%), including all ranges of atypia, such as atypical probably reactive, atypical indeterminate and atypical suspicious of malignancy.

The 56 atypical cases corresponded to 16 female and 40 male patients with median age of 66 years. Evaluation of atypical cases according to the TPS classification criteria provided the following results: 20 cases were benign and negative for high-grade urothelial carcinoma (HGUC), 26 were atypical urothelial cells (AUC) and 10 suspicious for HGUC. Diagnostic consistency between the four observers reached 91.48%. There was diagnostic discrepancy between observers in four of the 56 cases; in three cases, there was discrepancy between AUC and negative for HGUC, while in one case, the discrepancy was between AUC and suspicious for HGUC. Finally, the consensus was reached after discussion.

**Conclusions.** In our experience, the newly proposed Paris System for reporting urinary cytology furnishes useful and specific criteria; in detail, criteria for diagnosing AUC should include one major and one minor criterion. Specifically, the major criterion is the presence of non superficial and non degenerated urothelial cells with an increased nuclear cytoplasmic (N/C) ratio (>0.5), while minor criteria may include: (1) mild nuclear hyperchromasia, (2) irregular nuclear membranes or nuclear contour, (3) irregular, coarse, clumped chromatin. In addition, recent evidences have suggested a potential role to identify atypical urinary elements by some immunohistochemical markers, such as p53, p63 and CK20, even if more additional investigations are required.

# DERMOPATOLOGIA

## PRIMARY DIFFUSE CUTANEOUS PLASMACYTOMA: WHEN A CORRECT CLINICO- PATHOLOGIC APPROACH IS MANDATORY FOR PATIENT'S HEALTH. A CASE REPORT

Broggi[1], E. Martino[2], C. Conticello[2], E. Piombino[1], W. Saliba[1], L. Villari[3], R. De Pasquale[4], R. Caltabiano[1]

*[1] Department "G.F. Ingrassia", Section of Pathologic Anatomy, University of Catania, Catania, Italy; [2] Division of Hematology, Azienda Ospedaliero-Universitaria 'Policlinico-Vittorio Emanuele', Catania, Italy; [3] Division of Pathology, AOU "Policlinico-Vittorio Emanuele", Via Plebiscito 628, 95100 Catania, Italy; [4] Department of Dermatology, Policlinic Vittorio Emanuele Hospital, Catania, Italy*

**Objectives.** Primary cutaneous diffuse plasmacytoma (PDCP) is a rare disease [1] which arises primarily in the skin, so can be considered as a localized cutaneous extramidollary plasmacytoma (EMP) and should not be confused with secondary cutaneous plasmacytoma (SCP) in the context of MM [2]. After the first description by Stout and Frerichs in 1949 [3], according to a recent systematic review, only 68 cases of primary cutaneous plasmacytomas (PCPs) have been reported in literature, the majority of which were solitary lesions.
Clinically PDCP usually presents as purplish-blue cutaneous nodules with a predilection for the face, trunk, and extremities [1 2]. Diagnosis rests on histology and immunohistochemistry (IHC) and must include exclusion of underlying myeloma through laboratory, radiological, and bone marrow investigations.
**Materials and Methods.** On February 2015 a 76-year-old woman presented with cervical lymphadenopathy and four purplish plaques located to the left arm, two in the left deltoid region, one in the right elbow, one in the left elbow, one in the left wrist, one in the right breast and others in the two legs. Lesions were painful at the touch without itching.
Total body CT-scan revealed latero-cervical lymphadenopathy. Patient underwent FDG PET/CT imaging that demonstrated abnormal uptake in the previous-described sites. Ultrasound imaging of the neck showed some latero-cervical lymph nodes that were excised. A diagnosis of chronic granulomatous epithelioid necrotizing lymphadenitis tubercolosis-like was rendered. An infectious counselling was performed and the patient underwent to anti-tubercolosis therapy with isoniazid at the dosage of 300 mg/daily and rifampicin 600 mg/daily for 5 months. During this time, skin lesions increased in number and were more painful. Revaluation FDG PET/CT demonstrated an increased abnormal uptake also in the lung, as an evolution from the previous. Uncertain about the real nature of the infectious disease lesion, a dermatologic evaluation and a skin biopsy of the left arm was made.
**Results.** Histological examination of hematoxylin and eosin-stained sections showed the presence of perivascular and interstitial clusters of atypical oval-shaped cells with abundant cytoplasm, eccentric nuclei, "clock face" chromatin and sometimes prominent nucleoli, infiltrating the medium and deep dermis. Mitotic figures were seen and a lymphocytic reactive infiltrate mixed to neoplastic cells was noted. These cells were morphologically similar to mature plasma cells, so a specific immunohistochemical panel was performed and neoplastic cells were diffusely positive to CD138, MUM-1 and EMA. Immunohistochemical studies for kappa and lambda light chains revealed a monoclonal expression of immunoglobulin kappa lights chains. To complete the diagnostic iter, a bone marrow biopsy was performed and it was negative for MM localization (less than 10% plasma cells; no clonal restriction). There were not Bence-Jones proteins in urine. Normal value of serum creatinine (0.87 gr/dL), haemoglobin (12,2 gr/dL), serum calcium (9,4 mg/dL) at the blood analysis. Serum protein electrophoresis highlighted a lambda light chain spike.
Once the absence of other localizations of disease was confirmed, a clinico-pathologic diagnosis of
primary diffuse cutaneous plasmacytoma could be rendered.
Considering the extensive dissemination of the cutaneous involvement, and according to the good performance status and lack of comorbidities, patient received systemic therapy. It consisted of Bortezomib at the dosage of 1.3 mg/m2 subcutaneous at day 1,8,15 and 22, Melphalan given orally at the dosage of 14 mg at day 1, 2, 3 and 4, and Dexamethasone at the dosage of 20 mg at day 1-2- 8-9-15-16-22-23 (VMP).
Cycles of immunochemotherapy were well tolerated and no adverse effects were registered.
After nine cycles, FDG PET/CT showed complete disappearance of the skin lesions but persistence of nodal and lung lesions. Moreover, absence of the lambda immunoglobulin G spike at the serum protein electrophoresis. Patient completed therapy on October 2016 and referred an improvement in her quality of life; she is still in follow-up without any worsening of cutaneous lesions.
**Conclusions.** Histologically, a diffuse or nodular infiltration pattern can be recognized in PCPs and neoplastic cells may show different stages of plasma cells maturation process, from well differentiated to pleomorphic (similar to plasmablasts) features [3]. PCPs with plasmablast-like features are composed of neoplastic cells with higher nuclear/ cytoplasmic ratio, finely dispersed chromatin and more prominent nucleoli. Epidermotropism is usually absent in PCPs [3].
The main prognostic factor is clinical presentation (solitary vs multiple lesions), but is also important to consider patient's performance status and comorbidities which can impair on the compliance in the treatment.
Differential diagnosis of PCP includes MM with SCP, extramedullary plasmacytoma with secondary cutaneous involvement, other B-cell lymphomas of the skin, like marginal-zone lymphoma, infective diseases such as syphilis and Borrelia infections, and inflammatory dermatoses, including circumorificial plasmacytosis, with prominent reactive plasma cell infiltration. MM rarely involves the skin and because of the absence of distinctive histological features, only with clinical and laboratory examinations it is possible distinguish between SCP in MM and PCP [2]. Generally, EMP origins from the upper respiratory tract, especially from the naso- oropharyngeal and laryngeal district, and tends to keep localized, with the only onset of lymph node metastasis.
It is important to emphasize that neoplastic plasma cells in PCPs can be cytologically indistinguishable from reactive ones in infectious diseases, representing a potential diagnostic pitfall for pathologists, so IHC evaluation of mono- or polyclonal expression of immunoglobulin light chains, combined with the absence of an evocative history of infection or causal agent identification, are crucial for a diagnosis of malignancy [3].
Finally, PCDP is a rare disease which requires a wide multidisciplinary approach, which is strongly recommended to achieve a certain diagnosis of "true" PCP, in order to choose the optimal treatment.

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## A PECULIAR UNCOMMON CUTANEOUS TUMOURS: ANGIOSARCOMA OF THE HEAD AND NECK OF THE ELDERLY. A CASE REPORT

A. D'Amuri[1], F. Floccari[2], M. Filotico[1]

*[1] Anatomic Pathology Unit, "Card. G. Panico" Hospital, Tricase (LE), Italy; [2] Clinic Pathology Unit, "Camillo De Lellis" Hospital, Manfredonia (FG), Italy*

**Introduction.** We report the case of a 85-year-old man who presented a erythematous patch on the right cheek and extensive involvement of the scalp and face. The lesion continued to expand despite various local therapies. The histological investigation showed the dermal level numerous cavities with an irregular lumen, sometimes wide, sometimes fissuriform in which papillary vegetations often protrude. The lumen of the cavities, as well as, the axes of the papillary vegetations are papered by plump endothelial elements, hyperchromatic. These elements express strongly CD34 and CD31. Further studies also showed positivity for podoplanin, p53, c-myc and negativity for HHV8. On the basis of these elements the diagnosis of "Well-Differentiated Cutaneous Angiosarcoma of the head and neck of the elderly" was performed.
**Material and Methods.** A man of 85 years with an erythematous patch on the right cheek, which it has progressively expanded to reach the scalp and preauricular region. Upon palpation the lesion is infiltrated and hardened. The lesion continued to expand despite various local therapies. An incisional biopsy of a cutaneous lozenge is performed. The bioptic fragment is fixed in formalin and in paraffin embedded. Sections stained with HE and subjected to immunohistochemical investigation with CD34 and CD31 antibodies.
**Results.** Histological observation reveals the complete integrity of the epidermal lining. The dermis, on the other hand, appears to be dissociated by numerous cavities with an irregular lumen, sometimes wide, sometimes fissuriform in which papillary vegetations often protrude. The lumen of the cavities, as well as the axes of the papillary vegetations are papered by plump endothelial elements, hyperchromatic, distinctly atypical. These elements express intensely and extensively CD34 and CD31. On the basis of these elements the diagnosis of "Well-Differentiated Cutaneous Angiosarcoma of the head and neck of the elderly" is posed. The patient for further investigation and treatment is admitted to the National Cancer Institute in Milan (INT), where the histological diagnosis is confirmed, and further validated with other immunochemical and biomolecular researches.
**Discussion and Conclusions.** In 1964 Wilson-Jones described in detail this particular cutaneous angiosarcoma variant which is characterized by its particular clinical presentation. Two aspects characterize this lesion: the localization to the cheek with extension to the scalp, to the retroauricular region and the neck, the advanced age of the patient who is usually male. The lesion begins as an erythematous macula which progressively extends in a centrifugal manner giving the skin an edematous consistency. As the lesion progresses the color passing through the violet to the bluish, assuming, sometimes, the appearance of a hematoma. The plaque is progressively hardened and nodules may appear in its context and undergo ulceration. The centrifugal progress of the neoplasm is often very rapid, so that in a short time the whole cheek is occupied. Histopathological investigation reveals the lesion is more extensive than would appear on clinical examination. A wide variety of histological aspects can be found both in the various neoplasm and in those of the same neoplasm. From highly differentiated lesions Hemangioma/Lymphangioma-like, to classically papillary patterns, to solid poorly differentiate, epithelioid or spindle cell. in these solid lesions the differential diagnosis from carcinoma, melanoma, or atypical fibroxanthoma can be difficult. In this particular neoplasia to date no characteristic chromosomal aberration has been signaled. According to Manner et al. in post-irradiation skin angiosarcomas or associated with lymphedema, an amplification of the MYC gene would occur, which would not occur in primitive angiosarcomas. In experience of Requena et al. the MYC amplifications in angiosarcoma, as detected by either FISH or immunohistochemistry, are much more widespread in angiosarcomas than originally suggested by Manner. Remarkably, so far, almost all of our facial and scalp angiosarcoma cases have stained positive for MYC. We therefore suggest to include immunohistochemical MYC staining in any diagnostic vascular antibody panel. Immunohistochemical MYC staining is qualitatively equivalent to analogous FISH.

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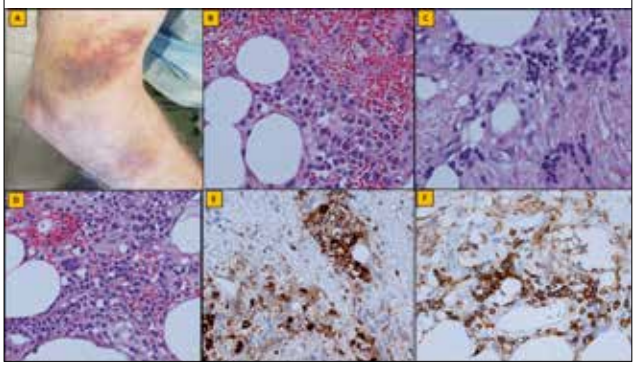
## EXTRAMEDULLARY HAEMATOPOIESIS PRESENTING AS HAEMORRHAGIC PANNICULITIS: REPORT OF AN ADULT CASE

G. Gallo[1], F. Azzali[1], S. Bigliardi[2], A.M. Cesinaro[1]

*[1] Department of Anatomic Pathology Azienda Ospedaliero-Universitaria di Modena, Modena; [2] Department of Oncology, Nuovo Ospedale di Sassuolo (Modena)*

**Aims.** Extramedullary haematopoiesis (EMH) in adult is a rare event, usually associated with myeloid disorders [1], and can be the first sign of chronic myelomonocytic leukemia [2]. We report a case of EMH presenting as a haemorrhagic panniculitis in a patient with apparently no myeloid disorders.
**Case report.** A 69-year-old man with 3 years- history of mantle cell lymphoma, classic type, localized in the colon and treated with R-BAC chemotherapy, in complete remission, presented spontaneous hematomas on the arms and legs, and subcutaneous firm, multiple "migrant" nodules, about 2,5 cm in diameter (Fig. 1A). Laboratory investigations revealed leukocytosis (16.200/mmc), neutrophilia (14.580/mmc) and thrombocytopenia (77.000/mmc). Bone marrow biopsy demonstrated normoblastic erythropoiesis with rich cellularity, and no atypia. No other symptoms were present. A deep incisional biopsy of a nodule from the thigh was performed for histology.
At scanning magnification, H&E stained slide showed dermal perivascular lymphocytic infiltrate, with diffuse haemorrhage and fat necrosis, akin to panniculitis. In haemorrhagic areas of subcutis, clusters of myeloid and erythroid cells, together with scattered megakaryocytes (Fig. 1B-1D) were visible. Fungal infection or lymphoma were not present. Immunohistochemical study revealed positivity for myeloperoxidase (polyclonal,

**Figure 1.** Clinical aspect of a lesion from the arm A). Biopsy of a nodule from the thigh: Clusters of cells in subcutaneous hemorrhagic adipose tissue **B)**. Constituted by erythroid **C)**. And myeloid cells **D)**, with scattered megakaryocytes. Immunohistochemical stain of myeloid cells for MPO (E) and CD4 (F).



prediluted, CELL MARQUE) and CD4 (clone SP35, prediluted, VENTANA) (Fig. 1 E-F) in myeloid cells, while CD34 (clone QBEnd/10, prediluted, VENTANA) resulted negative. All these characters suggested a diagnosis of EMH. The patient was further investigated for the presence of JAK2 mutation, that turned out to be absent. At last control, increased leucocytosis (19.000/mmc) and neutrophilia (16.530/mmc) were observed.

**Conclusions.** EMH is defined as the presence of haematopoietic tissue outside the bone marrow. In early fetal development, EMH is physiologic and involves the liver and, to a lesser extent, the spleen [1]. In postnatal period, EMH can present associated with congenital viral infections, including cytomegalovirus and Rubella, as well as haematopoietic disorders. In adults, EMH represents a pathologic finding. It occurs in benign haematologic disorders, haematopoietic neoplasms, stromal disorders of the marrow, non-haematopoietic tumours, infections, and disorders of the circulation [2]. EMH has been reported also as an early sign of myelocytic disorders [2].

Cutaneous EMH is a rare complication, resulting by migration of abnormal neoplastic haemopoietic precursor cells into the skin, with subsequent differentiation into divergent cell lineages [1 3 4]. Cutaneous EMH is often seen in the context of myelofibrosis evolving from chronic myeloid diseases, in polycythaemia vera or essential thrombocytosis. [1 3 5]. A unique case of acute myeloid leukaemia presenting as panniculitis has been described [2], but no cases presenting as EMH are reported in literature. Multiple molecular abnormalities have been identified in myeloproliferative neoplasms. The most common somatic mutations are in JAK2, detected in the majority of these above- mentioned cases [6]. Thus, the presence of cutaneous EMH should be a trigger to investigate the patient's haematological system. Although our patient was negative for JAK2, and, after 8 months of skin involvement, no signs of myeloid disorder are evident, a follow-up is still mandatory.

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## THE USEFULNESS OF CDX2 AND HER2 IN THE DIAGNOSIS OF SECONDARY PERI- ANAL PAGET DISEASE


C. Rossi[1], G Fumo[2], R. Zanniello[2], C. Ferreli[2], L. Atzori[2], G. Senes[1], G. Faa[1], L. Pilloni[1]

*[1] Department of Surgical Sciences, Division of Pathology, University of Cagliari, Italy; [2] Department of Medical Science and Public Health, Section of Dermatology, University of Cagliari, Italy*


**Aim.** Peri-anal Paget disease (PPD) is a rare cutaneous malignancy, first described by Darier and Couillard in 1893 as a location of Extramammary Paget's disease (EMPD) [1]. PPD presents as a peri-anal itchy, erythematous, eczematous skin lesion. PPD is classified in two categories depending on the presence of an underlying neoplasm: primary PPD, a local and relatively benign disease, not associated with an underlying neoplasm, and secondary PPD, strongly associated with an underlying colorectal or anal neoplasm which may occur synchronously or metachronously [2]. Differential diagnosis among primary and secondary PPD is necessary, given the differences in treatment and prognosis. However, the two types of PPD cannot be distinguished on histopathologic grounds alone. Immunoreactivity for cytokeratin (CK) 7 and BRST2 with no reactivity for CK20 and CEA was described in primary PPD, conversely in secondary PPD immunostaining for CK7, CK20 and CEA with no staining for BRST2 has been reported. Some studies [3-5] highlighted the possibility of immunoreactivity for CK20 and CEA in primary PPD and immunostaining for BRST2 in secondary PPD. Therefore, immunohistochemistry for CK 7, CK20, BRST2 and CEA is not sufficiently specific and sensitive in discriminating the two entities. Recent studies [4 5] have suggested the use of an expanded imunohistochemical panel, including CDX2 and HER2, which may be useful to differenziate the two types of PPD. The expected result in primary PPD is immunoreactivity for HER2 and no reactivity for CDX2, while secondary PPD show immunostaining for CDX2 and no reactivity for HER2. Here we report a case of PPD with an immunoprofile suggestive for secondary PPD.

**Materials and Methods.** A 73-year-old male patient consulted the dermatologist for a recent history of itchy peri-anal skin lesion. The clinical examination revealed an erythematous, eczematous, circular skin lesion around the anus (fig. 1). The dermatologist suspected psoriasis or chronic eczematous dermatitis. An incisional biopsy of the peri-anal skin lesion was performed.

**Results.** Histological examination of the biopsy showed large, round cells with a pale vacuolated cytoplasm and a large nucleus often displaced to the periphery of the cell (signet ring sign) scattered throughout the squamous epithelium, without evidence of dermal invasion. These cells, also known, Paget's cells showed immunoreactivity for CK7, CK20, CEA and CDX2 while did not show any reactivity for HER2 (fig. 2). The immunoprofile was more in keeping with a secondary PPD. Conclusions. PPD is a rare but clinically important disease. Recent publications [4 5] emphasize the role of an immunohistochemical panel in order to differentiate between primary and secondary PPD. Our case shows immunostaining for CDX2 and no stain-

**Figure 1.** Clinical examination shows an erythematous, eczematous, circular skin lesion around the anus.

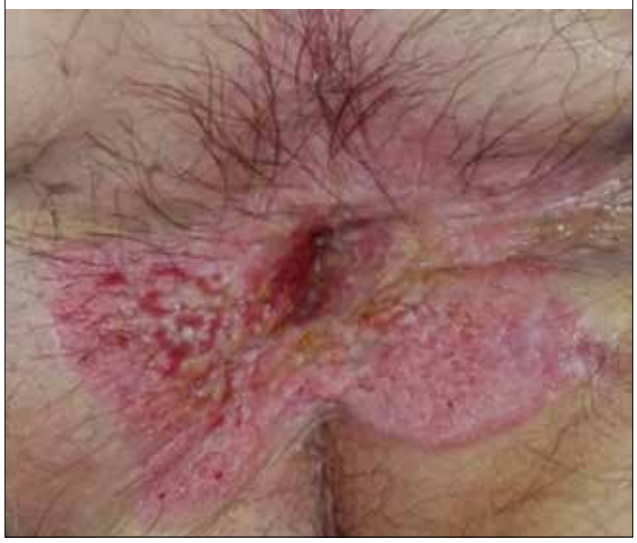
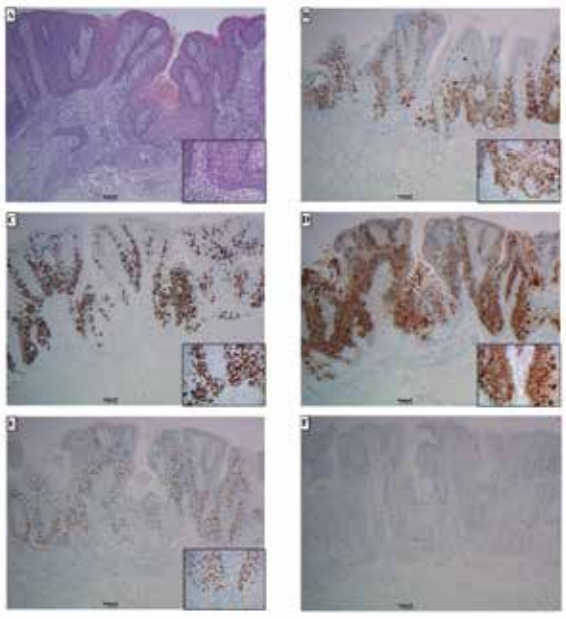


**Figure 2.** Peri-anal skin lesion. A) Hematoxylin and eosin shows Paget's cells scattered throughout the epidermis, without evidence of dermal invasion. B) CK7 highlights Paget's cells in the epidermis showing a strong cytoplasmic staining. C) CK20 highlights Paget's cells in the epidermis showing a strong cytoplasmic staining. D) CEA shows a cytoplasmic staining in Paget's cells. E) CDX2 highlights Paget's cells in the epidermis showing a nuclear staining. F) Paget's cells do not show any reactivity for HER2.



ing for HER2. According to the expected results reported in literature, this immunoprofile is suggestive for secondary PPD.

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## MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL FEATURES OF PIMARY CUTANEOU MUCINOU CARCINOMA: A CASE REPORT


A. Santarsiere, S. Esposito, R. Lucci, R. Luise, G. Carillo, A. Di Iasi, M. Manno, M. Santarsiere

*UOC Anatomia Patologica "A. Di Tuoro" PO "S.G.Moscati" Aversa-Caserta*


Primary cutaneous mucinous carcinoma (PCMC) is a rare low-grade malignant neoplasm derived from the eccrine glands [1]. These carcinomas most commonly arise in the head and neck region. It is most frequently between the ages of 50 and 70 years . The prevalence is higher in white patients (77.2%) than in Asians (12.7%) or in African Americans (10.1%) [2]. We report a case of PCMC of the scalp in a 86-year-old female.

A 86-year-old female patient presented to the clinicians with a coutaneous nodule on the scalp. The mass appeared benign in clinical examination, and under local anesthesia, the patient underwent a excision and primary closure.

Macroscopically the lesion appeared as a cutaneous lozenge of cm.2,4x1,6 seat of whitish nodular lesion of 1,5 cm.

The tissue was routinely processed in a formalin-fixed, paraffin-embedded material.

Histologic study of this first specimen revealed epithelial cell islands floating in a mucin lake (PAS +); the epithelial component showed a solid and cribriform growth pattern and the tumor cells revealed moderate nuclear atypia and a mitotic aspect.

Representative sections were cut for morphological examination and tissue blocks submitted for immunohistochemistry methods that were used to confirm the diagnosis.The lesion showed positivity for Ck35bE12, EMA, CEA.

Thus, a diagnosis ofPCMCwas rendered.

During a close follow-up over 1 year, the patient did not experience any postoperative complication or recurrence.

PCMC was first described by Lennox et al. [1] and is a rare subtype of sweat gland tumor. Controversy about the apocrine or eccrine origins of this tumor has existed, but most authors favor eccrine differentiation as the origin [3].

The tumor appears as an unencapsulated, nodular dermal tumor composed of solid and cribriform nests, cords and tubules embedded within a desmoplastic stroma.

Tumor cells expressed CK7, CK5/6, EMA, CEA, S-100, BerEP4 and c-kit and were negative for cytokeratin 20, estrogen receptor/progesterone receptor (ER/PR), androgen receptor and GCDFP-15 [4].

Primary mucinous carcinoma of the skin has a relatively good prognosis with lower rates of distant metastases (9.6%, commonly to regional lymph nodes), but local recurrence rate is higher at 29.4% [5]. The treatment recommendation has been surgical excision with a minimum of 10-mm margin [6].

mmunohistochemical studies will often help to distinguish Primary cutaneous mucinous carcinoma from other neoplasms.

Figura 1:HE/HE 5x.

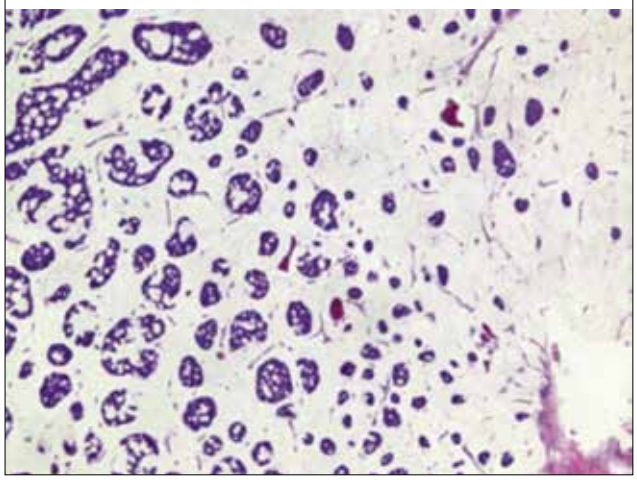
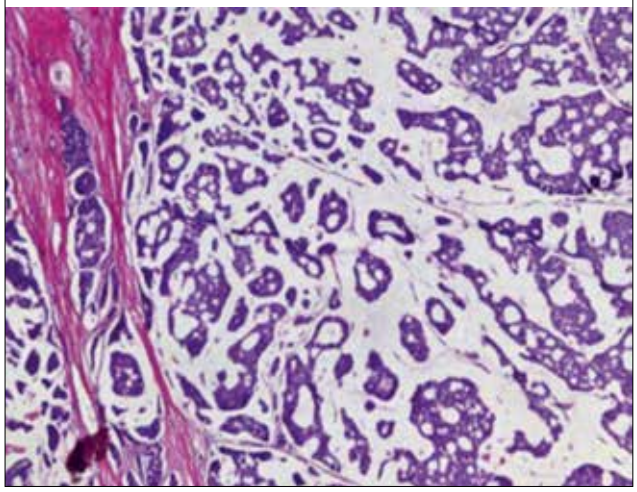


Figura 2 HE/HE 5x.



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## A RARE CASE OF DERMATOMYOFIBROMA

E. Unti, S. Giambalvo, N. Scibetta

*UOC Anatomia Patologica, ARNAS Ospedale Civico Di Cristina Benfratelli, Palermo*

**Introduction** and objectives. dermatomyofibroma is a rare benign neoplasm of skin derived from myofibroblasts, which was first described by Ugel in 1991 as "plaque-like dermal fibromatosis" in a series of 25 patients, then in 1992 by Kamino in a series of 9 cases. The neoplasm mainly affects young women, with an average age of 31 years, and is located predominantly in the skin of the shoulders, even if several other skin sites have been reported. Clinically, it is represented by a flat, oval, reddish-brown neoformation of firm, non-painful, palpation consistency, of dimensions between 1 and 2 cm. We report this case for its rarity.

**Materials, Methods and Results.** here we report the case of a 65-year-old woman who was examined at the Dermatology Clinic of our Hospital for a skin neoformation of the posterior region of the right shoulder, non-painful ,that had arisen for a year and did not change over time. The patient did not report trauma in that location, nor other concomitant diseases.

Clinical observation showed a flat lesion, of cm 1, of a elastic consistency, reddish-brown, which was removed surgically. Histologically the reticular dermis was the site of a proliferation of fused cells with eosinophilic cytoplasm and regular nuclei with one or two nucleoli, devoid of mitosis, arranged in bundles parallel to the epidermis, interspersed with connective fibers. The elastic fibers highlighted with trichrome by Van Gieson were intact. There was a slight scattered lymphocytic infiltrate of the dermis. The overlying epidermis looked thinner.

Immunohistochemically, the cells were positive for vimentin, smooth muscle actin, calponin, CD34, negative for desmin, CD117, HHV8, S100, ER, PGR, CD68 (PGM1). Based on these findings, the diagnosis of dermatomyofibroma was made. The patient was clinically reevaluated one year after surgical removal of the lesion and no local recurrences were noted.

**Conclusions.** dermatomyofibroma is a rare pathological entity, of which about 100 cases have been reported in the literature to date. The treatment consists in a simple excision. No local recurrences or metastases have ever been reported. Clinically it must be distinguished from dermatofibroma, scar, keloid, leiomyoma cutis, neurofibroma, granuloma annular. Histologically it shows a poorly defined proliferation of fibroblasts and myofibroblasts with positive immunophenotype for intermediate myogenetic filaments. Dermatofibroma is usually characterized by marked acanthosis and epidermal hyperplasia of the overlying epidermis, and the tumor cells are negative for SMA. The fused cells of the neurofibroma are positive for S100 and negative for SMA. The leiomyoma cutis cells have a pink cytoplasm and elongated nuclei with blunt ends, positive for SMA. We report this case as well as for its rarity, also to underline that if the site and the clinical aspect of dermatomyofibroma often allow to suspect it, the diagnosis must be confirmed by the peculiarity of the immunomorphological picture, to exclude skin diseases of a different nature.

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# EMATOPATOLOGIA

## AN UNUSUAL EPSTEIN-BARR VIRUS - ASSOCIATED NEOPLASM OF THE SPLEEN

A. Maglietta[1], C. Martella[2], M. Federico[3], L. Resta[1], G. Ingravallo[1]

*[1] Department of Pathology, University of Bari; [2] Laboratory P. Pignatelli Lifebrain, Lecce; [3] Department of Oncology, Haematology and Respiratory Disease, University of Modena and Reggio Emilia*

We report a morphologic variant of splenic Epstein-Barr virus (EBV)-associated inflammatory pseudotumor-like (IPT-like) follicular dendritic cell (FDC) tumor. To date, little more than 30 cases of splenic IPT-like FDC tumor have been reported in the English-language literature. Very rare cases of IPT-like FDC tumor have been identified outside the spleen or liver.

A 67 years old man without symptoms was found to have avascular, solid masses of the spleen with SUVmax of 6,2 measured by [$^{18}$F]FDG PET/CT. Blood chemistery tests showed high value of antibodies (IgG) against Epstein-Barr virus (EBV). The lesions were compatible with a primary tumor of the spleen. Laparoscopic splenectomy was performed to enable a definite diagnosis.

Grossly, the spleen misured 15x7x7 cm, the multiple splenic masses were well circumscribed, with a variable diameter from 7 to 25 mm. They often showed homogeneous yellow or grayish white cut surfaces. Histologically, the masses were well delineated from the surrounding parenchyma, with a fibrous pseudocapsule. There were numerous noncaseating epithelioid granulomas distributed throughout the lesion. The granulomas were composed of epithelioid histiocytes, mixed with occasional Langhans giant cells. Necrosis was present focally. The areas between the granulomas were densely populated by small lymphocytes, eosinophils, plasma cells without formation of lymphoid follicles and aggregated spindly to ovoid cells, which sometimes exhibited a fascicular pattern. The latter cells had pale to eosinophilic cytoplasm with indistinct cell borders, elongated to oval nuclei with thin nuclear membrane, finely dispersed chromatin, and centrally located nucleoli. Mitotic figures, however, were rare. Special stains for bacteria, acid-fast bacilli, and fungi were all negative. The lesions harbored only small numbers of CD20+ B cells and moderate numbers of CD3+ T cells. A small numbers of the atypical spindle cells showed reactivity for CD21 and CD35. In situ hybridization for EBVencoded RNAs labeled the nuclei of spindle cells.

Our case represents a rare example of granulomatous and eosinophil-rich variant of a splenic IPT- like FDC tumor. More recently, six cases of this variant were described. It is important to be aware of the granulomatous and eosinophil-rich variant of this tumor type mimicking reactive or infective conditions to facilitate the correct diagnosis.

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## GINGIVAL HYPERPLASIA AS CLINICAL MANIFESTATION OF A THERAPY-RELEATED ACUTE MYELOMONOCYTIC LEUKEMIA (T-AML) ARISING IN PATIENT WITH AFFECTED BY NON-HODGKIN DIFFUSE LARGE B-CELL LYMPHOMA (NHL-DLBCL) TREATED WITH R-CHOP THERAPEUTIC SCHEME

I. Panarese, A. Ronchi, I. Cozzolino, G. Toni, R.L. Sabetta, G. Ferrara, R. Franco

*Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università della Campania "Luigi Vanvitelli", Naples, Italy*

**Objectives.** Acute myeloid leukemia is malignat hematological neoplasia, characterized by clonal proliferation of myeloid blasts in the bone marrow and peripheral blood. Acute myelomonocytic leukaemia is an acute hematological neoplasia. The proliferation of both neutrophil and monocyte precursors is observed in this leukaemia. The bone marrow or peripheral blood contains at least 20% of blasts. In addition to their precursors, also monocytes and neutrophils are at least 20% of bone marrow cells. Acute myelomonocytic leukaemia can be an exemple of therapy-related acute myeloid leukaemia (t-AML). T-AML is considered a late complication of radiation therapy or chemotherapy. Usually the period of time between treatment of the primary disease and the onset of the t-AML is several months or years, on average 4 years, and appears to be related to the cumulative dose and type of chemotherapy or radiotherapy. In t-AML typical cytogenetic abnormalities are: t(9;11), complex karyotipes, -7 or 7q-, -5 or 5q-. Acute myelomonocytic leukaemia and acute myeloid leukaemia in general may be shown with oral signs as gingival hyperplasia. Gingival hyperplasia is caused by the infiltration of neoplastic cells and is an early manifestation of neoplasia. It is more common in acute myeloid leukaemia and in subtypes of this, for example acute myelomonocytic leukaemia, than in other types of leukaemia.

Diffuse large B-cell lymphoma is the most common subtype of non-Hodgkin lymphoma. It is an aggressive but curable lymphoma. The standard of care is rapresented by chemotherapy regimen R-CHOP. This combination includes: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

**Materials and Methods.** We submit a case of a 58 years-old female patient with a diagnosis of Non-Hodgkin Diffuse Large B-Cell Lymphoma (NHL-DLBCL) in 2010, treated positively with R-CHOP therapeutic scheme.

In 2016 the patient showed a diffuse gingival hyperplasia and high white blood cell counts in the peripheral blood. Therefore, a biopsy sampling of gingival mucosae was performed. We analyzed a greyish fragment of oral mucosa of 8 mm of diameter. The microscopic evaluation of the sample showed a slightly hyperplastic squamous epithelial layer and a massive infiltration of the chorion by a neoplastic proliferation, with diffuse growth pattern, composed by large cells with eosinophilic cytoplasm and round nuclei, with finely dispersed chromatin and prominent nucleoli. Occasionally cells with distorted nuclei and nuclear folds can be seen.

In the first instance the hypothesis of a relapse of the lymphoma in the anamnesis was evaluated, but the immunophenotype for the lymphoproliferative disease gave a negative result. On the basis of the serological data, the hypothesis of a leukemia was taken into consideration and an immunohistochemical panel containing stem and myeloid markers (CD68 +, lysozyme +, myeloperoxidase +, CD4 +, CD34-, CD117-) was performed.

**Results and Conclusions.** We report a case of a patient affected by a gingival hyperplasia and marked systemic leukocytosis and positive history of Non-Hodgkin lymphoma treated with R-CHOP scheme. For this reason, a recurrence of the previous haematological disease has been hypothesized, but the data in our possession have confirmed the hypothesis that it was a gingival localization by an acute myelo- monocytic systemic leukemia, probably related to the previous cytotoxic therapy against lymphoma.

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## A CASE OF BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM (BPDCN) ONSET WITH SPONTANEOUS SPLENIC RUPTURE

E. Unti, N. Arena, C. Rizzo, N. Scibetta

*UOC Anatomia Patologica, ARNAS Ospedale Civico Di Cristina Benfratelli, Palermo*

**Introduction** and objectives. BPDCN is a rare and aggressive haematological neoplasm that originates from a clonal proliferation of dendritic plasmocytoid cells, professional type I interferon producing cells. It is classified as a distinct entity among myeloid neoplasms in the 2016 revision of WHO Classification of tumors of Haematopoietic and Lymphoid Tissues. It tends to be a disease of older men. In about 90% of cases the onset of the disease is indolent, with multiple skin lesions and subsequent systemic involvement, while in about 10% of cases the disease begins with an acute leukemia accompanied or not by skin lesions. BPDCN is characterized by a widespread infiltration of immature lymphoblastic or myeloblastic-like cells, without lineage specific antigens and with a peculiar immunophenotype (CD4 +, CD56 +, CD123 +, TCL-1 +, CD 303 +).
We have reported a rare case of BPDCN, arising in a woman, characterized by splenomegaly, lymphadenopathy, bone marrow infiltration, without skin involvement.
**Materials, Methods and Results.** A 54–years old woman referred to our hospital for ten days history of pain to left hypochondrium, and an anemic aggravating condition. She did not refer previous pathologies or surgical interventions, nor recent traumas.
Ultrasounds and the CT scan showed splenomegaly and abdominal lymphadenomegaly, no cerebral involvement and hepatomegaly. Laboratory tests showed cytopenia, particularly anemia. There were not skin lesions.
Urgent splenectomy was performed.
The spleen, of 25x17x4 cm, showed numerous solutions of continuous, and capsular and intraparenchymal haemorrhagic spillages too.
On histological examination, the white pulp was extremely reduced, due to the abnormal expansion of the red pulp, which was widely infiltrated by small-medium sized, immature, monomorphic cells, with irregular nuclei and poor cytoplasm.
By immunohistochemistry the cells express CD56, CD4, CD123, CD7, CD33, CD43, CD 68-PGM1, CD 45RA , CD10 and they did not express CD20,
CD3, CD1a, CD23, CD34, CD25, CD138, CD117, TdT. EBV antigens were not found.
Bone marrow biopsy showed a markedly hypercellular marrow (90%), with analogous cells CD4+, CD 43+,CD 45+, CD 56+, CD123+, TdT-, CD 117-, CD34-, CD20-, CD3-.
The immunophenotype detected with flow-cytofluorimetry highlighted the lack of lineage-ssociated antigens, together with the expression of CD4, CD45RA, CD56, CD123, CD38, HLADR. Conventional cytogenetic analysis of bone marrow samples was performed revealing normal karyotype.
As a result of this, a blastic plasmocytoid dendritic cell neoplasm diagnosis was made.
The patient underwent cycles of ALL/lymphoma–type chemotherapy (HyperCvad), intrathecal prophylaxis, with excellent response.
She is currently in remission, six months after therapy, and waiting for allogenic hematopoietic stem cell transplantation (HSCT).
**Conclusions.** We have reported a rare case of BPDCN, arising in a woman, characterized by splenomegaly, lymphadenopathy, bone marrow infiltration, without skin involvement.
The peculiarities of this case consist precisely in the unusuality of the symptomatology at the onset (spontaneous rupture of the spleen).
The diagnosis of blastic plasmocytoid dendritic cell neoplasm was therefore based on the immunophenotype (CD4+, CD56+, CD123+) and on the cytofluorimetric data showing the expression of CD4, CD45RA, CD56 and CD123 and the absence of lineage-associated markers.
The double negative subset (CD34 e CD117) with extra medullary involvement , as in the case of our patient, has been defined as the “mature” one. Furthermore, in our case the CD10, unlike what is often reported, was positive. The CD 10 antigen is expressed by early pre B cells and T/NK cells precusors and IT is lost during lymphoid differentiation. This data, together with the involvement of lymphoid tissue, may not guide the diagnosis, in the absence of cytofluorimetric data and immunohistochemical characterization.

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# GINECOLOGIA

## PRIMARY HIGH-GRADE ENDOMETRIOID STROMAL SARCOMA OF THE OVARY: A CASE REPORT AND BRIEF REVIEW OF LITERATURE

A. D'Amuri[1], F. Floccari[2], G. Marzo[3], A. Morciano[3], M. Filotico[1]

*[1] Anatomic Pathology Unit, "Card. G. Panico" Hospital, Tricase (LE), Italy; [2] Clinic Pathology Unit, "Camillo De Lellis" Hospital, Manfredonia (FG), Italy; [3] Obstetric and Gynecology Unit "Card. G. Panico" Hospital, Tricase (LE), Italy*

**Introduction.** A 50 years old woman has been suffering from abdominal pain and intestinal transit disorders for several months. An abdominal ultrasound shows the presence of a mass at the right half of the abdomen. At the surgery, there is a mass in correspondence of the right ovary. The patient undergoes bilateral hystero-salpingo-oophorectomy.

**Material and Methods.** The surgical sample of the right ovary consists of a mass of 17 x 16 x 9 cm. The sectioned surface presents cystic areas with citrine liquid content alternating with areas of solid tan yellow appearance. The left ovary and the uterus do not show significant macroscopic alterations. Several fragments are taken in various areas of the right ovarian tumor mass. Fragments are taken from the left ovary, fallopian tubes, cervix, and the body of the uterus. The material was fixed in formalin and in paraffin embedded. The sections were colored with H&E and subjected to a panel of antibodies for immunohistochemistry. The normal structure of the ovary was no longer recognizable. Wide bands of fibrous tissue reminiscent of ovarian fibroma, delimit irregular areas in shape and size. In the context of these areas, it is present. a massive proliferation of roundish, small mononuclear cells. The elements are aggregated in a diffuse manner, usually compact, sometimes with aspects of a honeycomb. The proliferation winds between the fibrous bands with tongue-like extensions. There is also an abundant vascular component of the arteriolar type reminiscent of the spiral arterioles of the endometrial stroma. The results of the immunohistochemical investigations are: CD10 +; vimentin +; PGR +; ER +; CKAE1/AE3 +/-; WT-1 +/-; SMA +/-; inibin -/+; EMA -; desmin -; Cyclin-D1 -; PAX-8 -; CD117 -; p53 -; Ki-67 >20%. The morphological pattern and immunohistochemical profile favor the diagnosis endometrioid stromal sarcoma high grade of the ovary. The absence of a similar lesion at the uterine level or elsewhere allows the term primary to be added to the diagnosis.

**Discussion and Conclusions**. Ovarian neoplasms presenting the morphological characters of the endometrial stroma has been reported since the 1960, and later in the early 80. Only in 1984, in a series of 23 cases studied by Scully et al. did the term Endometrioid Stromal Sarcoma of the ovary (ESS) appeared in the literature. Under this term in the article, are included both lesions with exclusively ovarian localization, but also those associated with a similar synchronous or metachronous uterine lesion. In this publication, the morphological characters of the lesions are defined as completely superimposable to those of the uterine counterpart. The largest primitive ESS series of the ovary, report the study of 27 cases and to our knowledge, represents, to date, the most detailed study on the subject. The WHO 2003 classification of Ovarian Tumors poses these neoplasms between the Surface Epithelial-Stromal tumors and precisely among Endometrioid tumors giving them the following definition: "Endometrioid stromal sarcoma (ESS) is a monophasic sarcomatous tumour characterized by a diffuse proliferation of neoplastic cells similar to stromal cells of the proliferative endometrium. At its periphery, the tumour exhibits a typical infiltrative growth pattern". The WHO 2014 classification includes these neoplasms between Mesenchymal Tumors, giving the following definition: "A Mesenchymal Tumour identical to low- grade uterine endometrial stromal sarcoma". In terms of nosographic precision, the definition of 2003 seems to us more appropriate as the endometrial stroma is a Mullerian derivative which, in turn, originates from the celomatic mesoderm and not from the mesenchyme which, as is known, is that part of the mesoderm from which originate the connective tissues, bone, cartilage, blood vessels, and lymphatics and not the organs of the urogenital system. In a series of 20 primary extrauterine ESS we can see how the case series is distributed on all the organs of the celomatic area: ovaries, salpinges, pelvic cavity and abdominal wall. The same area of distribution of endometriosis. An association with endometriosis has been reported in about 50% of cases. But for this, a mandatory interrelationship between the two phenomena cannot be inferred. The extensive and detailed description of the morphologic pattern of these lesions reported in the aforementioned publication can be summarized as follows: the most frequent pattern is that of a widespread proliferation of small roundish elements with scant cytoplasm, closely packed. The proliferation is often intersected by a band of fibrous tissue reminiscent of the ovarian fibroma. Sex cord differentiation is present in about 25% of cases. Smooth muscle differentiation is present in about 20% of cases. The characteristic tongue-like infiltration and intravascular penetration are rarely seen in ovarian localization. Small vessels with the spiral arterioles characters of the proliferative phase, with an often dilated lumen and curvilinear trend (low-grade fibromixosarcoma-like), are disseminated in the proliferation. The recommended immunohistochemical panel is comparable to that adopted for similar uterine neoplasms : CD10 +; SMA +; desim -; H-caldesmon - or +; PGR +; ER +; vimentin +; WT-1 +; AR + or -; calponin + or -; βCatenin + or -; CKAE1/AE3 - or +. From the biomolecular point of view, there are two subsets of ESS, JAZF1-SUZ12 or equivalent genetic rearrangements and another characterized by YWHAE-FAM22 genetic fusion histologically of higher grade and clinically more aggressive. This second subset expresses consistently Cyclin D1, with coexistent negativity for CD10 and PGR. A wide range of primitive and secondary ovarian lesions (fibroma, thecoma, fibrosarcoma, mesodermal adenosarcoma, metastatic GIST) should be placed in differential diagnosis of these lesions, which in addition to being particularly rare, may contain aspects related to other entities (sex cord, thecoma) of the neoplastic ovary pathology. A recent survey of 14 patients yielded the following results. The average age of patients is around 49 years with a median of 51. Nine (64%) was classifiable as low-grade and 5 (36%) as high-grade. After a 65 months F.U., all low-grade Pts were alive. 33% of them had developed a relapse. Of high-grade Pts was alive without recurrence only 1 Pt, the other 4 develops relapses and two died due to the progress of the disease. The case observed by us can rightly be numbered in the rare case of this neoplasm that according to a recent review of the literature amounts to, to date, less than 100. In fact, the morphological parameters are respected both as regards the cellular morphology and its organization, the tongue-like character of the infiltration, the vascular component with the characteristic appearance of the spiral arterioles, and the fibrous component with the aspects of the ovarian fibroma. Immunohistochemistry showed diffuse and intense positivity for Vimentin, CD10, PGR, ER, focal and sporadic for CK, positive SMA on the level of the muscular

tunics of the vessels, even just sketched out, WT1 is weakly positive in scattered elements while exhibiting strong positivity in the muscular tunics of the spiral arterioles. Scattered elements from fibroblastic morphology exhibit positivity for inibin in the context of the fibrous bands. The Ki67 proliferation index stands at values below up of 20%. The morphologic pattern and the immunohistochemical profile suggest considering this case as a high grade.

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## mtDNA AND TYPE I ENDOMETRIAL ADENOCARCINOMA. OUR EXPERIENCE

L Duda[1], G Cormio[2], A Cormio[3], V Loizzi[2], T Lettini[1], R Rossi[1], L Resta[1]

*[1] Department of Pathology, University of Bari; [2] Department of Biomedical Sciences and Medical Oncology, University of Bari; [3] Department of Biosciences, Biotechnologies e Biopharmaceutics, University of Bari*

In the last years several researches studied the possible relationships between germline and somatic mutations of the mtDNA and the cancerogenesis and the progression of the human tumors. We studied in particular the mtDNA in endometrial type I adenocarcinoma.

In a general point of view, the total content of mtDNA and the CS (citrate synthase) activity may be considered as a good marker of the transition from proliferative, hyperplastic, atypical and carcinomatous endometrium. The content of mtDNA increases by hyperplastic endometrium, while the increase of CS activity (considered as a marker of the mitochondrial mass) is significant by atypical endometrium. The two markers do not correlate with the tumoral prognostic factors (grade, stage, myometrial invasion), probably because the high proliferation rate in more aggressive neoplasms reduces the number of mitochondria per cell and because in the high grade cancer the estrogen receptors are absent [1].

In the comparison between type I endometrial carcinoma samples and proliferative endometrial tissues we found in the neoplastic tissue an upregulation of the signaling pathway of the PPAR- coactivator-1 alpha (PGC-1a), the major regulator of mitochondrial biogenesis and its main cofactors (nuclear respiratory factors 1, NRF-1; mitochondrial transcription factor A TFAM) [2].

In the transition from endometrial hyperplasia to type I adenocarcinoma the mtDNA mutation was found in 69% of patients, whereas the nuclear genes were mutated in 56% of cases. Our hypothesis is that the mitochondrial mutations precede the nuclear mutations: the elevation of the ROS induced by the mtDNA mutation may damage the nuclear DNA by the consequent genetic instability and causes the tumor development. Among the genes evaluated, the PTEN gene seems to be precociously involved in 39% of cases. The eventual mutations of KRAS, TP53 and CTNNB1 genes were found in the neoplastic samples and not in hyperplastic ones, as witness of a late possible mutation [3].

The estrogen stimulation may induce some mtDNA mutation in 2 ways: a) increasing the mitochondrial ROS (reactive oxygen species) and consequently DNA damage; b) stimulating mitochondrial biogenesis with excessive DNA replication and possibility of error in the polymerase function. The latter mechanism seems to be the most frequent phenomenon [4].

The gene mutation of mtDNA may help the clarify the origin of tumors in patients with a contemporary endometrial and ovarian endometrioid neoplasia. If the same mutation is present in the uterus and in the ovary, it is very probably that the tumor has an unique source in the same clonal proliferation and the ovarian cancer is a metastasis of the endometrial cancer. If the mutation of mtDNA is different, a contemporary and independent origin of the two cancer is more probably [5 6].

Recently, the mitochondrial Complex I (CI) has been evaluated nondenaturing Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) and its activity has been reduced in adenocarcinatous samples as respect to control tissue. The western blotting analysis confirms that the reduction was correlate with the CI amount decrease. Our hypothesis about the progression is: mtDNA mutation, loss of CI, “oncocytic-like” transformation, neoplastic progression [7].

In conclusion, our research and other similar in literature permit to formulate the following hypothesis about the mitochondrial disfunction in type I endometrial cancer: “Hyperestrogenism may stimulate reactive oxygen species (ROS) production and increase mitochondrial biogenesis. ROS increase and excessive mitochondrial DNA (mtDNA) replication due to increased mitochondrial biogenesis may lead to mtDNA mutations. These mutations may affect respiratory complexes, in particular complex I, and may induce mitochondrial dysfunction that reinforces ROS production and stimulates mitochondrial proliferation in a vicious cycle. ROS increase activates the antioxidant response. Mitochondrial dysfunction may also increase mitochondrial fission in order to segregate damaged mitochondria components, which can then be degraded by proteolysis and mitophagy” [8].

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## STUDY OF THE ROLE OF GENE-GENE INTERACTIONS RS2013573, RS1079866 AND RS11031010 IN THE FORMATION OF ENDOMETRIAL HYPERPLASTIC PROCESSES

I.V. Ponomarenko[1], E.N. Krikun[2], M.I. Churnosov[1], R.F. Kapustin[3]

*[1] Belgorod National Research University, Department Biomedical Sciences, Belgorod, Russia; [2] Belgorod National Research University, Belgorod, Russia, Medical College; [3] Belgorod State Agrarian University Named After V.J. Gorin, Department of Morphology and Physiology, Maiskii, Russia*

Hyperplastic processes of the endometrium are characterized by proliferation of predominantly glandular tissue, which increases the ratio of the gland / stroma compared with the normal endometrium [1-5]. The aim of the study was to study the role of the combinations of rs2013573, rs1079866 and rs11031010 genes in the formation of endometrial hyperplastic processes among the population of the Central Chernozem Region of Russia. Material and methods: The study group consisted of 1501 individuals: 520 patients with hyperplastic endometrial processes and 981 women in the control group. Samples of patients and control included women of Russian nationality who are born in the Central Chernozem region of Russia and are not related to each other. The material for the study was venous blood in a volume of 6 ml, taken from the ulnar vein of the proband. Isolation of genomic DNA from peripheral blood was carried out by phenol- chloroform extraction. Polymorphism was studied by polymerase chain reaction using appropriate primers and probes on an IQ5 amplifier. Results: Genotyping of three molecular- genetic markers: rs2013573, rs1079866 and rs11031010. When studying the frequency distribution of genotypes at the studied loci among patients and in the control group, it was found that the Hardy-Weinberg equilibrium is satisfied for them (p>0.05). It was found that the combination of alleles A rs2013573 with G rs1079866 and A rs11031010 was registered among patients with hyperplastic endometrial processes (4.80%) statistically significantly less than the control group (9.22%, p = 0.001, OR = 0.50, 95% CI 0.31-0.79 ). Conclusions: Among women in the Central region of Russia, the combination of molecular-genetic markers A rs2013573 with G rs1079866 and A rs11031010 is a protective factor in the development of endometrial hyperplastic processes (OR = 0.50).

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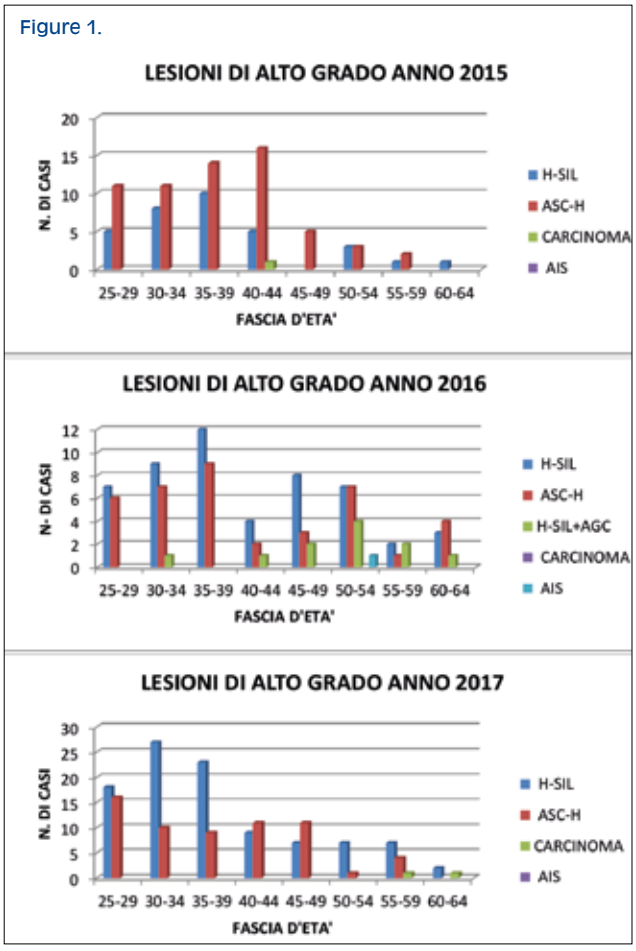
## HIGH- GRADE SQUAMOUS INTRAEPITHELIAL LESIONS IN YOUNG WOMEN

S. Stella[1], M. De Santis[1], D. Fuzio[1], M. Iacobellis[2]

*[1] Byologist; [2] Pathologist, Cytology-Screening Unit, P.O. Di Venere-ASL BA, Bari, Italy*

**Background.** There is an international discussion about the best age for the beginning of cervical screening whit HPV primary test. Even if our Regional Administration decided to begin at 30 years old, we have considered interesting the data of our screening in young women between 25- 30 years old.

Figure 1.




**Materials and Methods.** Figure 1.

**Discussion.** The theories about infection, viral persistence and progression are known (Figs. 2, 3).

**Conclusions.** This study is interesting because there is some more to know about progression concept, and probably sometimes there is a direct passage from infection to high grade lesions, without progression through a low grade lesion. Probably we have to genotyping the samples of this patients to know something more. Furthermore we have to give a special attention recruitment in screening of young women.

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Figure 2.

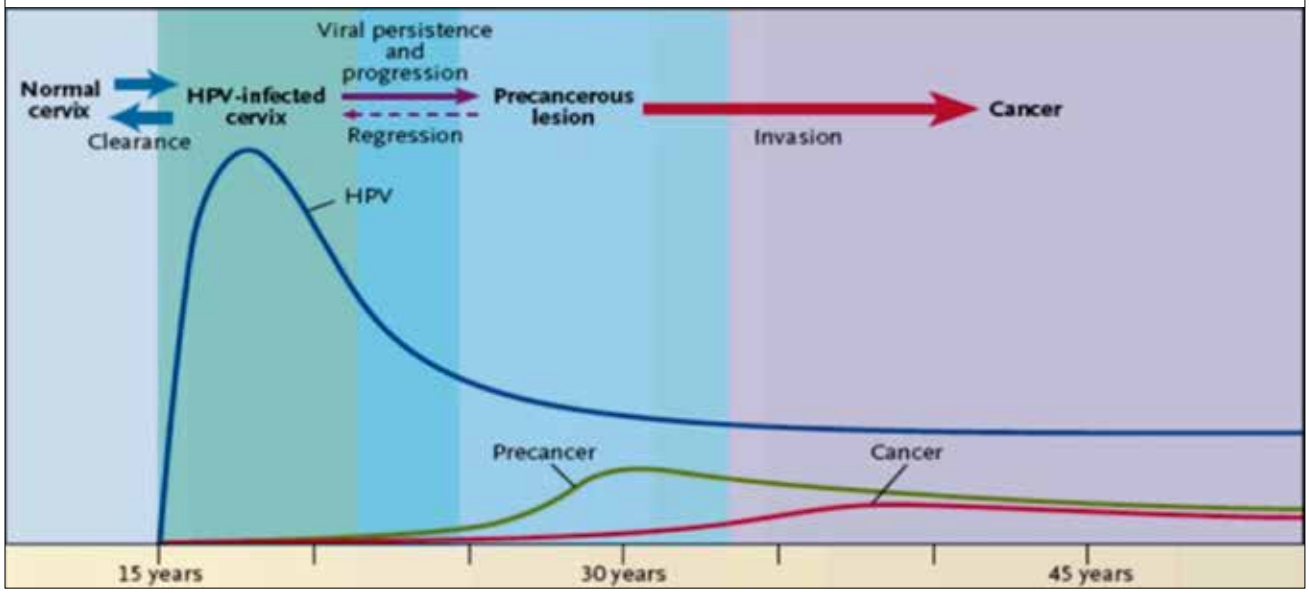
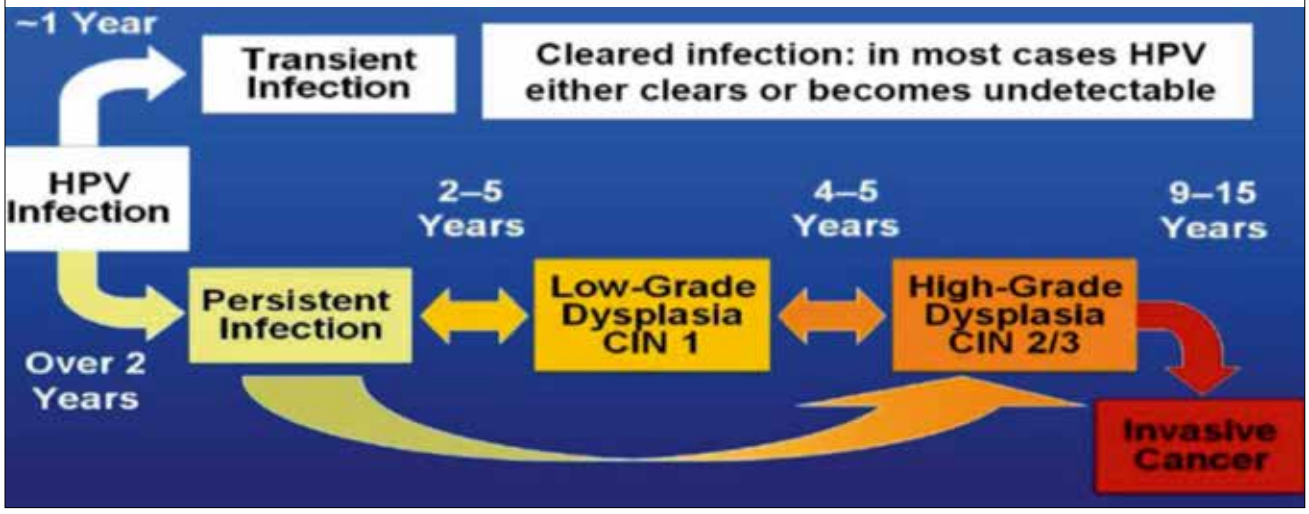



Figure 3.

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## OVARIAN CLEAR CELL CARCINOMA WITH YOLK SAC COMPONENT: REPORT OF A CASE

S. Tramontozzi[1], A. De Leo[1 2], D.D. Di Nanni[1], N.C. Salfi[1], D. Rubino[3], Anna M. Perrone[4], D. Santini[1]

*[1] U.O. Anatomia e Istologia Patologica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia; [2] Dipartimento di Medicina specialistica, diagnostica e sperimentale, Università di Bologna, Bologna, Italia; [3] Oncologia Medica Addarii, Policlinico S.Orsola-Malpighi, Bologna, Italia; [4] Oncologia Ginecologica, Policlinico S.Orsola-Malpighi, Università di Bologna, Bologna, Italia*

**Go/Background.** Yolk sac tumours (YSTs) of the ovary generally occur in childhood, adolescence and early adult life, and are rare over age 40. Recently, some studies have reported singular cases of YST of the female genital tract in postmenopausal women, associated with a somatic epithelial neoplasm. It has been supposed that the YSTs probably derive from the epithelial neoplasm through a process which has been referred to as neometaplasia or retrodifferentiation. The term 'somatically derived YSTs' has been proposed for these uncommon neoplasms.

Herein, we report the clinico-pathological and immunohistochemical characteristics of a rare case of clear cell carcinoma associated with YST component.

**Materials and Methods.** Clinical charts, radiological and histopathological findings of the patient were reviewed, and the morphological and immunohistochemical features were discussed and compared to those of similar reported cases.

Routine immunohistochemistry was performed on sections cut from formalin-fixed, paraffin-embeeded blocks with the following antibodies: cytokeratin 7 (CK7), paired box gene 8 (PAX 8), alfa-fetoprotein (AFP), hepatocyte nuclear factor

β (HNF1-β), napsin-A, Wilms tumour 1 (WT1), glypican-3 (GPC-3), progesterone receptor (PR), oestrogen receptor (ER), tumour protein 53 (p53), caudal type homeobox 2 (CDX2), GATA binding protein 3 (GATA 3), and hepatocyte paraffin 1 (HEP-PAR 1). Mismatch repair (MMR) protein expression (MLH1, MSH2, MSH6, and PMS2) were also evaluated.

**Results.** A 68-year-old postmenopausal woman presented with abdominal swelling and a pelvic mass. Ultrasound and Computed tomography (CT) examination found a solid and cystic mass (cm 16 x 11), with thick septa and without signs of carcinosis, suggesting an ovarian origin. At that time, her preoperative serum tumour marker levels were significantly high (CA19-9: 100 U/ml, CA125: 434 U/ml). The patient underwent total hysterectomy with bilateral salpingo- oophorectomy, omentectomy and lymphadenectomy. Gross examination showed a multicystic ovarian mass measured cm 18 in greatest dimension, adherent to the posterior uterine wall, with involvement of both fallopian tubes. Microscopically, the ovarian tumor was composed by clear vacuolated cells with hyaline globules and tubule-papillary patterns that were consistent with clear cell carcinoma. Admixed to conventional clear cell carcinoma, some areas of the tumour showed the presence of tall cylindrical cells with regular nuclei, polarized apical or subnuclear vacuoles, occasional goblet cells and subepithelial stromal rarefaction at the periphery that were suggestive for YST. Furthermore, multifocal aspects of mucinous differentiation were also present. Given the morphological overlap between the clear cell and glandular YST, a detailed immunohistochemical panel was performed to define the two components.

Interestingly, clear cell carcinoma was positive for CK7, PAX 8 (di?use in epithelial component and focal in YST), WT1, HNF1-β, napsin-A, while YST glandular areas had a heterogeneous expression AFP, GPC-3, GATA 3, and HEP-PAR 1.

Conclusion. Epithelial carcinomas associated with germ cell tumor are rare entities and constitute a challenging diagnosis for pathologists. A YST component should be suspected in tumours with an unusual clear cell morphology and a heterogeneous immunohistochemical profile. Furthermore, these neoplasms with YST component represent an aggressive disease, less sensitive to chemotherapy than de novo YST. Because of its prognosis, complete surgical staging and aggressive systemic therapy must be considered in an attempt to improve disease outcome. Future studies are needed to delineate the optimal systemic approach in the ?rst-line and salvage settings.

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# IMMUNOISTOCHIMICA

## IMMUNOHISTOCHEMICAL EXPRESSION OF PD-L1 IN NON SMALL CELL LUNG CANCER: A SINGLE INSTITUTION EXPERIENCE

M. Sonnessa[1], R. Sozio[1], D. Loisi[1], G. Giannone[1], R. Daprile[1], F. Colonna[1], R. Tinelli[1], G. Cristiani[1], C. Fasano[2], D. Galetta[3], P. Sardelli[4], F.A. Zito[1] , M.A. Botticella[1]

*[1] UOC Anatomia Patologica, IRCCS-Istituto Tumori "Giovanni Paolo II"; [2] UOC Anatomia Patologica, "Vito Fazzi" Lecce; [3] Oncologia Medica per la Patologia Toracica, IRCCS-Istituto Tumori "Giovanni Paolo II"; [4] Chirurgia Toracica, IRCCS-Istituto Tumori "Giovanni Paolo II"*

**Introduction.** Immunotherapy with checkpoint inhibitors is becoming a new standard of treatment for advanced non-small cell lung cancer (NSCLC). Some reports on high-profile clinical trials have shown the association of PD-L1 expression by immunohistochemistry (IHC) with higher overall response rates to the PD-1/PD-L1 axis blockade suggesting that PD-L1 expression may serve as a predictive marker, but also advantage in overall survival and in PFS [1].

To address the issue it is investigated the immunohistochemical PD-L1 expression, using commercial Clone 22C3 on the Omnis Dako Platform, in patients with NSCLC of a large territory of Puglia corresponding to the provincial area of Bari and Lecce.

**Material and Methods.** A total of 417 primary tumor samples of NSCLC were evaluated for immunohistochemical PD-L1 expression, using Monoclonal Mouse Anti-Human PD-L1, Clone 22C3, Isotype IgG1. The sample were consecutively obtained from patients who underwent surgical resection or bronchial biopsy at "Giovanni Paolo II" Cancer Institute of Bari and "Vito Fazzi Hospital" of Lecce between September 2017 and June 2018.

A minimum of 100 viable tumor cells present in the PD-L1 stained slide was considered adequate for PD-L1 evaluation.

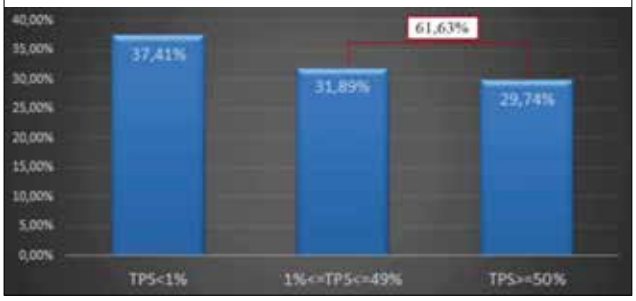
For staining procedure, the optimal condition on Dako OMNIS Platform, provide a 1:30 dilution factor in Dako Antibody Diluent. The following incubation time and temperature for Primary Antibody, Epitope retrieval and marked polymer are those expected by NordiQC protocol (NordiQC protocol 12 Dec 2017).

Tonsil is used as a positive control, and following the protocol described above, at 20X magnification, it is already possible to observe the epithelium of the well colored tonsillar crypts. Each staining run contained a positive control. Following the standard recommendation, PD-L1 expression is determined by TPS (Tumor Proportion Score) and classified into TPS <1% (negative), TPS 1 to 49% and TPS ≥ 50%.

**Results.** A total of 417 tumor samples were examined, of them 251(60,19%) were AC and 111(26,62%) were SCC. The majority of samples were retrieved from 335 (80,28%) male patients and from 82(19,72%) female. The mean age at diagnosis was 67 years.

The results of diagnostic assays revealed that expression of PD-L1 scores of TPS ≥ 50% , TPS 1 to 49% and TPS <1% were observed in 29.74% , 31.89% and 37.41% of the 417 archival samples, respectively (Fig. 1) .

Figure 1.




Using PD-L1 TPS ≥ 1% as the cut off, PD-L1 expression were classified into positive (TPS ≥1%) and negative (TPS <1%) groups [2].

The level of PD-L1 positive expression was compared in subgroups based on histology (adenocarcinoma or squamous carcinoma).

So with TPS ≥1%, 204 (48.92%) and 55 (13,19%) are respectively males and females.

Incidence of positive PD-L1 expression in squamous carcinoma tumors was 72 (64,86%) comparing to 156 (62,15%) in adenocarcinoma tumors.

**Conclusions.** Results achieved with 22C3 IHC assays, presented in this work, reveal that PD-L1 is expressed (TPS≥1%) in a significant proportion of NSCLC (61,63%). This value agrees with data reported in literature, for which in advanced NSCLC, the percentage of PD-L1 positive patients [Tumor Proportion Score (TPS) ≥ 1%] is found in around half of samples evaluated for PD-L1 [3].

**Some** reports comparing different PD-L1 IHC assays have been published and show similar results in terms of the PD-L1 staining performance using different clones (22C3, 28-8, SP263, E1L3N) and Platforms (Dako Omnis, Ventana Benchmark Ultra, Leica Bond III) with an almost 100% concordance rate for tumor proportion score (TPS) results [4 5].

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# PALEOPATOLOGIA

## CRANIAL INJURIES FROM 6TH CENTURY IN ALBA (CUNEO)

E. Fulcheri[1 2], F. Buffelli[2 3], A. Cinti[4], G. Minetti[5], R. Boano[4], V.G. Vellone[1], A. Bonsignore[6]

*[1] Histhopathology Unit, Dept. DISC, School of Medical and Pharmaceutical Sciences, University of Genova, Italy; [2] Fetal-Perinatal Pathology Unit, IRCCS-Istituto Giannina Gaslini, Genova, Italy; [3] Ph.D course in Paediatric Science, Fetal-Perinatal and Paediatric Pathology, University of Genova, Italy; [4] Department of Life Sciences and Systems Biology, School of Anthropology, University of Torino, Italy, [5] Radiology Unit, San Martino Teaching Hospital, Genova, Italy; [6] Legal and Forensic Medicine Unit, Dept. DISSAL, School of Medical and Pharmaceutical Sciences, University of Genova, Italy*

**Introduction.** Archaeological excavations carried out between 2007 and 2011 in the Cathedral of San Lorenzo (Alba, CN) have unearthed about 350 burials.

**Materials and Method.** Based on stratigraphic evidences and archaeological data it was possible to make a good chronological contextualization of anthropological material from 6th to 18th centuries, according to the different phases of use of the cemetery.

**Discussion.** The beginning of the cemetery use is documented by 16 tombs dated to the 6th century. Among these burials, one of the oldest (T 276) contains two male skeletons, placed in front of the entrance of the church. The typology of the tomb and its position, in a privileged place, suggest the hypothesis that it was destined to important persons in the society of that time. The younger individual (around 30-35 years old) shows signs of three dramatic unhealed perimortem cranial injuries to the left parietal and occipital bones explained as interpersonal violence in hand-to-hand combat. Sword has penetrated the bone in all its thickness with three different trajectories.

**Conclusions.** Side, direction and position of the wounds suggest that the person was struck while lying face down on the ground.

# PATOLOGIA APPARATO DIGERENTE, PANCREAS E FEGATO

## A RARE CASE OF SYNCHRONOUS MUCINOUS COLONIC ADENOCARCINOMA AND MULTIPLE SMALL INTESTINAL ADENOCARCINOMAS IN A YOUNG WOMAN

M.R. Campanino[1], A. Travaglino[1], A. Corvino[1 2], F. Corvino[3], L. Radice[1], F.P. D'Armiento[1]

*[1] Department of Advanced Biomedical Sciences, University of Naples "Federico II", Italy; [2] University of Naples "Parthenope", Naples, Italy; [3] Department of Vascular and Interventional Radiology, AORN "A. Cardarelli", Naples, Italy.*

**AIM.** Colonrectal cancer (CRC) is the third most common cancer in the United States [1]. Colon is the most common site for multiple primary malignant tumors. The reported incidence of multiple primary cancers in extracolonic sites among CRC patients range from 2.4% to 8.7%. In this regard, the most common extracolonic site of synchronous multiple primary cancers among CRC patients is stomach (Asia) and lung/breast (Europe) [2]. In reverse, malignant small intestine tumors are very rare, and they account for 0.1–0.3% of all malignancies and 1–3% of all gastrointestinal malignancies [3 4]. Synchronous cancers in the small and large bowel with different histological characteristics are rare. We present their occurrence in a young patient.

**Material and Methods.** A 29-years-old woman was admitted to the department of gastroenterology of "Federico II" Hospital of Naples-Italy with vaginal bleeding, changes in bowel habits, and moderate weight loss. Her past medical history was not otherwise significant. Her father died of colon cancer shortly after diagnosis at the age of 40. The other family members were healthy. Laboratory studies revealed an iron-deficiency anemia and abnormal liver function tests. Tumor marker tests showed elevated serum Carcinoembryonic antigen (CEA) and serum CA19-9 values and normal serum CA125 values. Ultrasound (US) scan of the abdomen showed the presence of bilateral adnexial complex big masses. Ovarian masses were characterized by anechoic cystic portions and hypoechoic solid components with pronounced vascularity at Color-Doppler and Power-Doppler examination. In addition, multiple well-defined, relatively homogeneous, hyperechoic nodular lesions of variable size suggestive of liver metastases were found in both lobes of liver. Therefore a liver biopsy was performed. Pathological examination of a biopsy specimen revealed a well-differentiated adenocarcinoma with cells arranged in a glandular acinar pattern and focal mucin production; however, the morphologic pattern was not specific to define the site of metastases origin. Immunohistochemical analysis, showing a citokeratin pattern CK20+, CK7−, CEA+, CDX2+ and CA125-, was instead helpful to suggest a primary tumour localization in the gastrointestinal (CRC). Computer tomography (CT) scan showed marked and asymmetric thickening of caecum and the proximal portion of ascending colon and a moderate distension of terminal ileum. Furthermore, CT views clearly depicted bilateral huge and oval- shaped ovarian masses with a complex internal structure and vivid enhancement of multiple intralesional solid portions and confirmed the presence of multiple hypovascular liver metastases. Following that, a full-length colonoscopy was performed which showed a stenotic malignant tumor almost totally obstructing the lumen of caecum and the proximal tract of ascendent colon, infiltrating the colonic wall. Surprisingly, at laparotomy, as well as the previously mentioned malignant growth in the large bowel, other three partially obstructing lesions in the ileum were discovered. In total, the cecum, the ascending colon, the hepatic flexure, the first one third of the transverse colon, and a long part of small bowel were resected. In addition, an extended regional lymphadenectomy, hysterectomy with bilateral salpingophorectomy, and a total omentectomy were performed. Histological examination revealed the colonic lesion was a mucinous adenocarcinoma of right colon with mucinous lakes found to be more than 50%. It was diffusely infiltrating full thickness the colonic wall with focal expansion into the surrounding mesenteric adipose tissue, locoregional lymphnode metastates and peritoneal. The histology of three ileal lesions, instead, were proved to be well differentiated adenocarcinomas of small bowel. Histologically these tumors were clearly different from that seen in the large bowel which showed abundant mucin production with formation of mucinous lakes. The histology of the bilateral ovarian tumors and nodules in the omentum was similar to that of the colon cancer.

**Conclusions.** This is a rare case of a primary adenocarcinoma of large bowel already with ovarian, hepatic, and peritoneal metastases developed "synchronously" with three primary adenocarcinomas of small bowel (ileum). This type of association has not been described yet in literature.

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## PD-L1 IN GASTROINTESTINAL SOLID TUMORS

A. Cavalcanti, F. Ignazzi, A.M. Dipinto, F. Valentini, S. Coletta, F. De Michele, M.L. Caruso

*Department of Pathology, IRCCS Gastroenterologico "S. de Bellis", Castellana Grotte, Bari, Italy*

**Purpose.** The immune escape mechanisms of GI tumors is not yet well characterized. The aim of this study was to elucidate the roles of programmed cell death ligand 1 (PD-L1) in solid tumors of gastrointestinal tract.

**Methods and Results.** We investigated PD-L1 immunohistochemical expression in 68 neuroendocrine neoplasms (NENs) and 63 colorectal cancers (CRCs) and its correlation with grade, gender, primary site, histological type, lymph nodes status, MSI and peri-and intra-tumor immune cells. In particular the PD-L1 positivity rate in NEN and signal intensity are directly correlated ($p< 0.001$) with grade increase, in particular from NENs G1 to NENs G3. Therefore, high grade tumors are characterized by significant PD-L1 expression in both the tumor and infiltrating immune cells ($p<0.001$), reflecting an unfavorable environment for T cell-mediated tumor aggression. In CRCs the PD-L1 expression on neoplastic cells was associates with right sided tumors (13; 81%) and high grade, medullary histological type (10/11), MSI status (12; 75%) and marked tumor-infiltrating immune cells. Despite these results being encouraging, it is clear the most MSI CRCs do not respond to chemotherapy as well as the platinum and oxaliplatinum - based

chemiotherapy could not the best choice for the group togheter corresponde of GEP-NEN G2-G3. Therefore, there is an urgent need to develop novel and effective systemic therapies. In this era of personalized medicine using targeted biological agents, biomarkers predictive of response to therapy are central to treatment decision making. Moreover, the prognostic role of PD-L1 remains controversial, because of natural tumor heterogeneity, variability in the assays, different histological grade/type, and cutoff values rancing from 1% to 10% . The expected different PD-L1 expression in various systems of the human body (gastrointestinal, pulmonary, skin) need strict evaluation criteria for PD-L1 standardization.

**Conclusions.** In conclusion, PD-L1 might be a useful prognostic biomarker to approach immunotherapy treatment in solid GI tumor jet occur in melanoma, renal and lung cancer.

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## DIFFERENTIATED-TYPE EARLY GASTRIC CANCER : A STUDY OF MUC5AC AND CDX2 EXPRESSION

A. Cavalcanti, A.M. Ignazzi, F. Valentini, A.M. Dipinto, A. Panarese, M.L. Caruso

*Department of Pathology, IRCCS Gastroenterologico "S. de Bellis", Castellana Grotte, Bari, Italy*

**Aims.** Despite the gradual reduction of incidence and mortality over the years, carcinoma gastric remains one of the most common human cancers death in Europe. Gastric cancer (GC) is a heterogeneous disease with different phenotypes and varying prognoses and responses to treatmen. Histologically, GC cases are classified into two main types, the intestinal and diffuse types, on glandular structure presence, as described by Lauren, corresponding to the differentiated and undifferentiated types of Nakamura et al. Intestinal and diffuse GC types show distinct clinical characteristics and type-specific genetic and epigenetic alterations. This classification, although dated, is still widespread due to its immediacy and simplicity but is inadequate to recent chemotherapeutic and surgical approach. In particular the Lauren classification not distinguish intestinal or gastric glandular origin in the intestinal/differentiated type tumor. Recent advances in mucin immunohistochemistry have shown that Differentiated/Intestinal Type tumor may be subclassified into three groups based on their mucin phenotype: foveolar, intestinal and combined. We used a mucin (MUC5AC) and an ontogenetic (CDX2) antibody important in differentiation and development antibody for subclassification of Differentiated Type Early Gastric Cancer (DEGC).

**Methods and Results.** Paraffin-embedded specimens of 63 ECG tissues, collected from January 2009 to December 2017, were evaluated for MUC5AC and CDX2 to clarify the phenotypic expressions and correlated with clinicopathological characteristics. MUCA5C was defined as gastric foveolar phenotypic markers (G-type) , while CDX2 as intestinal phenotypic markers (I-type). The criteria for the classification in G and I-type mucin phenotypes were as follows: G-type included those in which 20% of cells were stained positively for gastric foveolar type markers showing negativity for intestinal markers and the opposite for I-Type. DEGCs that were stained positively for both G and I-type markers (combined) were classified as M-type, and those that were stained negatively for both were classified as N-type (Fig. 1). In particular in DEGCs we found 22 (68%) I–type, and 11 (32%) GI-type.

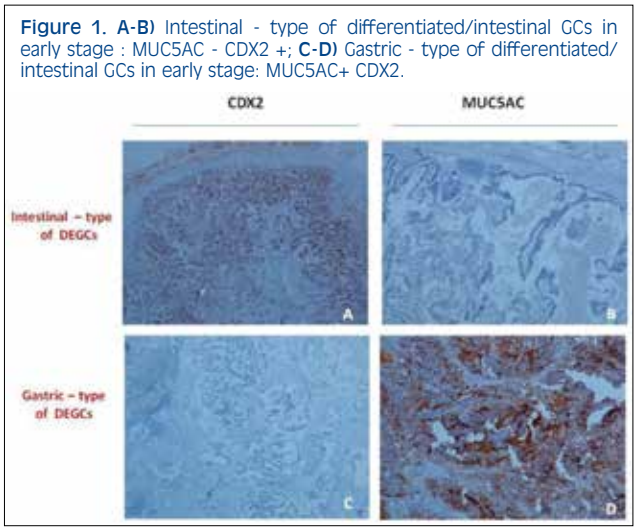


**Figure 1. A-B)** Intestinal - type of differentiated/intestinal GCs in early stage : MUC5AC - CDX2 +; **C-D)** Gastric - type of differentiated/ intestinal GCs in early stage: MUC5AC+ CDX2.

**Conclusions.** The important to discriminate these phenotypes is also due to the fact that early gastric foveolar-type differentiated adenocarcinomas is more aggressive, tend to be significantly larger tumors and exhibit higher rates of submucosal invasion than intestinal-type EGC which is reserved endoscopic treatment. Moreover incidence of gastric foveolar-type differentiated adenocarcinoma appears to be 7.9%-23.9% and closely associated to MSI status. Whereas CDX2 is expressed in the very early stage of gastric carcinogenesis, infact the loss of CDX2 occur as signal of tumor progression from early to advances GC that representing the shift to gastric phenotype expression. CDX2-positive GCs had a significantly better outcome than CDX2-negative GCs. In conclusion, it is important to discriminate this two phenotype for prognostic and therapeutic implications, CDX2 and MUC5AC were a sensitive markers for assessing Gastric or Intestinal phenotype in DECGs. The precise identification of G-foveolar type should be reflected guide line prognostic treatment in endoscopic mucosal resection.

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## IMMUNOHISTOCHEMESTRY PD-L1 DETERMINATION IN PAN-NENS

A. Cavalcanti, F. Ignazzi, A.M. Dipinto, F. Valentini, S. Coletta, F. De Michele, M.L. Caruso

*Department of Pathology, IRCCS Gastroenterologico "S. de Bellis", Castellana Grotte, Bari, Italy*

**Aims.** Neuroendocrine neoplasms (NENs) are rare, heterogeneous and ubiquitous tumors, commonly localized in the gastrointestinal tract, lung and pancreas. The clinical behavior of NEN is highly unpredictable, in fact, low grade cases can unexpectedly be associated with metastases. NENs have a common phenotype but vary in origin, morphology, function, molecular profile, aggressiveness, type, site specific prognosis and response to treatment. The last WHO NEN classification dated 2010 (previous 1980; 2000; 2004) is attempted to cope with these differences. Several new scientific, biomolecular and prognostic evidences, results of more recent studies (NORDIC RADIANT) and some considerations due to difficulties of correctly set some case demand a new system classification. In 2017, WHO published, exclusively for pancreatic tumors (PanNENs), a new classification where NENs is subclassified in well differentiated (NET) and poorly differentiated histology (NEC) only on the basis of morphology. The second change regard the cut-off of Ki67 index in G1 cases. In the pancreas, G3 NEN and NEC overlap in their proliferation index, making difficult the choose the most efficient therapeutic strategy based on the histological differentiation of the tumor cell and their proliferative index. Currently, the Ki67 expression level is associated with different prognoses and can only partially indicate the most efficient pharmacological strategy in G3 NETs/ NEC because the broad interval indicated by Ki67 expression for G3 disease (21–100%) may include a variety of different neoplasms, with potentially different responses to therapies. Therefore, there is an urgent need for a better characterization of G3 NENs/ NEC. Clinically, the introduction of G3 PanNETs category is important because these usually show a poor response to the first–line platinum base chemotherapy , while they response favorable to surgery for resectable disease and to somatostatin analogs in metastatic disease. Actually, immunotherapy, which allows immune cells to attack cancer cells, results in long-term survival in patients with several different tumors, such as melanoma, non- small cell lung cancer, and renal cell carcinoma. Like a lot of other tumors, NENs possess immune escape mechanisms, but very little has been done to characterize the crucial aspect of PD-L1 expression in these tumors. In this regard, in our previous work we highlighted the role of programmed cell death ligand 1 (PD- L1) in GEP-NEN: PD-L1 expression was significantly associated with a high-grade WHO classification (G3) becoming the new gold standard for G3 NEN discrimination.

**Methods and Results.** In this study we evaluated the cell membrane PD-L1 expression in tissues from 77 patients with NENs, in particular 13 were PanNENs and were classified as follows: 5 grade 1 (38%), 6 grade 2 (47%), and 2 grade 3 (15%). Among these, 5 (38%) resulted negative PD-L1 expression while 8 were positive (62%). Importantly, PD-L1 expression was absent in all cases with WD-NENs (G1), while PDL1 positivity in tumor cell membranes was detected in 6 G2 cases (83%) and 2 G3 cases (100%). PD-L1 expression was significantly associated with a high-grade WHO classification (G3) ($p< 0.001$) but not with gender, primary site, or lymph nodes status. PD-L1 positivity rate and signal intensity are directly correlated ($p< 0.001$) with a grade increase from G1 to G3. In particular in G3 cases, we have observed, confirming the goal of new classification, a dichotomy between the morphology (WD and PD-NENs) and Ki67 index. Moreover, our study demonstrated a significant association with the grade and PD-L1 expression levels in immune-infiltrating cells ($p< 0.001$). In particular, G3 tumors are characterized by strong PD-L1 expression in both the tumor and infiltrating immune cells ($p<0.001$), reflecting an unfavorable environment for T cell- mediated tumor aggression.

**Conclusions.** These findings suggest that NENs might acquire resistance to immune surveillance by up-regulating PD-L1 and inhibiting peri and intra-tumoral infiltrating lymphocytes. Here we demonstrate that PD-L1 is currently the best-known biomarker for G3 NENs, in particular G3 PanNENs, becoming the new gold standard for G3 NENs discrimination. Furthermore, immunotherapy might be a valid alternative or support to other therapies in G3 PanNENs. Pharmacological approaches using anti-PD-1 antibodies may become the logical choice for the treatment of G3 cases with a poor prognosis.

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## VERY RARE LIVER NEOPLASM: THE LYMPHOEPITHELIOMA-LIKE (LEL) HEPATOCELLULAR CARCINOMA. A CASE REPORT

A. D'Amuri[1], V. Moretti[1], F. Floccari[2], M. Filotico[1]

*[1] Anatomic Pathology Unit, "Card. G. Panico" Hospital, Tricase (LE), Italy; [2] Clinic Pathology Unit, "Camillo De Lellis" Hospital, Manfredonia (FG), Italy*

**Introduction.** A 62-year-old woman with HBV-related hepatopathy has been suffering for a few months of pain and a sense of weight in the right hypochondriac site. A CT scan reveals a lesion of about 5 cm between the VI hepatic segment and right colon. A rectum-colonoscopy demonstrates near the right colonic flexure an ab extrinseco compression that dislocates the bowel and makes the endoscope's progression difficult. Antibodies to HBV = positive. An exophytic neoformation with a diameter of 5 cm, well capsulated at the level of the VI hepatic segment, is found in the laparoscopic procedure.

**Material and Methods.** Numerous fragments are taken from the surgical sample, fixed in formalin and included in paraffin. The sections are stained with H&E and subjected to silver impregnation according to Gomori. A large panel of antibodies is used for immunohistochemistry.

**Results.** The surgical sample consists of a globose neoplasm, polypoid in shape with a diameter of about 5 cm and a large peduncle implanted on a triangular flap of hepatic parenchyma. The neoformation has a smooth and shiny surface, of a reddish-brownish color. At the section, the surface is reddish-brownish, opaque grainy, intersected by coarse fibrous bands. The normal liver structure is no longer recognizable. Just below the

Glisson's capsule are ductular biliary structures. The tissue is crossed by wide bands of fibrous tissue surrounding grossly nodular areas. The bulk is made up of disorderly proliferation of globose-polyhedral epithelial elements, of clear neoplastic meaning, with a pink, foliaceus, cytoplasm. The nucleus is voluminous, frequently nucleolate and hyperchromatic. There is a mitotic and highly atypical activity. Occasionally in some areas a vaguely trabecular structure is still recognizable along with a few abortive tubular formations. These cells are dissociated by a massive lymphoid infiltrate consisting of elements of small and medium size, sometimes with plasmacytoid appearance that occupy the sinusoidal spaces, up to render scarcely visible the epithelial component. In correspondence of fibrous bands, the lymphoid infiltrates aggregates into nodular formations. There are also large areas of steatosis and coagulative necrosis. The argyrophilic network is fragmented. As a consequence of this phenomenon, the epithelial component is fragmented into a small group of the few cells or even isolated elements. The immunohistochemical investigations, demonstrates the existence of two epithelial cell lines: one, majority, expressing CKs AE1/AE3, Hepatocyte, TTF1, AFP, CD10, and another, minority expressing CK7 and CK19. The diffuse lymphoid component mainly expresses elements with phenotype T: CD3, CD4, CD5, CD8, while the phenotype B: CD20 is limited to the nodular aggregates in the fibrous bands and CD138 to the plasmocytoid elements. The morphological and immunohistochemical data allow placing the lesion in the field of the so-called Lymphoepithelioma-like Hepatocellular Carcinoma.

**Discussion and Conclusions.** The lymphoepithelioma-like (LEL) hepatocellular carcinoma (or inflammatory hepatocellular carcinoma) only recently been recognized as an entity in itself. The first report dates back to 1995. The definition of Lymphoepithelioma-like has been present in the literature since 1996. To date, 86 cases have been reported, in 59 of which the epithelial component shows the morphological characters and the immunophenotypic profile of the hepatocellular carcinoma (HCC) and in 27 those of cholangiocellular carcinoma (CC). On the basis of similar lesions occurring in various sites (lung, urinary bladder, vagina, uterus, skin, etc.), all referring to the basic model in nasopharyngeal localization, from the earliest observations has sought and found an association with EBV. Between LEL–HCC and LEL-CC significant differences have reported. The male sex prevails in the LEL-HCC (64%), while the Female prevails in the LEL-CC (66%). The Caucasians prevail in LEL-HCC (65%), while Asians prevail in the LEL-CC (92%). EBV is reported in 2% of cases of LEL-HCC and in 74% of cases of LEL-CC. Cirrhosis is present in 46% of cases of LEL-HCC and in 19% of cases of LEL-CC. Positivity for HBV is present in 30 and 40% of cases, respectively, while HCV is 34% and 7%. The better prognosis of the LEL-HCC group could attribute to the antitumor effect induced by the cellular immunity of CD8 + and CD4 + T lymphocytes, and partly by the humoral immunity of B cells which formed lymph follicles. In a report of 8 LEL-HCC cases, free from viral infections of any kind, the lymphoid infiltrate is made up mainly of T cells CD4 +, CD8 +. The immunophenotypic evaluation of our case is substantially consistent with the literature, with the exception of the extent of a lower expressivity of CD8 + lymphocytes. From the literature it is clear that the phenomenon of lymphoid infiltration can affect both types of hepatobiliary neoplasms following with their epidemiological distribution. As well it is evident that in the majority of cases, they were poorly differentiated neoplasms. The immunophenotypic evaluation of an epithelial component of our case highlights the expression of antigens related to HCC and also antigens related to CC. The poorly differentiated morphology was further altered by the massive lymphoid infiltration, which did not help in identifying two distinct components. The expression of specific hepatocellular antigens (Hepatocyte, CD10, $\alpha$Fp) is sufficient to affirm the presence of a hepatocellular differentiation, just as it cannot be said for CC because CK7 and CK19 do not explicitly state a biliary differentiation of a proliferation as these antigens can also be expressed by the hepatocellular component. It should be stressed, however, that the lumen of the rare and abortive tubular structures present is delimited by smaller cuboidal cells expressing antigens CK7 and CK19. This finding would favor the possibility of the existence of a true cholangiocellular component. Liver tumors of mixed hepatocellular and biliary phenotype were, until recently, referred to as combined hepatocellular and cholangiocarcinoma (mixed hepatocellular-cholangiocarcinoma). Under these terms were classified, both "collision" tumors, which coexist in the same organ two distinct tumors hepato and cholangiocarcinoma, both single tumors with mixed morphologic features of both hepatocellular and biliary differentiation. The International Consensus Panel advises reserving the denomination of Myxed Hepatobiliary Tumors only to single tumors with myxed morphologic features, distinguishing in it two subgroups according to the presence or absence of stem cells. No case of LEL-myxed epatobiliary carcinoma (hepato-cholangiocellular carcinoma) according to the previous definition, hitherto been reported in the literature. A single report of myxed LEL-carcinoma describes a case in which a well- differentiated cholangiocellular component coexisted with one second poorly differentiated. Immunohistochemical studies revealed that both components were immune reactive for CKAE1/AE3, CK7, CK19, and, focally, for monoclonal CEA. Both components were negative for CK20 and HePar1. The case of our observation, however, can rightfully be classified as a LEL Myxed Hepatobiliary Tumor without stem cells, as immunophenotypically the two components are well represented and in some areas a trabecular architecture and some tubular formation, CK7 and CK19, can be identified. To the best of our knowledge, this would be the first case of LEL Myxed Hepatobiliary Tumor reported in the literatures.

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## UNIVERSAL SCREENING TO IDENTIFY LYNCH SYNDROME: TWO YEARS OF EXPERIENCE


E. Leoni, A.M. Chiaravalli, I. Carnevali, N. Sahnane, D. Furlan, F. Sessa, M.G. Tibiletti

*UO Anatomia Patologica Ospedale di Circolo ASST-settelaghi Varese; Centro di ricerca per lo studio dei tumori eredo-familiari, Università dell'Insubria Varese*


Colorectal cancer (CRC) is the third most common cancer in men and women and it is estimated that approximately 3% of all CRC are due to inherited conditions. The most common inherited CRC is Lynch syndrome (ORPHA 144) (LS) an autosomal dominant genetic disorder associated with greatly increased risks for developing CRC and endometrial cancers.

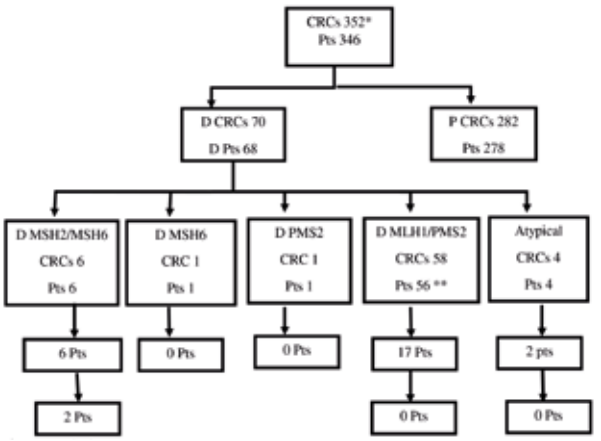


**Figure 1:** Results of FLOW-CHART for Lynch syndrome identification. * 6 patients had 2 CRCs; ** 2 patients had 2 CRCs D defective; P proficient.

LS is due to germline pathogenetic variants in the DNA mismatch repair genes such as MLH1, MSH2, MSH6, PMS2 and EPCAM.

Recent data showed that identifying patients affected by LS at high CRC risk using germline molecular testing in CRC affected patients and in their healthy relatives is an efficient prevention model to reduce mortality and morbidity of CRC and other LS-related cancers.

Standard population CRC screenings fail to provide early detection or prevention for most LS CRCs as they tend to occur at young ages. Usually, screening for LS is confined to a subset of patients selected on the basis of clinical criteria including family histories, early age of CRC diagnosis and or presence of CRC with MMR defects (Amsterdam and Bethesda criteria). Recently this approach has been criticized for the not optimal sensitivity and studies evaluating the efficacy of universal molecular testing of CRC revealed that as many as 28% of individuals with LS are not identified using clinical criteria.

Different platforms should be used for LS universal testing including germline molecular analysis, somatic microsatellite instability (MSI), immunohistochemical analysis (IHC) on CRC. Moreover, BRAF mutation and/or MLH1 methylation analyses have been suggested to have a clinical utility to distinguish sporadic from LS-related CRCs.

According to these findings, Lombardy Region in 2015 improved LS screening network (Delibera n°4498 3/6/2015) recommending to include MMR IHC expression in all diagnostic reports of resected CRC as universal screening and to suggest Cancer Genetic Counselling for all patients affected by a MMR defective CRC.

Here we reported two years of experience of IHC universal screening to identify LS patients in Ospedale di Circolo, ASST Settelaghi in Varese (Italy).

**Materials and Methods.** In this prospective study, a cohort of 352 consecutive cases of surgical CRC diagnosed from 1st September 2015 to 31stAugust 2017 from 346 patients (6 patients had two CRCs) was routinely evaluated for MSH2, MLH1, MSH6 and PMS2 protein expression using immunohistochemical approach. The mean age of patients at CRC diagnosis was 71 years (range 35-92). Microsatellite instability (MSI) using pentaplex PCR at NR21, NR22, NR24, BAT25, BAT26 mononucleotide loci was investigated in all these tumors.

All MLH1 defective CRCs were examined for MLH1 promoter methylation by MS-MLPA (ME011, MRC-Holland) and for BRAF V600E mutation by pyrosequencing (Anti-EGFR response BRAF status, Diatech Pharmacogenetics) and immunohistochemistry (VE1 antibody).

**Results.** A MMR defect was identified in 70 out of 352 CRCs (19.9%) from 68 patients, in detail 6 out of 70 (8.6%) CRCs showed MSH2/MSH6 defects, 58 out of 70 (83%) showed MLH1/PMS2 defects, two CRCs showed absence of PMS2 (1.4%) and MSH6 (1.4%) expression, respectively. Four cases were classified as atypical showing unusual pattern of MMR expression, including clonal absence of immunoreactivity or defect for more than 2 proteins.

**MSI** was detected in all MMR-deficient CRC except for one case classified as atypical showing focal immunohistochemical defect for MSH6. Among the 61 MLH1 immunonegative tumors, MLH1 promoter hypermethylation was detected in 56 (92%) cases, two of which showed high levels of methylation suggestive of biallelic MLH1 methylation. V600E BRAF mutation was found in 41 out of 61 (67%) CRC by both the applied methods. Cancer Genetic Counselling was offered to all 68 patients affected by MMR defective CRC and 25 patients affered to this service (compliance of 40%). Taking into account family history and somatic analyses molecular germline testing was offered to 10 patients: 2 patients carried MSH2 germline mutations, 2 patients were suspected for MLH1 primary epimutation. In summary 2 patients resulted affected by LS and 2 patients were suspected for LS. Figure 1 summarized results of our flow-chart.

**Conclusions.** Using combined somatic approaches in order to ascertain MMR defect we identified 2 out of 25 (8%) LS patients and also two cases suspected for Lynch Like syndrome. Interestingly one out of two LS patients did not show clinical criteria for genetic testing. Our results suggest that universal screening including BRAF V600E and, above all, MLH1 methylation analyses is an efficient approach to identify high risk patients for CRC and other LS- related cancers especially in a cohort of old aged patients.

## AN UNUSUAL CASE OF SQUAMOUS CELL CARCINOMA OF THE RIGHT COLON

S. Pizzolitto, S. Battista, C. Rizzi, G. De Maglio, V. Angione, P. Cataldi, M. Costantini, A. De Pellegrin, M. Turina

*Pathology Department, S. Maria della Misericordia Hospital, Udine, Italy*

**Introduction and Aim of the study.** Squamous cell carcinoma (SCC) of colon cancer is an unusual entity.

We report the case of a patient with a SCC of the colon. A 76-year-old women underwent a right colectomy. In the anamnesis the patient had a left nefrectomy for clear cell carcinoma (nuclear grading 2, acc. Fuhrman Staging pT1b(m) acc. TNM VIIth ed.) with negative follow up and a lung amartochondroma.

Grossly, in supravalvular site, at 9 cm from the colonic margin of surgical resection, we reported an ulcerated neoplasia, occupying 9/10 of the circumference for an extension of 5 cm; the lesion infiltrated the wall and the perivisceral adipose tissue.

**Histological report.** squamous and adeno-squamous ulcerated carcinoma of the large intestine with moderate differentiation and marked nuclear pleomorphism, infiltrating the wall and the perivisceral adipose tissue. Three out of 25 lymph nodes isolated were metastatic: one showed squamous cell differentiation

and the other two had glandular histotype. Surgical margins of resection were free from neoplastic localizations.

**Material and Methods.** The formalin fixed sample was dissected, processed and paraffin-embedded. The sections were stained with hematoxylin and eosin.

Molecular characterization has been performed separately on squamous component of the lesion, glandular/mucinous differentiation area and metastatic lymph nodes.

KRAS/NRAS/BRAF analysis has been performed with Mass Spectrometry system with CE-IVD Myriapod® Colon status kit (Diatech Pharmacogenetics) on Sequenom MassARRAY® System (Agena bioscience).

Immunohistochemical evaluation of mismatch repair (MMR) proteins has been performed with a panel of four antibodies: MLH1 (clone M1, Ventana), PMS2 (clone EPR 3947, Ventana), MSH2 (clone 44, Ventana) e MSH6 (clone G219-1129, Ventana) on BenchMark/VENTANA Ultra platform (Ventana).

**Results.** Molecular profiling of different areas of the colon resection specimen shows mutation c.1799T>A p.(Val600Glu) in BRAF gene in all different components. Any other alteration in KRAS, NRAS and PIK3CA genes have been detected.

Immunohistochemical profiling of MMR panel revealed nuclear positivity for all markers, suggesting a microsatellite stable phenotype of the tumor.

**Conclusions.** To the best of our knowledge, this is the first described case of squamous carcinoma of the right colon with BRAF mutation.

## MULTIPLE HISTOLOGICAL FEATURES DETERMINE RISK ASSESSMENT OF pN+ IN COLORECTAL CARCINOMA: HOW THE UNITY IS STRENGTH

G.L. Rampioni Vinciguerra[1], G. Antonelli[2], S. Angeletti[2], N. Noccioli[1], M. Pesce[1], E. di Giulio[2], A. Vecchione[1], E. Pilozzi[1]

*[1] Pathology Unit; [2] Endoscopy Unit, Santo Andrea Hospital, University of Rome Sapienza, Italy*

**Objective.** Implementation of colorectal cancer (CRC) screening programs has greatly increased the number of CRCs that are diagnosed and treated in the early stage. Endoscopic resection has been shown to be safer than surgery, although the risk of regional lymph node metastasis (pN+) may compromise its success. Despite many histological features have been linked to increased risk of pN+, a great variability was reported, due to the lack of standardisation and inter-observer agreement [1 2].

The aim of our study is to establish which histological risk factors are more useful to assess the risk of pN+ in early CRC.

**Material and Methods.** From 2008 to June 2018, 127 cases of CRC infiltrating the submucosal space (defined as pT1 according to AJCC 2017), were collected. Patients with both endoscopic removal (39 cases) and incisional biopsy (88 cases) of polyps/flat lesions followed by surgical hemi-colectomy were included. The absence of surgical specimen and the presence of synchronous CRC higher in pT stage were considered as exclusion criteria.

A panel of macroscopic and histological features were evaluated and reported according to the literature: type of associated adenoma, tumour grading, percentage of carcinomatous component, Haggitt and Kikuchi classification, depth of submucosal invasion, tumour budding, lympho-vascular and perineural invasions [3-7]. Proper immunohistochemical stains were performed in doubtful cases. Continuous data were reported as mean±SD and compared using student's. Categorical variables were expressed as number (percentage) and compared using the chi-squared test.

**Results.** Our record was composed by 127 cases of pT1 CRCs distributed as follows: 40,2% females; 59,8% males. Mean age at diagnosis was 69,8 yo; SD: ±9,8. The 6,3% of patients resulted pN+: (37,5% females; 62,5% males. Mean age 67,2 yo; SD: ±9,0).

All of these tumours arose from left bowel and showed a villous component in the associated adenoma.

Tumour size and Grading did not correlate with nodal status. A percentage of invasive carcinoma higher than 50% and presence of lymphovascular invasion were significantly associated with pN+ (p= .0014 and p= .0325, respectively). Interestingly, tumour budding expressed in four grades [5] showed a linear correlation with pN+ (r =0.9927). Haggitt and Kikuchi levels correlated with pN stage. Their re-organisation in a two-scale system (Haggitt score 1-2 versus 3-4; Kikuchi level 1 versus 2-3) maintained the same prognostic value.

The depth of submucosal invasion was initially analysed separately for each macroscopic type of adenoma (peduncolated/sessile polyps and flat lesions). For each group, the average of tumour invasion was above 3mm. Curiously, considering this value as a cut-off, all CRCs with pN+ infiltrated more than 3mm independently by the adenoma they originated from.

**Conclusions.** According to our study, approximately the 6,3% of early CRCs spread to local lymph-nodes. With the aim of avoiding useless surgery, a careful risk evaluation of CRCs endoscopically removed is mandatory.

All the histological factors needs to be considered. Haggitt and Kikuchi classification, presence of a large invasive component, lymphovascular invasion and tumour budding appeared as factors more reliable than other canonical variable like tumour size and Grading.

Moreover, the use of a two-scale system to report Haggitt and Kikuchi score and the introduction of a threshold for submucosal invasion should be introduced, in order to simplify routine diagnostic procedures and increase the concordance between pathologists.

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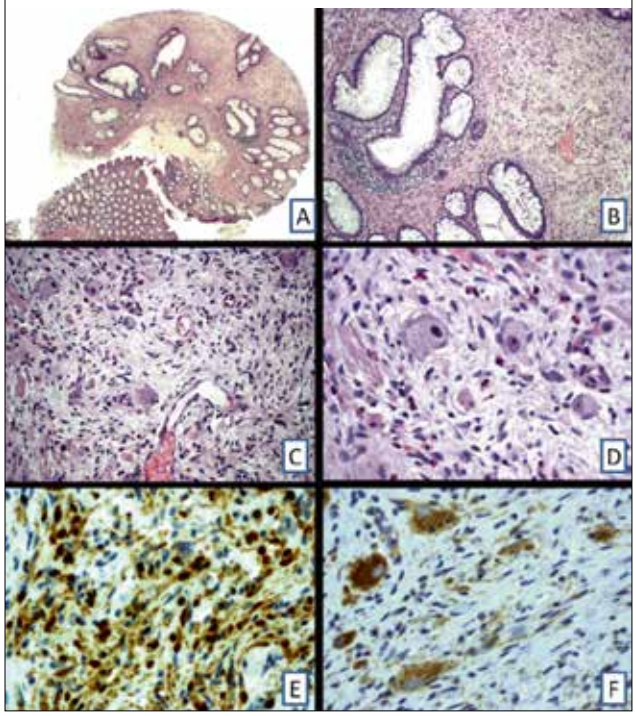
## SOLITARY COLONIC POLYPOID GANGLIONEUROMA: A RARE CLINICOPATHOLOGICAL ENTITY

M. Salemme[1], A. Cominotti[1], E. Chirico1, S. Negri[1], P. Fontana[1], G. Cengia[2]

*[1] Department of Pathology, Manerbio Hospital, Brescia, Italy; [2] Gastroenterology and Endoscopy Unit, Manerbio Hospital, Brescia, Italy*

**AIMS.** Ganglioneuromas (GNs) of the gastrointestinal tract are very rare hamartomatous tumors derived from the autonomic nervous system and composed of a benign proliferation of nerve ganglion cells, nerve fibers and supporting cells of the enteric nervous system (glial cells) [1]. Shekitka and Sobin [2] divided gastrointestinal GNs into three different groups: polypoid GNs, ganglioneuromatous polyposis and diffuse ganglioneuromatosis. Colonic polypoid GNs are usually asymptomatic or associated with vague symptoms of abdominal pain, constipation and bleeding. They often present as incidental colonoscopic discovery of solitary, small, sessile or peduncolated polyps, not associated with peculiar endoscopic features. Histological analysis shows an hypercellular and expanded stroma composed of spindle-shaped cells (S100-positive Schwann cells) admixed with variable numbers of ganglion cells (neuron-specific enolase and synaptophysin-positive cells), that displaces and distorts colonic crypts [3]. Polypoid GNs are usually treated with complete endoscopic excision and do not require long-term follow up. Here we report a case of solitary colonic polypoid ganglioneuroma, to date the thirty-first case reported in the literature (Tab. I) with a well-characterized histology.

Figure 1.




**Materials and Methods.** Our patient is an asymptomatic 67-year-old female who underwent a colonoscopy because of positive fecal occult blood test. Endoscopic examination revealed a 4 mm sessile polyp in the descending colon which was removed by excisional polypectomy.

**Results.** Microscopically, at low magnification histological appearance of the specimen resembled a juvenile polyp (Fig. 1A, 1B, hematoxylin-eosin, original magnification X2,5 and X10), with a disturbed crypt architecture characterized by elongated, cystic glands displaced by an hypercellular and expanded lamina propria. At higher magnification abundant spindle-shaped cells admixed with scattered ganglion cells were identified in the stroma (Fig. 1C, 1D, hematoxylin- eosin, original magnification X20 and X40). Immunohistochemistry demonstrated that spindle cells were reactive to S100 protein (Fig. 1E, hematoxylin-eosin, original magnification X40), while ganglion cells were synaptophysin-positive (Fig. 1F, hematoxylin-eosin, original magnification X40). Taking together, the morphological features were consistent with solitary colonic polypoid ganglioneuroma.

**Conclusions.** We report a case of solitary colonic polypoid ganglioneuroma, to date the thirty- first case reported in the literature with a well-characterized histology. Colonic polypoid GNs are very rare entities, often asymptomatic and incidentally detected on endoscopy. Histologically they are hamartomatous polyps composed of collections of spindle and ganglion cells. Due to the benign nature of these lesions, a long-term follow up is not needed.

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Table I. The table summarizes the cases of solitary colonic polypoid ganglioneuromas reported to date in the literature with a well-characterized histology.

| Authors | Year of publication | Number of cases | Sex and age of patient | Location of the lesion |
|---|---|---|---|---|
| Shekitka KM and Sobin LH | 1994 | 24 | Not available | Left colon/rectum (12 cases) Right colon (4 cases) Transverse colon (1 case) Colon, not otherwise specified (7 cases) |
| Srinivasan R and Mayle JE | 1998 | 1 | Male, 54 y/o | Hepatic flexure |
| Ozawa T | 2008 | 1 | Female, 32 y/o | Descending colon |
| Rabjerg M and Kolodziejczyk A | 2012 | 1 | Female, 70 y/o | Sigmoid colon |
| Abraham G and Prakash SR | 2015 | 1 | Male, 43 y/o | Cecum |
| Herman M, Abed J et al | 2015 | 1 | Male, 57 y/o | Sigmoid colon |
| Ofori E, Ona M et al | 2017 | 1 | Male, 65 y/o | Ascending colon |

## CD8+ T CELL DENSITY AND PD-L1 EXPRESSION IN GASTRIC CANCER

A.M. Valentini, F. Di Pinto, S. Coletta, E. Cavalcanti, A. Ignazzi, F. De Michele, ML. Caruso

*Department of Pathology, National Institute of Gastroenterology "S. de Bellis", Research Hospital,via Turi 27,70013 Castellana Grotte, Italy*

The programmed death receptor 1 (PD-1) protein is a cell-surface receptor on T lymphocytes that, with its ligand programmed death ligand 1 (PD-L1) on tumor cells, helps to evade immune recognition. Precision therapies targeting the PD-1/PD-L1 pathway have the potential to restore this anti-tumor immunity and then immunotherapy have been revealed to be effective in some cancers such as malignant melanoma and non-small lung cancer [1]. Although the expression of PD-L1 on the surface of tumor cells, as evaluated by immunohistochemistry, may potentially serve as a predictive factor to identify patients who would benefit from immunotherapy, not all PD-L1 positive patients respond well. Therefore, PD-L1 expression on tumor cells is currently considered an imperfect predictor of response to immune checkpoint inhibitors therapy [2]. In this perspective, the researchers' attention has turned to the study of the tumor microenvironment, especially the degree of tumor immune cell infiltration. A recent immunological classification of tumors into 4 microenvironment immune types (TMITs) on the basis of their PD-L1 status and low/high CD8+T cell density has already been proposed and validated in melanoma [3-5]. To date few studies have described this classification in the gastric cancer.

We evaluated PD-L1 expression in tumor microenvironment and we quantified tumor infiltrating CD8+T cells density in 60 gastric cancers, from 38 males and 22 females underwent to curative gastrectomy at National Institute of Gastroenterology "S de Bellis", Castellana Grotte, Italy, between 2014 and 2016. The tumor site was proximal (cardias, corpus and fundus) for 32 patients and distal (antrum/pylorus) for 28. Gastric carcinomas were classified according to Lauren as follows: 30 diffuse and 30 intestinal.

Serial 4 micron thick sections from formalin fixed paraffin embedded samples were cut onto glass positively charged slides and advanced for immunohistochemical staining. CD8 (MAb C8/144B, Dako, Glostrup, Denmark, diluted 1:200) and PD-L1(clone E1L3N, Cell Signaling Technology, Danvers, MA, USA) immunohistochemical stainings were carried out on the automated autostainer (Dako, Glostrup, Denmark) after antigen retrieval in EDTA buffer (pH 8). Dako Real Envision (Dako, Glostrup, Denmark) was used as visualization reagent and the 3,3'-DAB as chromogen, according to the manufacturer´s instructions.

PD-L1 positivity was defined by the presence of at least 5% of positive cells with membrane staining of any intensity [6]. For the evaluation of CD8, a pathologist selected five field in the intratumoral and peritumoral areas. Absolute numbers of positive cells were counted and median amount of CD8+T lymphocytes was used to classify low/high CD8+T cells gastric cancers [7].

Among 60 patients, six cases (10%) showed PD-L1 immunoreactivity on tumor cells. No difference was seen between gender patients and tumor histotypes. CD8+ lymphocytes were present both within tumor-cell nests and intratumoral and peritumoral stroma. The score of the CD8+ T cells allowed to identify 20% of gastric cancers with high and 48 ( 80%) with low CD8+ density. The specimens PD-L1 positive showed more frequently high CD8+T cells density than negative ones ( 66.6% vs 14.8%, $p<0.05$). Based on the results of immunohistochemical expression of PD-L1 on tumor cells and CD8+ density, we categorized the patients into TMITs I-IV (3). The number and proportion of each TMIT were as follow: type 1 ( PD-L1+/CD8+hgh), 4 (6.7%) (Figure 1); type II (PD-L1-/CD8+low) 46 (76.7%); type III ( PD-L1+/CD8+low), 2 (3.3%); type IV (PD-L1-/CD8+high), 8 (13.3%).

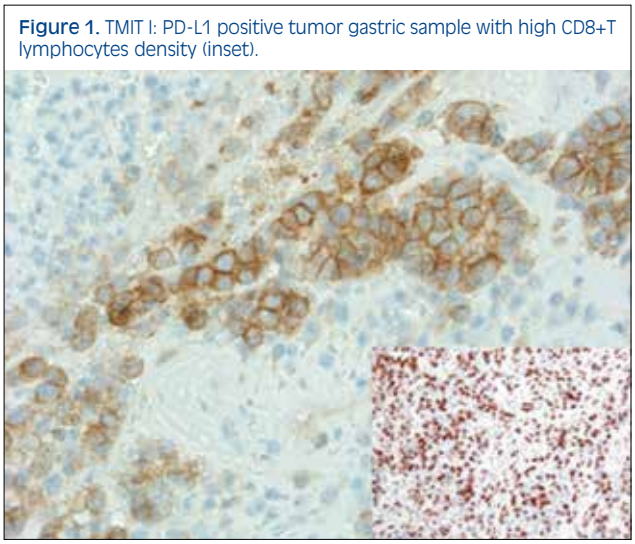

**Figure 1.** TMIT I: PD-L1 positive tumor gastric sample with high CD8+T lymphocytes density (inset).

Gastric cancer has limited treatment in the locally and metastatic setting. Immunotherapy with immune checkpoint inhibitors may be a new chance, especially for HER2 negative patients. At this aim, four groups of tumors have now been proposed on the basis of PD-L1 status and presence of low/high CD8+ T cells density [3-5]. These include type I ( PD-L1pos with high CD8+ T driving adaptive immune resistance), type II ( PD-L1 negative with low CD8+T cells indicating immune ignorance), type III ( PD-L1pos with low CD8+ T cells density indicating intrinsic induction) and type IV (PD-L1 negative with high CD8+T cells density indicating the role of other suppressors in promoting immune tolerance. The proportion of various cancers that fit into each of these types,, likely depends on the genetic aberrations and oncogene drivers as well as the tissue they arise in [5]. Malignant melanoma has been extensively studied in this regard and an high proportion of type I and II microenvironments are described [5]. Clinical studies demonstrated that approximately 38% of patients with advanced melanomas with a type I profile are sensitive to anti-PD-L1 treatment. Conversely, melanoma patients that fall within type II tumor microenvironment, unfortunately, are not responsive to checkpoint blockade [5]. These informations, generated by the same methodologies are not yet available for gastric cancer.

In agreement with previous studies, we have shown that the majority of gastric cancers belong to TMIT II (76%) ie immune ignorance type. A low level of CD8+T cell density might partially explain the high mortality of gastric cancer patients and restrict the use of antibodies that target immune checkpoints. We found 7% of TMITs I, 3% of TMITs III and 13% of TMITs IV. Gastric cancers that fall into type I are associated with Epstein Barr virus infection and MSI status, both characterized by heavy lymphocytic infiltration. TMIT I status implies the adaptive immune escape responses, and based on many previous studies [4], there is a good chance that gastric cancers with this signature can be reversed by immune checkpoint blockade. Type III represents the least numerous category demonstrating that the constitutive expression of PD-

L1 on tumor cells . This group could include those patients who, while showing positive PD-L1 on the neoplastic cells, do not respond to the therapy [5]. Type IV contain high CD8+T cell density, but no obvious adaptive resistance. It was reported that, in patients with stage III malignant melanoma, the most predictive marker of clinical response to PD-1 blockade was the density of CD8+T cells, not PD-L1 expression itself. This could better explain the responsiveness to therapy for patients with negative PD-L1 on tumor cells..

In conclusion, we suggest that the classification of gastric tumors in the TMITs I-IV, based on PD-L1 expression and CD8+T cell density provides useful information on the sensibility to PD-L1 blockade.

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## IMMUNOPHENOTYPICAL CLASSIFICATION OF GASTRIC CANCERS


A.M. Valentini, F. Di Pinto, S. Coletta, E. Cavalcanti, A. Ignazzi, F. De Michele, M.L. Caruso

*Department of Pathology, National Institute of Gastroenterology "S. de Bellis", Research Hospital,via Turi 27,70013 Castellana Grotte, Italy*


**Introduction and Objectives**. Gastric cancer (GC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide. The overall survival of GC patients remains poor, in fact the five years' survival is less than 30% [1]. The genomic landscape of GC is highly heterogeneous and a deep understanding of the molecular factors involved in the development of GC is needed in order to identify new biomarkers to diagnose GC in early stages and develop more effective therapeutic strategies. To date, only two targeted treatments are available: Trastuzumab (anti-HER2) and Ramucirumab (anti-VEGF). The overexpression of HER2 is a therapeutic target for the eligibility of patients to Trastuzumab treatment. To allow the identification of new possible therapeutic targets, in 2014 The Cancer Genome Atlas Project (TGCA) [2] proposed a molecular classification, that was approximated in 2016 by Gonzales RS et al. by using a less expensive and widely available method such as immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) [3].

They [3] classified the GCs in four groups: EBV positive (EBER positive), unstable microsatellite (MLH1 loss), chromosomal instability (p53 aberrant), genomically stable (EBER neg /no MLH1 loss , no aberrant p53). We tried to modify the Gonzales' classification using also the status of HER2 to define the group with chromosomal instability (CIN).

**Materials and Methods**. We stained 30 gastric cancers, from 20 males and 10 females underwent to curative gastrectomy at National Institute of Gastroenterology "S de Bellis", Castellana Grotte, Italy, between 2015 and 2016. The tumor site was proximal (cardias, corpus and fundus) for 17 patients and distal (antrum/pylorus) for 13. GCs were classified according to Lauren in 8 diffuse and 22 intestinal. Serial 4 micron thick sections from formalin fixed paraffin embedded samples were cut onto positively charged slides. MLH1 (MAb ES05, Dako, Glostrup, Denmark, diluted 1:50), p53 (MAb DO-7, Thermo Scientific, Fremont CA, USA, 1:500), HER2 (Policlonal antibody, Dako, Glostrup, Denmark, diluted 1:200), PD-L1 (clone E1L3N, Cell Signaling Technology, Danvers, MA, USA, diluted 1:200) IHC stainings were carried out on the automated autostainer (Dako, Glostrup, Denmark). MLH1 and p53 immunohistochemical stainings were carried out after antigen retrieval in EDTA buffer (pH 8), HER2 staining was carried out after antigen retrieval in 10 mM Citrate buffer (pH 6). Dako Real Envision (Dako, Glostrup, Denmark) was used as visualization reagent and then the 3,3'-DAB as chromogen, according to the manufacturer´s instructions. For evaluation of HER2 scoring, criteria based on the report by Hofmann et al. [4] were adopted. Only IHC score 2+ cases were subjected to CISH for the evaluation of HER2 gene amplification. HER2 CISH method was carried out using ZytoDot2C SPEC ERBB2/CEN17 probe kit (Zytovision Bremerhaven, Germany). Sections were subjected to denaturation at 80°C for 5' and to hybridization with HER2 probe at 37°C overnight in the Thermobrite Hybridizer (StatSpin, Norwood, MA, USA). The HER2 gene amplification was detected with sequential incubation with Anti-DIG/DNP-Mix, HRP/AP-Polimer-Mix, AP-Red solution, HRP-Green Solution. For the evaluation of HER2 status, the cases with IHC 3+ and cases with IHC 2+/ gene amplified were regarded as HER2 positive, the remaining cases were HER2 negative. EBV CISH method was carried out using RNAscope 2.5 Assay (Advanced Cell Diagnostics, Hayward, CA, USA). Sections were treated with RNAscope Pretreatment Reagents and hybridized with EBER RNAscope Probe (Advanced Cell Diagnostics, Hayward, CA, USA) at 40°C for 2 hours in HybEZ Hybridization System (Advanced Cell Diagnostics, Hayward, CA, USA). The EBER signal was detected with RNAscope 2.5 HD Assay-Brown (Advanced Cell Diagnostics, Hayward, CA, USA).

Tumors with complete loss of staining of MLH1 in tumor cell nuclei were defined to be MMR deficient (MMR-D) while tumors with retained nuclear staining were deemed MMR proficient (MMR-P). Strong p53 nuclear expression in al least 50% of tumor nuclei was interpreted as aberrant. For evaluation of HER2 scoring, criteria based on the report by Hofmann et al.(4) were adopted. PD-L1 positivity was defined by the presence of at least 5% of positive cells with membrane staining of any intensity (5).

**Results and Conclusions.** We categorized 30 GCs specimens in four groups: group 1 (EBER positive) 2 (6,7%), group 2 (MMR-D) 5 (16,67%), group 3 (aberrant p53 and/or HER2 positive status) 17 (56,67%), and group 4 (the remaining GCs, that were EBER negative, MMR-P, no aberrant p53, HER2 negative) 6 (20%). Group 1 ( two samples) represents the least numerous category as aforementioned studies [2 3]. Both samples were proximal, diffuse, with N1 status. We found a significant

association between EBER positivity and PD-L1 immunohistochemical expression on tumor cells (2/2, 100%, p<0,05), in accordance to TGCA Network [2].

In agreement with findings of TGCA Network [2] and Gonzales et al [3] we collected 16,67% samples in the group 2. These tumors were prevalently distal, intestinal, with N1 status.

Seventeen (56.67%) samples belong to group 3 (CIN) vs 38% and 49.8% of others studies [2 3]. They were prevalently proximal, intestinal, with N1 status. The highest percentage of samples in this group is due precisely to the addition of HER2 positive cases.

Twenty percent of the samples, against 19% and 38% of other studies [2 3], belong to group 4 and correspond to the group of genomically stable GCs of the molecular classification.These samples were intestinal type and with N1 status.

In conclusion, this classification offers an alternative inexpensive and widely available method than the molecular one. Unlike Gonzales et al. [3], we have also taken into account, in addition to the p53 state, also the HER2 state to better define the CIN group. In fact, the TCGA data showed that only 71% of their CIN tumors harbored a p53 mutation and that HER2 amplification was commonly present in these GCs.

Because Trastuzumab is only effective in a small percentage of patients, it is possible that this type of classifications may help to identify new possible therapeutic targets.

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# PATOLOGIA DEI TRAPIANTI

## KIDNEY DONATION AFTER CIRCULATORY DEATH (DCD): TOWARDS THE DEFINITION OF HISTOLOGIC INCLUSION AND OUTCOME CRITERIA

M. Novario[1], P. Morbini[1 2], C. Schena[2], G. D'Ambrosio[2], C. Rossi[1], M. Abelli[3], M. Gregorini[4], M.T. Rampino[4], M. Zanierato[5], M. Paulli[1 2]

*[1] Università degli Studi di Pavia; [2] S.C. Anatomia Patologica, Ospedale Policlinico San Matteo, Pavia; [3] S.S.D. Chirurgia Trapiantologica Addominale, Ospedale Policlinico San Matteo, Pavia; [4] S.C. Nefrologia, Ospedale Policlinico San Matteo, Pavia; [5] S.S.D. Coordinazione Donazione e Trapianti, Ospedale Policlinico San Matteo, Pavia*

**Objectives.** Renal transplantation represents the most reliable treatment for patients affected by terminal chronic renal insufficiency, but it is strongly limited by the lack of donor organs. Such situation forced the medical community to find new ways to increase the pool of donations, such as kidney donation after circulatory death (K-DCD). Our Institution was the first italian center to perform DCD kidney transplantation ("Alba program") [1]: our experience demonstrated other criteria, beyond the currently employed Remuzzi score are required to stratify the outcome of such particular and pioneering grafts. The objective of this study is to find reliable and easily applicable morpho-histologic and immunohistochemical parameters to help determine whether the organ is eligible to transplantation and predict the occurrence of delayed graft function (DGF) or primary non function (PNF).
**Materials and Methods.** During the period 2007-2017, 37 potential donors were enrolled in the program (34 man and 3 female; mean age 50y): 31 DCD class II and 3 DCD class III [2], for which normothermic regional perfusion (NRP) was undertaken; 3 cases were discharged due to inefficient extracorporeal cardiac life support (ECLS). In 13 cases the procedure was stopped or even not started at all, more often (9 cases) because of macroscopic vascular malperfusion or flow instability at the perfusion machine during NRP. All cases were biopsied for histological examination. Paraffin-embedded tissue slides from all the selected cases were analyzed for morphological evaluation with Hematoxilin-Eosin, PAS, Masson trichrome and Giemsa stains. The evaluated parameters were Remuzzi score, congestion of glomerular and interstitial capillaries, acute tubular injury (ATI) [3], microthrombi in glomerular and peritubular capillaries [4 5], cortical necrosis and inflammatory infiltrate ("lympho-epithelial pseudolesions"). ATI was graded using the parameters proposed by Oppong et al. [6]. Inflammatory infiltrate was characterized by immunohistochemical reactions (CD3, CD20 and CD138). Collected data were therefore statistically correlated with graft function and the PNF/DGF group was compared with the "normal"-functioning one by Student T test.
**Results.** In our cohort, no organ showed signs of cortical necrosis. Acute tubular necrosis (ATI) was the principal lesion recognized in all biopsies, always graded mild to severe: nevertheless, no statistically significant difference was found between the two groups. We did not find any microvascular alteration (i.e. microthrombi) neither in the peritubular vessels nor in the glomerular capillaries. The inflammatory infiltrate, always mild to poor, was predominantly composed by T-lymphocytes (CD3+) with only very rare B-lymphocytes (CD20+) and plasmacells (CD138+); no evidence of aggression of tubules (tubulitis) nor arteries (arteritis) was noted.
We did not find any statistically significant difference in the expression of histo- morphological parameter between PNF/DGF and normally functioning grafts.
**Conclusions.** Our study demonstrates how, at the current state of art, no histo-morphologic parameter let us predict the outcome of DCD kidney transplant. Such results must consider the limited number of PNF-patients (n=1), that clearly needs an expansion of the cohort. In our experience, if no cortical necrosis is seen, and in the absence of significative increase of flow-resistance at the perfusion machine (resistance >1.0), DCD-donor kidneys should be considered fully eligible for transplantation. Our perspectives are now to enlarge the number of patients, in order to increase our knowledge on the etiology of PNF in DCD organs and to study other possible markers, in particular at molecular level: transcription factors and cytokines such as HIF-1$\alpha$ may be promising targets [7 8 9 10].

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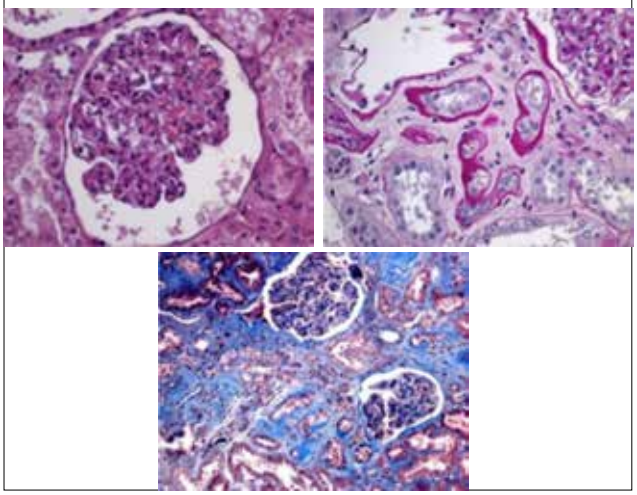
Figure 1.



# PATOLOGIA ENDOCRINA

## SO CALLED GOBLET CELL CARCINOID OF THE PERIAMPULLARY REGION PRESENTING WITH BILIARY OSTRUCTION

A.C. Camboni, A. Manca, A. Fancellu, Maria A. Fedeli, M. Meloni, F. Tanda

**Objective.** We describe a very unusual neuroendocrine tumour in an uncommon localization investigated by light microscopy, immunohistochemical and electron microscopical techniques and showing evidence of bidirectional differentiation. As far as we know the present case is the third to confirm this already described entity showing evidence of biliary obstruction.

**Background.** So called Goblet Cell Carcinoids (GCC) are very unusual tumours characterized by a double mucinous and endocrine differentiation [1] as shown by immunohistochemistry (IHC) and Electron Microscopy (E.M), which show evidence of divergent differentiation of the tumour cells. They are described in the gastrointestinal tract (GIT), mostly in the appendix though very rarely are reported also in the colon and exceptionally in the duodenum [2-4].

They mostly present clinically as appendicitis or represent an occasional finding.

**Case report.** We present a case of an 81-year-old man who was admitted to the emergency room because of abdominal pain and was investigated for obstructive jaundice (total bilirubin values: 20,5 mg/dl). Computerized Tomography-scan (CT-scan) with contrast and Endoscopic Retrograde Cholangiopancreatography (ERCP) showed a nodular lesion suspicious for malignancy localized in the papillary region with dilatation upstream of the common bile duct. A biopsy was performed and a stent was placed. After diagnosis of so called GCC, the patient was submitted to a duodenocephaolopancreasectomy.

**Materials and Methods.** The samples were fixed in 10% neutral formalin and embedded in paraffin; standard sections of 4 micrometri were stained with Hematoxylin and Eosin (H&E), Alcian Blue-PAS stains. For immunohistochemical examination, sections were stained with neuroendocrine markers and proliferative index Ki67. Immunolabeling was performed by the avidin-biotin horseradish peroxidase complex method. Some of the sections were treated with double histochemical and immunohistochemical analysis with Alcian Blue/PAS and Chromogranin A antibody. Tissue blocks for electron microscopy were taken from the formalin-fixed specimen and postfixed in 2,5% glutaraldehyde. These samples were then processed according to the usual techniques and studied with a Zeiss 109 Electron Microscope.

**Results.** The histologic and immunohistochemical characteristics found in the small biopsies were identical to those observed in the surgical specimens.

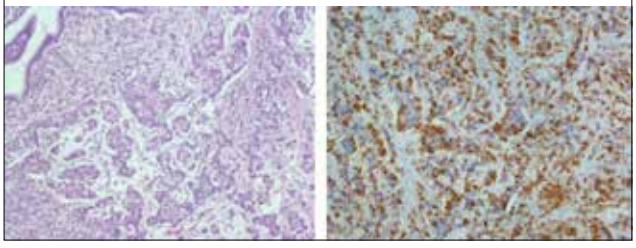
Grossly, the neoplasm involved only the periampullary region. It appeared as a whitish mass measuring 3 x 2 cm, extending to infiltrate the entire duodenal wall sparing pancreatic parenchyma. The light microscopy (L.M.) showed an epithelial type proliferation originating in the mucosa of the papilla of Vater and diffusely infiltrating the lamina propria in the periampullary region extending to the muscularis propria. The neoplasia consisted of two type of atypical cells, each representing about the 50% of the components: cells with hypercromic nuclei and eosinophilic cytoplasm, finely granular, arranged in trabecular structures and goblet and signet ring cells with eccentric nuclei and vacuolated cytoplasm, arranged in solid or very occasionally glandular structures with mucin production (Fig. 1a). Very rare mitoses were seen. The histochemical stains showed mucinous tumor cells representing about 30 % of the cells strongly cytoplasmic Alcian Blue positive next to cytoplasmatic granular cells strongly PAS positive representing about 10% of the cells while about 60% of the cells were positive for both Alcian and PAS.

On immunohistochemistry the neoplastic cells were strongly immunoreactive for neuroendocrine markers such as chromogranin A and synaptophysin. About 10% of the cells were positive for both Alcian Blu/PAS and Chromogranin A (Fig. 1b). The proliferative index Ki-67 was 70% in less differentiated areas but ranging from 10% up to 60% in areas where both components, goblet cells and neuroendocrine cells were recognized. The ultrastructural investigations confirmed the presence of a cellular population with two different properties: cells with cytoplasm rich in mucin vacuoles next to cells whose cytoplasm showed typical neurosecretory electron-dense, membrane bound 250 micrometri in size granules and cells presenting both features.

Our diagnosis was GCC, infiltrating the muscularis propria, without nodal metastasis, stage pT2N0 (AJCC TNM 8h ed.).

**Conclusions.** We describe an unusual localization of a so called GCC. Only 3 cases have been previously reported in the duodenum and the present case represents the third occurring in the papilla of Vater and presenting with biliary obstruction suggesting that papilla is the site mostly involved in the duodenum and such a diagnosis has to be considered in the differential diagnosis of tumour causing biliary obstruction The proliferating cells represent an evident pattern of divergent differentiation with two components seen both, morphologically with solid and trabecular pattern closely intermingled with goblet and signet ring cells and that with immunohistochemical investigations that show about 50% of the cell expressing neuroendocrine markers.The ultrastructure also confirmed these data: cells with typically dense neurosecretory granules, mixed with cell rich in mucin vacuoles without neurosecretory granules and other cells with both aspects. Such a double divergent differentiation suggests interesting support to the histogenetic hypothesis of the origin of the neuroendocrine tumour of the GI tract [5]. This rare entity can be easily recognized also in small biopsies and therefore we suggest to consider it into account when differential diagnosis are made studying biopsies from (GI) tract tumors. The prognosis and therapy of such tumours is challenging, Very few cases at the moment have been described and more are needed to identify the aggressiveness of such tumours. On the basis of the morphology and immuniohistochemistry of the described cases a surgical complete surgery has to be done but the medical treatment should be probably similar to others neuroendocrine tumours according to the Ki67 value and not to a classic adenocarcinoma of the GIT.

Figure 1a-1b.



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## AMYLOID GOITER: AN HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 5 CASES FROM A SINGLE INSTITUTION

C. De Marco MD, G. Carnassale MD[2], C. Brunelli MD, T. Musarra MD, E.D.a Rossi MD PhD, MIAC, C.P. Lombardi MD[2], G. Fadda MD, MIAC

*[1] Division of Anatomic Pathology and Histology; [2] Div. of Endocrine Surgery, Fondazione Policlinico "A. Gemelli", IRCCS, Rome Italy*

**Introduction.** Amyloidosis refers to a variety of conditions in which amyloid proteins are abnormally deposited in organs and/or tissues. Involvement of the thyroid gland by amyloid is a relatively common phenomenon, but a clinically significant enlargement of the thyroid owing to amyloid deposition is a rare occurrence.
**AIM.** The aim of our work is to investigate the biological behavior of amyloid goiter through himmunoistochemistry (HIC) analysis and relate it with clinical features of disease, including difficulties in preoperative diagnosis, management and correlation with rheumatological conditions and review the relevant literature.
**Material and Methods.** We describe five cases of amyloid goiter during the last ten years (3 women and 2 male) with a mean age of 56.6 yo (range: 38-77yo); two female patients have respectively a story of rheumatoid arthritis and systemic lupus erythematosus.
**Results.** Immunohistochemical analysis showed diffuse and intense positivity for AA Amyloid stain; CD34 and CD31 stains highlighted an increased vascular proliferation strictly associated with the amyloid deposition; IgG4 and B Amyloid were negative.
**Conclusions.** Amyloid goiter should be suspected in all patients with a progressive bilateral thyroid enlargement, presenting with a euthyroid state, and a concomitant history of chronic inflammatory and/or rheumatological disease. With a correct diagnosis the patient's prognosis can be predicted and the goiter correctly managed before surgery.

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## PRIMARY EPITHELIOID ANGIOSARCOMA OF THE THYROID GLAND: REPORT OF A CASE FROM AN ITALIAN NON-ALPINE AREA

R. Nenna[1], D. Di Clemente[1], T. Addati[1], V. Losito[1], G. Elicio[1], A. De Lucia[1], L. Bonomo[2]

*[1] C. D. Inchingolo Anatomic Pathology Unit, [2] ASL BT, Andria, Italy*

**Background.** Angiosarcomas are uncommon soft tissue neoplasms that account for less than 1% of all sarcomas. Thyroid angiosarcoma is rare, most of affected patients living in the mountainous Alpine regions, where the tumor may comprise as much as 16% of all thyroid malignancy. Its predilection in these regions has been linked to iodine-deficient goiter, since most thyroid angiosarcomas arise in multinodular goiters. We presente a case of epithelioid angiosarcoma, diagnosed in our department, which affected thyroid in a patient from a non-Alpine location.
**Methods.** A 66 year-old woman in wheelchair for multiple sclerosis with a 10-year history of multinodular goiter but negative anamnesis for malignant neoplasms at february 2018 presented with abrupt enlargement of the goiter. Physical examination showed a 7 cm mass within the right thyroid lobe. A ultrasound of the neck revealed a hypoechoic well circumscribed solid nodule whit remarkable vascularization that measured 7x4,5 cm arising from the right thyroid lobe. The nodule was classified EU-TIRADS 4. Two fine needle aspiration (FNA) of the right thyroid were made: the first one yielded only blood (TIR1 sec. AIT-Siapec alias C1 sec. Bethesda) but the second highlighted an cellular atypia souspicius for malignancy (TIR4 sec. AIT-Siapec alias C5 sec. Bethesda). A computed tomographic scan of the neck revealed there was significant mass effect, with displacement of the airway to the left. There was no invasion of adjacent structures and lymphadenopathy. The results of thyroid function tests were within normal limits. The patient underwent a thyroidectomy. Macroscopically, the thyroidectomy specimen contained in right lobe a well circumscribed nodule that measured 7x4,5cm of hemorrhagic appearence grossly confined within the thyroid. Minute colloid nodules were present in left lobe. On Micro-histologic examination, the right nodule consisted of a central malignant neoplastic proliferation of large epithelioid cells with enlarged pleomorphic nuclei and prominent nucleoli lined vascular-like spaces containing red blood cells and a residual periferic rim of follicular adenoma. Mitotic figures and siderotic stromal deposition were frequently seen. Recent hemorrhagic infarction and necrosis tumoral were present. No involvement of the capsular thyroid and no extraglandular tumour spread were observed. On immunohistochemical examination, the tumor cells stained strongly for Vimentin and endothelial markers (CD31 and Factor VIII), but stained focally for CKAE1/3 and CK19. The cells tumor were negative for CD34, Thyroglobulin, Calcitonin, Cromogranin, Actin Muscolar HHF35 and S-100. Was made diagnosis of Epithelioid Angiosarcoma arisen in follicular adenoma of right lobe of thyroid. The diagnosis was confirmed in second opinion by prof. A. P. Dei Tos. At present the patient is well and no distant metastases have been detected.

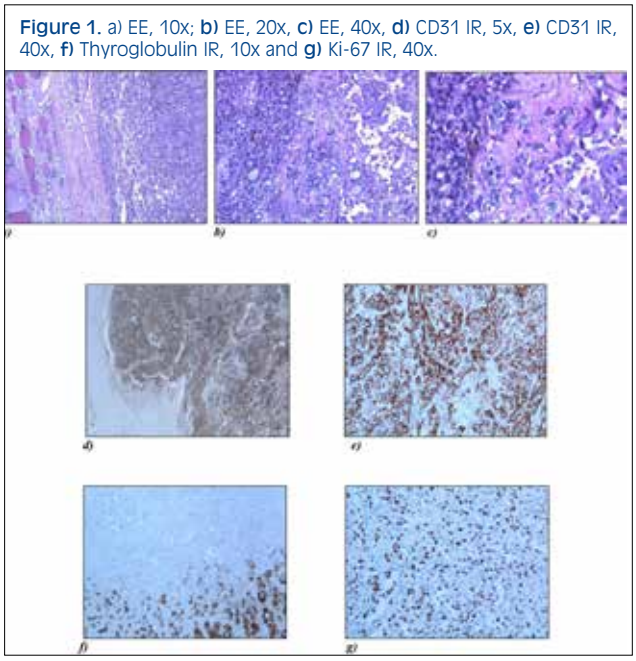

**Figure 1.** a) EE, 10x; b) EE, 20x, c) EE, 40x, d) CD31 IR, 5x, e) CD31 IR, 40x, f) Thyroglobulin IR, 10x and g) Ki-67 IR, 40x.

**Conclusions.** There has been considerable controversy about the true existence of thyroid angiosarcomas. Some authors opine that most of the reported cases were actually anaplastic or undifferentiated carcinomas with angiomatoid features and their epithelial nature can be demonstrated after examination of multiple sections. Mills et al. believe that epithelioid angiosarcomas are ''transitional'' tumors with both epithelial and endothelial differentiation displaying variable mesenchymal neometaplasia, being analogous to ''carcinosarcomas'' of the female genital tract, gastrointestinal tract, and head and neck regions. However, the expression of cytokeratin by endothelial cells is likely to be genuine, because non neoplastic endothelial cells and epithelioid vascular neoplasms in other anatomic sites have expressed cytokeratin. The expression of cytokeratin and close morphologic mimicry of epithelioid angiosarcomas with anaplastic carcinomas highlight the importance of using a broad panel of immunohistochemical stains in the diagnostic workup of these tumors. To date, CD31 is considered the most sensitive and specific marker for endothelial differentiation, being expressed in 90% of angiosarcomas.

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## ECTOPIC PARATHYROID MICROSCOPIC THYMOMA IN A PATIENT AFFECTED BY MEDULLARY CARCINOMA OF THE THYROID: A DIAGNOSTIC PITFALL.


A. Ronchi[1], M. Montella[1], I. Panarese[1], G. Toni[1], G. Roccuzzo[1], S. Lucà[1], M. Nasti1, E. La Mantia[1], F. Zito Marino[1], I. Cozzolino[1], R. Franco[1]

[1] *Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy*


**Objectives.** Ectopic thymic tissue is rarely found in parathyroid location. The pathogenesis of the ectopic parathyroid thymus is not completely elucidated, but it may be due to the incomplete migration of thymus during embryogenesis. An intimate commingling between parathyroid and thymic tissue has been sometimes reported, up to cases of really direct fusion of parathyroid and thymic tissues (thymus-parathyroid unit). Herein, we describe a case of microscopic thymoma developed in ectopic parathyroid thymus, in a patient affected by medullary carcinoma of thyroid.

**Materials and Methods.** We describe a case of 53 years old woman, who had undergone to Thyroid Surgery Unit, due to the presence of a nodule in right lobe of thyroid, associated to conspicuous elevation of serum calcitonin. The patient underwent total thyroidectomy and selective central compartment lymph-node dissection.

**Results.** Gross examination of the thyroid confirmed the presence of a nodule in the right lobe, measuring 2.2 centimeters. Microscopic and immunohistochemical features of the neoplasm were consistent with the diagnosis of medullary carcinoma of the thyroid. Microscopic examination of the central compartment tissue showed a complex commingling of thymic, parathyroid and lymph- node tissue. In the context of the thymic tissue, a small, unencapsulated nodule of oval and spindle epithelial cells was evident. The differential diagnosis included a metastasis by medullary carcinoma and a microscopic thymoma occurred in ectopic parathyroid location. This latter was our diagnosis.

**Conclusions.** Ectopic parathyroid thymus is a rare entity, with unknown exact epidemiology and pathogenesis. Very rare case of papillary carcinoma of thyroid metastasizing to ectopic cervical thymus have been reported. On the other side, the ectopic thymic tissue may undergo the same pathological abnormalities, like the normal thymus. The occurrence of microscopic thymoma is an ectopic parathyroid thymus is an exceeding rare event, which complicates the diagnostical evaluation of a patient affected by medullary carcinoma of the thyroid.

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# PATOLOGIA FETOPLACENTARE

## MEDIAL CALCIFIC SCLEROSIS OF PLACENTAL VESSELS AS A PUTATIVE PATHOGENESIS OF FETAL THROMBOTIC VASCULOPATHY

A. Bonsignore[1], F. Buffelli[2 3], F. Pesce[2], V.G. Vellone[4], R. Paoletti5, E. Fulcheri[2 4]

*[1] Legal and Forensic Medicine Unit, Dept. DISSAL, School of Medical and Pharmaceutical Sciences, University of Genova, Italy; [2] Fetal-Perinatal Pathology Unit, IRCCS-Istituto Giannina Gaslini, Genova, Italy; [3] Ph.D course in Paediatric Science, Fetal Perinatal and Paediatric Pathology, University of Genova, Italy; [4] Histhopathology Unit, Dept. DISC, School of Medical and Pharmaceutical Sciences, University of Genova, Italy; [5] Department of Obstetrics and Gynaecology, IRCCS-Istituto Giannina Gaslini, Genova, Italy*

**Introduction.** Fetal Thrombotic Vasculopathy (FTV) is a pathological entity of the placenta clearly identified and nosographically well known so far. This is a quite common lesion of main amniocorial or stem vessels of the placenta, which may cause significant consequences on the fetus or, in extreme cases, able to determine the death. Particularly, it deals with endoluminal thrombi of different sizes which must show some specific characteristics in order to be properly diagnosed (first of all they should be at different ages and stages). Precisely, these characteristics make it possible to perform a differential diagnosis between FTV and post-mortem vascular modifications.The injuries occurring during the third pregnancy quarter determine a small cluster of terminal villi lacking fetal capillaries and showing bland dense collagenization of the villous stroma, while on the fetus they are responsible for variable damages: among them, ischemic lesion to the Central Nervous System (CNS). In recent years the attention of neonatologists and gynecologists has been progressively increased in an attempt to identify early lesions in the form of anomalies or alterations in placental flows or to identify predisposing or causal maternal conditions of FTV. The relevance of this argument is confirmed by the fact that Armed Forces Institute of Pathology (AFIP), in the third volume of "Placental Pathology", has devoted 11 pages discussing such a topic. From the pathological anatomy perspective, the lesion or its evolving phases are easily identifiable. Significant difficulties and great uncertainties, however, still do exist in the definition of lesions etiopathogenesis.

**Materials and Method**. In the IRCCS Giannina Gaslini Institute of Genova, Italy, from 2015 to 2017, 9 cases of FTV (5 males and 4 females) were diagnosed. The cases had different gestational ages, particularly: 1 case at 41 weeks, 2 cases at 39 weeks, 1 case at 38 weeks, 2 cases at 34 weeks, 1 case at 31 weeks and 1 case at 30 weeks (dichorionic diamniotic twin pregnancy). In the considered cases, one has suffered brain damage while others (most recently diagnosed) have not yet completed the long-term follow-up. The above-mentioned case presented a dramatic picture of thrombosis of the upper right arm vessels with consequent areas of ischemic necrosis of the muscles and skin; extensive bilateral hemispherical malacic lesions also coexisted, while histopathology of placenta did not reveal other specific pathological conditions. As single cases of autoimmune maternal disease are reported in the literature, while some others have not yet been identified with certain pathogenetic factors, it appears interesting to point out two cases showing a Medial Calcific Vasculopathy (MCV) of amniochorial, stem and cord blood vessels. MCV, also called medial calcific sclerosis or Muncheberg sclerosis, consists in the calcification of the internal elastic lamina and the medial tunica of muscular arteries without significant involvement of the intima or lumen of the vessel. As it is considered associated with a dysregulation of calcium mobilization, it is more commonly associated with chronic kidney damage, hyperparathyroidism, osteoporosis or diabetes mellitus in the adult. Histological features are generally as follows: Dystrophic medial calcification of small to medium-sized muscular arteries, typically centered on internal elastic lamina (IEL). Calcification may be focal, nodular, or extend circumferentially mild to moderate intimate thickening may be present disruption or reduplication of IEL sometimes seen inflammation generally absenti in two of the above nine cases of FTV, a MCV was found. Particularly: 31-year-old woman at the 41st gestational week, with a history of a previous abortion, with development of gestational diabetes confirmed at histological examination; 40-year-old woman, at the 38th gestational week, primipara, with disentanglement/dismetabolism disorder. The lesion was studied through immunohistochemistry (CD34 and CD31) in order to demonstrate the integrity of the endothelium above calcification areas; Desmin and Smooth Muscle Actin, at the same time, demonstrated the injuries to the muscle component in the tunica media.

**Discussion.** FTV is now considered to be an emerging pathology that draws attention not only to pathologists and researchers but also to clinicians involved in the care process of the infant who often has serious injuries to the CNS as well as to the Medical Doctors for the obvious repercussions of such a damage about subject's health and validity. The contribution, even isolated, of some case reports may be important to share information, between the scientific community, about the pathogenesis of injuries which are still confused and doubtful. In placental histopathology, the calcification of the vessel wall is usually associated with vascular thrombosis affecting both cord blood and amniochorial vessels; this represents, together with the calcification of the thrombus, the evolution of a life-threatening pattern. In the presented cases, the calcification of the tunica media of the placental vessels was located not only in the thrombotic areas but also in far regions which showed an intact endothelium. In other areas, the endothelium was damaged with fibrin deposition and inflammatory cells: in these cases vascular damage was associated with calcific medial sclerosis evolving in major disorders with endothelial damage and initial thrombotic phenomena. Endothelial damage was therefore the triggering cause of thrombi formation that outlined the overall aspect of FTV. In both cases, the pathogenetic hypothesis was confirmed by the fact that – at level of the most important thrombotic lesions – there were neither wall damage nor calcification. If it is not possible to find a certain cause for MCV, it may be relevant in the placental pathology to associate it with maternal diabetes or abnormal mobilization of calcium.

**Conclusions.** It is believed that MCV can be considered, besides maternal autoimmune disease and vessel calibre alteration, one of the causes of FTV. In the presented cases, the association with a maternal dismetabolic state is also noteworthy.

## SUDDEN INTRAUTERINE UNEXPLAINED DEATH SYNDROME AND SUDDEN INFANT DEATH SYNDROME. IT'S TIME TO CHANGE

M.R. Campanino[1], A. Travaglino[1], M. Paternoster[2], G. Vitagliano[1], V. Graziano[2], F.P. D'Armiento[1], M. D'Armiento[1]

*[1] Department of Advanced Biomedical Sciences, Pathological Anatomy Section, University Federico II, Naples, Italy; [2] Department of Advanced Biomedical Sciences, Legal Medicine Section, University Federico II, Naples, Italy.*

**Aims.** On 7 October 2014, the Minister of Health signed the

decree of approval of the diagnostic protocols on 22 November 2014 (G.U. n. 27) to complete the provisions of the law of 2 February 2006, n. 31 about adoption diagnostic protocols "Discipline of the diagnosis of the victims of sudden infant death (SIDS) and of unexpected death of the fetus" (G.U. No. 34 of 10 February 2006). Indeed the Stillbirths contribute substantially to perinatal mortality in developed countries with a prevalence ranging between 4 and 6 per 1000 births. Despite careful evaluation during pregnancy of fetal well-being, about 25–50%, remains without a clear cause, coding for a new entity called Sudden Intrauterine Unexplained Death Syndrome (SIUDS). Moreover, an important cause of early sudden death is represented by sudden infant death syndrome (SIDS), which is still considered the most important basis of mortality during the first year of life [1-3]. The term sudden infant death syndrome (SIDS) was first proposed in 1969 in order to focus attention on a subgroup of infants with similar clinical features whose deaths occurred unexpectedly in the postnatal period. Today the definition of SIDS refers to death in a seemingly healthy infant younger than 1 year of age whose death remains unexplained after a thorough case investigation including a complete autopsy, review of medical and clinical history, and death scene investigation [4-6].

**Materials and Methods.** Between 1 January 2014 and 13 August 2018, at Department of Pathology, University of Naples Federico II, Italy, 423 perinatal diagnostic findings were made, of which 5 referred to newborns, 92 referring to fetuses after the 25th week. Of these 18 were requested by external structures, in light of the decree law of October 2014 concerning the unexpected child death and the fetus that makes it mandatory diagnostic confirmation. The cases that came to our observation from external structures consisted of a newborn and 17 fetuses. The newborn turned out to be a carrier of a congenital disease; the fetuses were found suffering from viral diseases, serious placental infections. The diagnostic findings were made according to the diagnostic protocol "diagnostic check"on deceased fetuses also without apparent cause after the twenty-fifth week of gestation pursuant to art. 1, paragraphs 1 and 2 of the law February 2, 2006 n. 31. Unfortunately, the Campania Region has not yet implemented the implementing decrees and our institution is the only one, for the moment, on the road to accreditation. The diagnostic protocol of the SIDS included: general framework and method of study of unexpected infantile sudden death (SUID-SIDS), SUID medical-legal investigation, unexpected infantile sudden death (SUID), infectious molecular diagnosis in SUID cases, assessment toxicology, genetic examination in SIDS victims, genetic counseling in SIDS, further genetic and cytogenetic investigations to be carried out in case of suspected SIDS [7]. All diagnostic findings were made after seeking parental consent and all cases of SIUD were referred to patients who died in health care facilities. 8 cases were sent to other structures. The deceased (SIDS-SIUD) at home have all followed the process of judicial autopsy because of Campania Region has not yet promulgated the implementation of decrees law and it has not yet identified a reference working group of experts.

**Conclusions.** The diagnostic finding of all these cases is the prerogative of the pathologist of the pathologist, the coroner intervenes only in case of suspicion of crime; this implies that where the law is correctly applied, on cases of deaths outside the healthcare environment, the medical examiner almost always intervenes. In order to make this organizational model fully operational, the involvement of the judiciary and law enforcement agencies that can intervene on the place of death is demonstrated by means of adequate awareness and on the basis of a shared training path aimed at building a relationship of profound trust between the operators. Furthermore, in order to ensure the diagnostic confirmation by competent personnel on SUID/SIDS cases it is necessary to stipulate an intervention protocol with the competent local Public Prosecutor's office which ensures a homogeneous and quality path based on the indications contained in this document also on the basis of existing regional experiences. This is only implemented in some Italian regions.

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## A UNIQUE CASE OF PLACENTATIONDISORDER: PLACENTA ACCRETA AND TOTAL PLACENTA PREVIA WITH CONCOMITANT EPS


V. Fiorentino[1], M. Palumbo[1], F. Pierconti[1], S. Triunfo[2], I. Pennacchia[1], V. Arena[1]

*[1] Institute of Pathology; [2] Institute of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Fondazione Policlinico A.Gemelli Rome, Italy*


**Background.** Massive obstetric hemorrhage is still the leading cause of pregnancy-related death, and placenta previa accreta remains one of the major predisposing factors. With the increasing rates of cesarean delivery and uterine curettage for abortion, both placenta previa and accreta are steadily increasing in frequency. Therefore, more cases of placenta previa accreta can be expected in obstetric practice [1]. In several recent series, placenta accreta has emerged as the major indication for peripartum hysterectomy, accounting for 40% to 60% of cases [2]. This clinical case report describes a unique case of placentation disorder which took the form of placenta accreta and total placenta previa with concomitant EPS. Exaggerated placental site (EPS) usually presents as an incidental microscopic finding in curettages after spontaneous or therapeutic abortion, but may also be seen in otherwise normal pregnancies. Histologically, it is characterized by exuberant infiltration of the myometrium by intermediate trophoblasts at the implantation site: it presents as increased invasive interstitial trophoblast of the inner third of the myometrium, sometimes with atypia, especially when associated with complete hydatidiform mole. Cords, nests, and diffusely infiltrative individual trophoblast cells may be seen, but there are no confluent masses of cells. Reliable quantitative histologic criteria are lacking, but non molar cases have a Ki-67 proliferative rate near zero. EPS is benign and asymptomatic, with no risk of recurrence per se, but must be distinguished from trophoblastic tumors and placenta increta [3].

There have been few reports describing its clinical course and the ultrasonogram and magnetic resonance imaging (MRI) characteristics have not been reported previously.

**Objectives.** We herein report an unusual case of placenta accreta and total placenta previa with concomitant EPS.

To the best of our knowledge, this is the first reported case of this combination of conditions: both clinicians and pathologists must be aware of this rare possibility to assure the patient a correct management and really effective therapeutic options.

**Material and Methods.** Surgical specimens were submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques, embedded in paraffin and stained with hematoxylin and eosin. Immunohistochemical staining was used to examine the expression levels of Ki67.

**Results.** A 51-year-old primigravida was referred to our tertiary hospital for further management, because of bleeding and placenta previa centralis.

The patient had a history of uterine leiomyomas, for which she underwent two LPT myomectomies in 2011 and 2015. Balloon occlusion of main hypogastric arteries trunks was performed without immediate complications. A caesarean delivery was carried out and the fetus was extracted alive and vital. Manual removal of the placenta was difficult and the placenta was extracted fragmented because of the presence of areas of strong adhesion without a complete cleavage plan between placenta and myometrium. However, due to the concomitant presence of bleeding and uterine atony, we performed a total hysterectomy and bilateral salpingectomy with ovarian preservation as the best option for survival. The resected uterus measured 13.5x24.3x12.3 cm, was edematous and presented an area of parietal thinning and partly hemorrhagic appearance at the level of the body, posteriorly. Histological examination showed an edematous myometrium in an organ with placental residues which, at the level of the samples taken at the posterior wall, showed villi in close continuity with the myometrium without decidual interposition as by accretism. Moreover, in some samples the presence of intermediate trophoblastic elements was mostly observed in single elements in close proximity/adhesion to myometrial fibers as from "exaggerated placental site" (EPS). The absence of positivity for Ki67 and the prevalence of trophoblastic elements in the form of individual elements confirmed this diagnostic orientation. Our case was diagnosed as EPS with evidence of placenta accreta according to the presence of villi in close continuity with the myometrium without decidual interposition.

**Conclusions.** Placenta accreta and increta often present as a morbidly adherent placenta, leading to postpartum hemorrhage. In high risk patients with prior caesarean section and/or low implantation, they may be diagnosed by ultrasound or MRI, with emphasis on an abnormal retroplacental zone and chaotic intraplacental blood flow (blood lakes). In contrast to EPS, morbidly adherent placentas may have interstitial and endovascular trophoblast in the outer half of the myometrium with a substantial risk of morbidity (or even mortality) and a definite risk of recurrence [3].

Nevertheless, Liu et al. 2013 [4], Takebayashi et al. 2014 [5] and Sidhu et al. 2018 [6] reported cases of EPS in term pregnancy presenting as postpartum hemorrhage: EPS should be considered in cases of postpartum hemorrhage caused by uterine inertia not responding to medical management. This should be confirmed by histopathological examination and followed up with serum beta-hCG levels. In our case, the concomitance of accretism, placenta previa and EPS may have exacerbated postpartum hemorrhage.

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## PLACENTAL METASTASES FROM MATERNAL LUNG CARCINOMA: A CASE REPORT


V. Fiorentino1, I. Pennacchia[1], S. Triunfo[2], S. D'Ippolito[1], V. Arena[1]

*[1] Institute of Pathology, [2] Institute of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Fondazione Policlinico A.Gemelli Rome, Italy*


**Background.** Cancer complicates 1 in 1000 pregnancies. Lung cancer, the leading cause of cancer deaths in males for decades, has recently become one of commonest causes for women too. Lung cancer is the second most common cancer type in women but it is also the most lethal. As women delay the start of their family, the co-existence of cancer and pregnancy is increasingly observed. Nevertheless, lung cancer during pregnancy remains a rather uncommon condition with less than 70 cases published in recent years. Non-small cell lung cancer (NSCLC) is the most common histological type accounting for 80–85% of all gestations lung cancer, 10–15% are small cell lung cancer (SCLC) and fewer that 5% are carcinoids of the lungs. Overall survival rates are low. Chemotherapy and/or targeted treatment have been used with poor outcomes. The disease has been also found to affect the products of conception with no short- or long-term consequences for the neonate.

**Objectives.** We herein report the clinicopathologic findings of a pregnant woman with poorly differentiated solid carcinoma of the lung with squamous differentiation and placental metastases.

**Material and Methods.** Surgical specimens were submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques, embedded in paraffin and stained with hematoxylin and eosin. Immunohistochemical staining was used to examine the expression of CAM5.2, AE1/3, p63, p40, CK7, TTF1, CDX2, chromogranin and synaptophysin.

**Results.** A 36-year-old primigravida was referred to our hospital for further management because of dyspnoea, cough and hemoftoe. A chest x-ray showed a right pulmonary hypodiaphany for which an empiric antibiotic therapy and corticosteroids were undertaken without benefit. Due to the persistence of the aforesaid symptomatology, various microbiological tests were carried out with negative results. A bronchoscopy was performed and showed a bilateral neoplastic flow infiltrating the main and lobar bronchi. Histological examination of biopsies demonstrated a poorly differentiated carcinoma, squamous histotype (CK AE1/AE3 and p40 positive, TTF1, CK5/6, chromogranin and synaptophysin negative). The subsequent chest CT scan without contrast confirmed the presence of a voluminous neoformation of the ower right lobe that measured 8 cm. At

30 weeks and 3 days she underwent a caesarean section and delivered a live infant. Her postpartum course was uneventful. The histological examination of the placental parenchyma documented the presence at the level of the intervillous space and close to the villous cytotrophoblast, of scattered micro-foci of metastatic cell groups from poorly differentiated epithelial neoplasia with solid growth pattern. Immunohistochemistry revealed a positivity of the malignant cells for CAM5.2, AE1/3, p63 and p40. CK7, TTF1, CDX2, chromogranin and synaptophysin were negative. This combination of markers identifies a squamous differentiation.

**Conclusions.** Lung cancer in pregnancy is rare and a few cases have been reported in literature. Placental metastases are extremely uncommon in these cases and can lead to fetal involvement by lung tumor. It is important to report all cases of lung cancer occurring in pregnancy with subsequent close clinical surveillance of the infant as all cases have a different clinical picture.

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# PATOLOGIA MAMMARIA

## 2007, 2013 AND 2018 ASCO/CAP HER 2 TESTING GUIDELINE IN BREAST CANCER, JUMP IN THE FUTURE OR RETURN TO THE PAST? A CASE REPORT

T. Addati, R. Nenna, V. Losito, D. Di Clemente, G. Elicio, A. De Lucia, C. D. Inchingolo

*Anatomic Pathology Unit, L. Bonomo, ASL BT, Andria, Italy*

**Beckground.** Currently the use of anti-HER2 directed therapy is established on the identification of HER2-postive breast cancers. Immunohistochemistry (IHC) and in situ hybridization (ISH) are the most common (and FDA-approved methods) for detecting HER2-positive breast cancers. While IHC and ISH are generally robust testing modalities, both can be subject to various pre-analytic, analytic and post-analytic issues/problems. In response to these issues/problems, ASCO and CAP formed a joint HER2 Guideline Committee. First released in 2007 and updated in 2013, the recommendations by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) human epidermal growth factor receptor 2 (HER2) testing Expert Panel are upgrade in 2018. What are the consequences in clinical practice?

**Aim.** We considered an emblematic case and classified it by applying the HER2 criteria of the guidelines of 2007 [1], 2013 [2] and finally the latest of 2017 [3].

**Methods.** On October 2015, a 74-year-old female patient with a 12x8 mm left breast nodule of the internal equatorial quadrant arrived to our attention. Biopsy was classified with diagnostic category B5 or positive for CDI (G2) sec. European Breast Guidelines 2006. Intraoperative evaluation, with OSNA method, of two sentinel lymph nodes of the left armpit was performed and both were positive to micrometastasis (+). Clinical decision was an equatorial left internal quadrantectomy without axillary dissection. HER2 status was assessed on biopsy and quadrantectomy sample both. Immunoistochemical panel of ER, PgR, and Ki-67 was assessed.

HER2 expression and amplification (FISH analysis) were conduct on definitive histological sample (using ASCO/CAP 2013 guideline).

**Results.** On definitive histological nodule were diagnosed a Moderately Differentiated Nas- infiltrating Ductal Carcinoma (G2, Elston-Ellis sec.) with a post-operative pathological staging: pT1c, N1 (sn), Mx - G2 -IIA Stadium (AJCC, 2010). The carcinoma presented a positive expression of ER (100%), PgR (<5%) and a proliferative index - Ki-67 - of 20% (Fig. 1).

According to HER2 status (Fig. 1), applying ASCO/CAP 2007 guideline [1], the protein score was (1+): weak / moderate intensity, incomplete membrane staining in the 60% of the tumor cells. HER2 IIC was Negative, you must not precede with Her2 molecular test.

Applying ASCO/CAP 2013 guideline [2], the protein score was (2+): weak / moderate intensity, incomplete membrane staining in the 60% (cut off was 30%) of the tumor cells. HER2 II resulted Equivocal, you must precede with HER2 molecular test. FISH test revelled an HER2/CEP17 ratio of 1.65, with HER2 copy number/cell = 5.22 and CEP17 copy number/cell = 3.16. FISH result would have been Negative according to 2007 guideline, but according to 2013 guideline it would become Positive, because even if HER2/CEP17 ratio was 1.65, HER2 copy number was ≥4 and ≤6 and another aspect you must evaluate: heterogeneity. In fact, the presence of an amplified population (HER ≥ 6) were assessed in 36% of the cells.

Applying ASCO/CAP 2018 [3] guideline this case remain HER2 immunoscore 2+: weak / moderate intensity, incomplete membrane staining in the 60% (cut off was 30%) of the tumor cells, and you must precede with HER2 molecular test. Paradoxically, FISH test would return Negative with a comment [3], in fact our case would belong to Group 4 (Figg. 2-3).

**Conclusions.** Finally, according to 2007 guideline our case would be a double negative (IIC = 1+; FISH = not amplified), the patient would not be a candidate for Trastuzumab therapy.

According 2018 guideline our case would remain equivocal to the IIC (2+) but would return negative (with a comment) to the FISH, so the patient would not be a candidate for Trastuzumab therapy.

According 2013 guideline our case was equivocal to IIC (2+) and positive to FISH, the patient was treated with hormone therapy, chemotherapy and immunological (trastuzumab) therapy and her follow-up is good (2015-2018 no relapse).

Where is the truth?

**Figure 1.** ER (100%), Ki-67 (20%) and HER2 (2+).

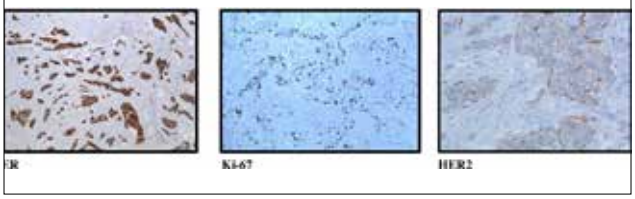



**Figure 2.** Group 4, according ASCO/CAP 2018.

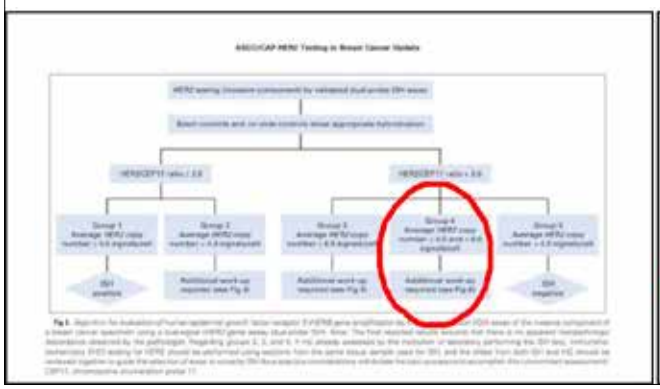
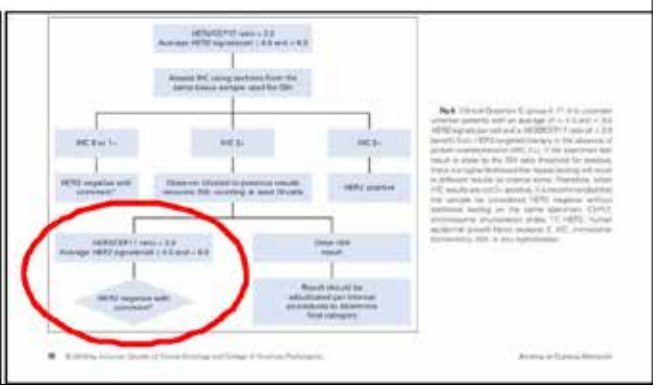


**Figure 3.** Reclassification of Group 4, according ASCO/CAP 2018.



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## THE LONG NON-CODING RNA *HOTAIR* IS A MARKER OF TAMOXIFEN RESISTANCE IN POSTMENOPAUSAL WOMEN WITH HORMONE RECEPTOR- POSITIVE EARLY BREAST CANCER

M. Cantile[1], F. Collina[1], G. Aquino[1], M. Brogna[1], S. Cipolletta[1], M. De Laurentiis[2], G. Botti[1], M. Di Bonito[1]

*[1] Pathology Unit, Istituto Nazionale Tumori-Irccs-Fondazione G.pascale, Naples, Italy; [2] Department of Breast Surgery and Cancer Prevention, Istituto Nazionale Tumori- Irccs-Fondazione G.pascale, Naples, Italy*

**Background.** LncRNAs are an emerging class of regulatory RNAs abnormally expressed in many tumor diseases. Recently many studies have reported that drug resistance is strongly modulated by lncRNAs through changing genomic stability and promoting the translation of genes involved in cell survival, proliferation, and drug metabolism [1]. HOTAIR is the first lncRNA able to promote tumor progression and associated with poor prognosis in breast cancer (BC) [2] and its aberrant expression is strongly related to drug resistance, both to chemotherapy and biological drug [3-9], in different solid tumors, including breast cancer [10].

**Aim.** A recent *in vitro* study highlighted that HOTAIR expression was increased in tamoxifen- resistant breast tumor cells compared to primary, hormone-naïve tumor cells [10]. To verify its potential role in the acquisition of tamoxifen resistance we decided to analyze its expression on patients who developed resistance within two years, comparing them with those without disease progression.

**Methods.** We selected a large series of hormone receptor-positive early breast cancer patients included in a multicentre, open-label, randomised, phase 3 trial named "FATA-GIM3". Patients were treated with oral tamoxifen (20 mg per day) for 2 years followed by oral administration of one of the three aromatase inhibitors for 3 years (switch strategy). We made a TissueMicroArray with the selected samples and analyzed the expression of the lncRNA HOTAIR with an RNA probe (RNAscope) using ISH method. The expression of HOTAIR has been correlated not only with the acquired resistance to tamoxifen, but also with the other clinical-pathological parameters and with the survival of BC patients.

**Results.** HOTAIR has an expression level varying between 0 to > 10 copies per cell. We used a semi-quantitative scoring utilizing the estimated number of dots present within each cell boundary, and we categorized staining into 4 scores as previously reported [11]. *HOTAIR* is highly upregulated in the tumors of tamoxifen-resistant breast cancer patients compared to tissues from without disease progression patients. Moreover**,** HOTAIR high expression is strongly correlated with grade, lymph nodes and distant metastases, and poor survival of BC patients.

**Conclusions.** HOTAIR is an important prognostic marker in postmenopausal women with hormone receptor-positive early breast cancer and its aberrant expression is able to identify patients who develop resistance to endocrine therapies. Since lncRNAs are molecules with high stability in biological fluids [12] the detection of HOTAIR in the blood of BC patients could represent a useful prognostic tool especially for the prediction of therapeutic response and for monitoring of treatment.

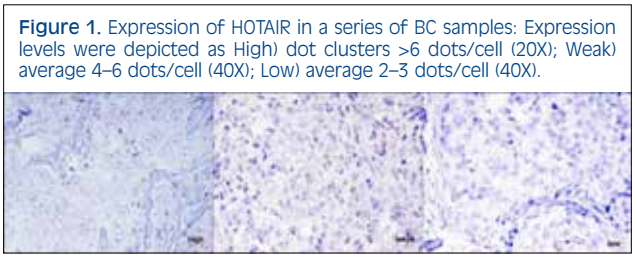

Figure 1. Expression of HOTAIR in a series of BC samples: Expression levels were depicted as High) dot clusters >6 dots/cell (20X); Weak) average 4–6 dots/cell (40X); Low) average 2–3 dots/cell (40X).

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## P16/CDKN2A DELETION AND VIMENTIN IMMUNOREACTIVITY: PUTATIVE MARKERS OF INCREASED AGGRESSIVENESS IN TRIPLE-NEGATIVE BREAST CANCER.

E. Cascardi, C. Caporusso, C. Vicenti, M.G. Mastropasqua, F. Caputo, C. Giardina, A. Napoli, G. Serio, E. Maiorano

*Anatomia Patologica, Dipartimento dell'Emergenza e dei Trapianti d'Organo (DETO) – Università degli Studi di Bari Aldo Moro – Bari*

**Introduction.** Triple-negative breast cancer (TNBC) is a heterogeneous group of malignancies sharing lack of estrogen receptor (ER) and progesterone receptor (PR) expression, and of human epidermal growth factor receptor 2 (HER2) overexpression or amplification in tumor cells. It represents only a small percentage (10-24%) [1-3] of all breast tumors, but the management of TNBCs still is a challenge because of its aggressive clinical course and poor prognosis, with short time to relapse and metastatic spread [2 4] Conventional chemotherapy, both in the neoadjuvant and adjuvant settings, remains the first treatment option for most TNBC patients, since they cannot benefit of hormonal and anti-HER2 targeted therapies [5]. Nevertheless, not all TNBC patients fully respond to standard therapeutic regimens and many of them tend to develop drug resistance [6]. Histologically, the majority of TNBCs are grade 3 invasive ductal carcinomas of no special type (NST) [5 7 8] with basal-like features, expression of basal cytokeratins (CK5/6, CK14, CK17), EGFR, and mesenchymal markers such as caveolin 1 and 2 and vimentin [3 7 8]. Omics technologies shed more light onto the biological complexity of this breast cancer subgroup, helping to clarify the molecular basis of TNBC and to identify novel targetable biomarkers, to possibly improve patients' outcome [6 9-11]. $p16^{INK4A}$, encoded by the CDKN2A gene at 9p21, is an important tumor suppressor, which controls cell cycle progression by inhibiting the CDK4/6-mediated phosphorilation of the retinoblastoma protein (pRb), thus preventing the start of the G1/S phase transition. Homozygous deletion of $p16^{INK4A}$ in breast cancer has been associated with unfavorable tumor features, such as high grade, high proliferation rates, lymph node metastases and triple- negative phenotype [12]. Loss of p16 expression also has been linked to breast cancer stem cell features and neoadjuvant chemotherapy resistance in TNBC [13]. Interestingly, it has been reported that breast cancer stem cells, a small cell population with the ability of self-renewal and differentiation potential, contribute to therapeutic resistance and to increased metastatic potential, by facilitating epithelial-mesenchymal transition (EMT) [14].

**Objective.** The aim of this study was to assess the presence of p16/CDKN2A deletion in TNBC histologic sections and to correlate it with the immune-expression of vimentin, one of the key genes regulating EMT and tumor aggressiveness in breast cancer.

**Materials and Methods.** Twenty 4% formalin-fixed, paraffin-embedded tissue specimens of TNBC, diagnosed from January 2015 to January 2016 at the Operative Unit of Pathological Anatomy of the Policlinico of Bari were selected for the study. p16/CDKN2A deletion was analyzed by dual-color fluorescent in-situ hybridization (FISH) for p16/CDKN2A and chromosome 9 (CEP-9); homozygous deletion was defined as >30% of nuclei showing no p16/CDKN2A and at least one CEP-9 signal. Immunohistochemistry was used to evaluate the expression of vimentin: cytoplasmic staining in tumor cells was considered positive.

**Results.** FISH analysis for p16/CDKN2A revealed that 80% (16/20) of TNBC samples showed homozygous deletion. Strong cytoplasmic vimentin positivity was detected in 55% (11/20) of cases. Interestingly, 45% (9/20) of tumors with loss of functional p16 also showed vimentin positivity. One breast cancer patient with bilateral vimentin-positive tumors, showed p16 deletion in one tumor only.

**Conclusions.**

- FISH analysis highlighted consistent p16/CDKN2A homozygous deletion in TNBC, which may facilitate epithelial / mesenchymal transition of tumor cells
- Vimentin immunoreactivity frequently is associated with p16/CDKN2A homozygous deletion in TNBC
- The simultaneous occurrence of p16/CDKN2A homozygous deletion and vimentin- positivity may correspond to increased aggressiveness and therapeutic resistance in TNBC.

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## THE ROLE OF CD73 IN PREDICTING THE RESPONSE TO NEOADJUVANT TREATMENT IN TRIPLE NEGATIVE BREAST CANCER

B. Cerbelli[1], A. Botticelli[2], A. Pisano[1], M.G. Pignataro[1], G. Naso[3], M. Monti[4], P. Ascierto[5], L. Costarelli[6], V. Magri[3], Maria Mauri[7], D. Campagna[6], M. Nuti[8], L. Fortunato[9], C. Della Rocca[1], G. D'Amati[1], P. Marchetti[2]

*[1] Department of Radiological, Oncological and Pathological Sciences, Sapienza, University of Rome, Rome, Italy; [2] Oncology Unit, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy; [3] Oncology Unit, Sapienza University of Rome, Rome, Italy; [4] Department of Surgical Sciences, Sapienza University of Rome, Rome, Italy; [5] Melanoma, Cancer Immunotherapy and Development Therapeutics Unit, Istituto Nazionale Tumori-IRCCS Fondazione "G. Pascale", Naples, Italy; [6] Department of Pathology, San Giovanni-Addolorata, Rome, Italy; [7] Department of Oncology, San Giovanni-Addolorata, Rome, Italy; [8] Cell Therapy Unit and Laboratory of Tumor Immunology, Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy; [9] Breast Unit, San Giovanni-Addolorata, Rome, Italy*

**Objectives.** Immune system plays a key role in tumor surveillance and escape. Tumor infiltrating lymphocytes (TILs)

and PD-L1 seem to predict clinical outcome and pathological response (pR) after neoadjuvant chemotherapy (NAC) in breast cancer [1 2]. Recently, CD73 has been proposed as a prognostic biomarker associated with better disease-free survival and overall survival in patients with triple negative breast cancer (TNBC) [3]. CD73 catalyzes the conversion of AMP to adenosine, which leads to an immunosuppressive microenvironment. Indeed, adenosine inhibits CD8+ lymphocytes and dendritic cells while enhancing M2, myeloid suppressor cells and Treg activity [4].

We aimed to investigate the role of CD73 and PD-L1 expression, as well as the percentage of stromal TILs, in predicting the pathological response to NAC in TNBC. To this purpose we retrospectively analysed 61 pre-NAC biopsies, comparing CD73 and PD-L1 expression on both neoplastic and inflammatory cells and stromal TILs with the response to neoadjuvant chemotherapy.

**Materials and methods.** We enrolled 61 pts with TNBC who had received NAC (EC for 4 cycles followed by Paclitaxel q7 for 12 cycles or q21 for 4 cycles) between January 2013 and June 2017 at our Institutions. We performed immunohistochemistry for PD-L1 (Ventana SP142 clone), CD73, CD20, CD3, CD4, CD8, CD68 and N-CAM, in basal paraffin-embedded biopsies. The expression of both CD73 and PD-L1 on tumor cells was evaluated quantitatively (percentage of positive cells) and qualitatively (intensity of staining). The percentage of tumor-infiltrating immune cells positive for PD-L1 and CD73 was also recorded. Statistical analysis was performed with Mann Whitney test, univariate, and multivariate logistic regression models.

**Results.** The median age of the patients was 49 y (range 28-74). In most of the patients (59 cases, 96.7%) the diagnosis was ductal carcinoma NST, G3. The clinical stage before NAC was as follow: cT1 13.1% (8 pts), cT2 75.4% (46 pts), cT3 4.9% (3 pts), cT4 6.6% (4 pts). Thirty-two patients (52,5%) had nodal disease at presentation. After NAC 29 patients (47.5%) underwent mastectomy and 32 (52.5%) conservative surgery. Twenty-three patients (38%) showed pathological complete response (pCR). The median expression level of CD73 on tumor cells in pre-NAC biopsies was 40%. In 29 cases (48%) the percentage of positive cells was under and in 32 cases (52%) over this value ("low CD73" and "high CD73", respectively). Five out of 61 patients presented PD-L1 expression levels higher than 25%, a cut-off value that significantly predicts the pCR according to our previous results [5].

**Fourteen** out of the 29 "low CD73" patients (48%) achieved pathological complete response (pCR); on the contrary only 9 out of 32 "high CD73" patients (28%) showed pCR. Four out of 5 patients (80%) with high PD-L1 expression achieved pCR. At univariate analysis a significant association was found between pCR and both CD73 (p=0.011) (expressed as continuous variable) and PD-L1 (p=0.035). At multivariate analysis a significant association was found only between pCR and CD73 expression (*p =0.027*) while no association was found with TILs and PD-L1.

**Conclusion.** Low CD73 expression seems to be associated with pCR in TNBC. This result extends our previous observations on the association between high PD-L1 value and pCR, providing further insights on the role of immune environment in TNBC. Moreover, these preliminary results suggest the possibility of using CD73 inhibitor plus anti-PD-1 in those patients with high expression of CD73.

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## CYSTIC ADENOMYOEPITHELIOMA OF THE BREAST WITH TUBULAR AND SOLID PATTERN


G. Cerrone, C. Rossi, G. Senes, R. Murru, G. Faa, A. Ravarino

*Department of Surgical Science, Division of Pathology, University of Cagliari, Cagliari, Italy*


**Background and Aims**. Adenomyoepithelioma (AME) of the breast is a relatively rare biphasic tumour, usually benign, but rarely associated to malignant transformation of the epithelial or myoepithelial component [1]. AME of the breast affects predominantly adult women of all ages. It is characterized by the proliferation of glandular epithelial and myoepithelial cells, representing one of the myoepithelial lesions of the breast. These lesions were classified by Tavassoli in 1991 into myoepitheliosis, malignant myoepithelioma and AMEs, with the latter being further divided according to the growth and cytological pattern into tubular, spindle cell and lobulated type [2]. Immunohistochemical reactivity for anti-p63, anti-smooth muscle actin (SMA), anti-calponin, anti-34βE12, anti-cytokeratin 5/6 (CK5/6), anti-CK14, anti-S100 and anti-CD10 antibodies has been described in myoepithelial cells[1]. Epithelial cells are immunostained with anti-CK8/18, anti-CK7 and anti-EMA antibodies[3]. Here we report a case of cystic AME with a tubular and solid pattern in a 37-years-old Indian woman.

**Matherials and Methods**. A 37-years-old woman was referred to our hospital complaining of a lump in the external upper quadrant of the right breast. Clinical and ultrasound examination revealed a cystic lesion with coarse margins of 15x12x10 mm almost entirely filled with an oval hypoechoic vascularised mass. Following the pathological examination of the needle core biopsy of the mass, a lumpectomy was performed.

**Results.** Microscopically, the needle core biopsy displayed areas of connective tissue surrounding small nests of epithelial and myoepithelial cells showing no atypia, mitosis, necrosis nor tumour infiltrating lymphocytes. The described cells were immunoreactive with anti-CK AE1/AE3, anti-p63, anti-CK5, anti-estrogen receptor (ER), anti-S100 and anti-CK8/18 antibodies. Morphological and immunohistochemical features resembled a probably benign myoepithelial lesion. Macroscopic examination of the surgical specimen revealed a 15 mm cyst filled with a tumour, localized close to the medial and the deep margin of resection. Microscopically, the cyst was lined by cuboidal cells and surrounded by a partial fibrous capsule. The tumour, even though quite well circumscribed, focally extended through the fibrous capsule and into the adjacent mammary gland (Fig. 1A). It exhibited a partially solid pattern (Fig. 1B), in the presence of a prevalent tubular pattern. It was composed by eosinophilic cells, sometimes with clear cytoplasm. Mild cytologic atypia was observed, in the absence of mitoses and necrosis. Immunohistochemical evaluation revealed a lesion of biphasic nature,

resulting epithelial cells positive with anti-CK7, anti-CK8/18 (Fig. 1C), anti-ER and anti-progesteron receptor antibodies and myoepithelial cells with anti-CK5 (Fig. 1D), anti-SMA, anti-S100, anti-p63 and anti-CD10 antibodies. Both components were immunostained with anti-CK AE1/AE3 antibody. Tumour cells were not immunoreactive neither with anti-desmin antibody nor with anti-CD34 antibody. We diagnosed a cystic AME with tubular and solid pattern.

**Conclusions.** AMEs of the breast are uncommon, usually benign tumours, with a wide heterogeneity at the gross and microscopic pathology. In the literature, AMEs arising in small cysts were observed in a minority of cases [3]. Here we described a case of cystic AME, with mixed tubular and solid pattern. The latter, composed of nests of variable dimensions, may be the result of a significant myoepithelial proliferation occluding or compressing ducts, resulting in their microscopic absence[4]. The solid pattern with a haphazard mixture of epithelial and myoepithelial cells may also be related to the proliferation of the common suprabasal progenitor cells, which can differentiate into both components with no duct formation [4]. The diagnosis of AME in a needle core biopsy may be challenging because of its microscopic variability. In our case, the presence of cellular nests without evident ducts surrounded by collagenous tissue could have led to the wrong diagnosis of invasive carcinoma of the breast of no special type, basal-like carcinoma or malignant myoepithelioma of the breast. However, those diagnoses were excluded because of the described morphological and immunohistochemical characteristics of neoplastic cells conversely supporting the diagnosis of a benign myoepithelial lesion.

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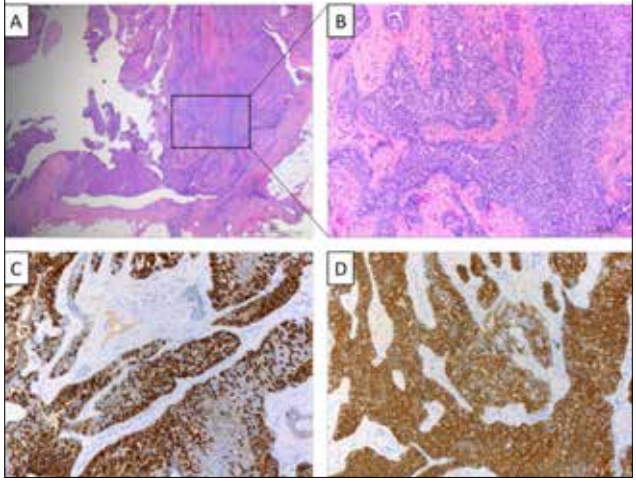
Figure 1. Microscopic examination showing cell proliferation inside a cyst, surrounded by partial fibrous capsule invaded by the proliferation (A). Tumour cells with mild atypia and no mitoses disposed in tubular and solid pattern (B). Biphasic nature of the lesion revealed by immunohistohemistry: epithelial cells reacting for anti-CK8/18 antibody (C) and myoepithelial cells staining with anti- CK5 antibody (D).




## THE ROLE OF BREAST CANCER SCREENING IN EARLY DETECTION OF POTENTIAL AGGRESSIVE DCIS IN YOUNG WOMEN


L. Domeniconi[1], S. Tramontozzi[1], G. Saguatti[2], M.C. Cucchi[3], M.G. Cattania[1], M.P. Foschini[1].

*[1] Department of Biomedical and Neuromotor Sciences, University of Bologna, Section of Anatomic Pathology, Bellaria Hospital, Bologna, Italy; [2] Unit of Senology, Bellaria Hospital, Bologna, Italy; [3] Unit of Breast Surgery, Bellaria Hospital, Bologna, Italy.*


**Background.** Ductal carcinoma in situ (DCIS) of the breast represents an heterogeneous group of neoplastic lesions confined to ductal-lobular unit, that differs in histological appearance and biological potential. DCIS constitutes about 20% of breast cancer cases. DCIS can be graded according to morphological features; cases showing high nuclear grade are related to higher risk to develop aggressive invasive carcinoma. A debate is ongoing in the medical literature about efficacy of breast cancer screening programs. Aim of the present study is to evaluate the DCIS incidence, according to patients age, in a series of screen detected breast cancers.

**Materials and methods.** All cases classified as B5 (of ductal type) on vacuum assisted biopsies (VAB) at Bellaria Hospital's Breast Unit between February 2014 and February 2018 were retrieved. Cases were divided into four groups according to the age (40-49, 50-59, 60-69 and >70). In each group, the number DCIS and invasive duct carcinoma (IDC) were evaluated. In addition, DCIS were further subdivided according to the nuclear grade.

**Results.** The total number DCIS and IDC was 680. Results are summarized in Table I.

**Conclusions.** The present data show that most of the screen detected cancers are DCIS, and most of the DCIS (81%) are high nuclear grade, therefore lesions that can transform into aggressive invasive carcinoma. The percentage of high grade DCIS is higher in the groups of younger women who undergo to breast cancer screening (groups 40-49 and 50-59). In older women the percentage of invasive IDC increases progressively, thus suggesting a development from a pre-existing DCIS.

In conclusion, the present data support the importance of breast screening programs.

The effectiveness of mammography screening for women ages 40 to 49 years still is questioned, and few studies of the effectiveness of service screening for this age group have been conducted. The effectiveness of mammography screening for women ages 40 to 49 years still is questioned, and few studies of the effectiveness of service screening for this age group have been conducted.

Table I.

| Group | n. cases | DCIS | DCIS g1 | DCIS g2-g3 | IDC |
|---|---|---|---|---|---|
| 40-49 | 158 | 108 (68,3%) | 33 (30,5%) | 75 (69,5%) | 50 (31,7%) |
| 50-59 | 177 | 110 (62,1%) | 24 (21,8%) | 86 (78,2%) | 67 (37,8%) |
| 60-69 | 210 | 97 (46,1%) | 8 (8,2%) | 89 (91,8%) | 113 (53,9%) |
| 70-74 | 135 | 59 (43,7%) | 6 (10,2%) | 53 (89,8%) | 76 (53,3%) |
| Total | 680 | 374 (55%) | 71 (19%) | 303 (81%) | 309 (45%) |

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## PROGNOSTIC ROLE OF TFEB, CARM1 AND SIRT1IN BREAST CANCER TREATED WITH CHEMOTHERAPY

N. Nardini[1], L. Viola[2], A.P. Londero[3], S. Bertozzi[4], C. Cedolini[4], S. Marzinotto[1], C.D. Loreto[1], L. Mariuzzi[1]

*[1] Institute of pathology, S. M. della Misericordia Hospital, Udine; [2] Radiology Department, University of Campania "Luigi Vanvitelli", Naples, Italy; [3] Clinic of Obstetrics and Gynecology, S.M. della Misericordia Hospital, Udine; [4] Breast Unit, S.M. della Misericordia Hospital, Udine*

**Aim.** The role of autophagy in cancer is an area of active study [1]. Research on breast cancer has revealed a role of autophagy in drug resistance [2-5]. Targeted inhibition of autophagy may be sufficient to restore sensitivity to chemotherapeutic drugs and promote death of breast cancer cells. The aim of this study was to evaluate the expression of TFEB, CARM1 and SIRT1(three protein involved in the regulation of autophagy pathway) [6] in a group of patients affected by infiltrating breast cancer, treated with adjuvant and neoadjuvant chemotherapy, in order to determine whether they play a role in drug resistance.

**Methods.** Of the 4504 patients affected by breast cancer and operated at the Hospital Clinic of Udine University Hospital from 2002 to 2016, 894 were treated with chemotherapy. The study sample was selected from these 894 patients. Our study is therefore composed of a random sample from these 894 patients (sub-cohort) and of all women with recurrence within 12 months of follow-up after chemotherapy. All samples were evaluated by expert pathologists and for each sample were defined: histotype, grade, molecular subtype and pathological stage (TNM).

In order to test the protein expression, the Tissue MicroArray (TMA) method was adopted. On the obtained TMA sections, immunohistochemical investigations were performed to evaluate the expression of reactivity to the following antibodies: TFEB, CARM1 and SIRT1. The expression of the three antibodies was evaluated by two pathologists; for each core the percentage of positive tumor cells was evaluated, as well as the intensity of expression according to a gradation by levels. In 25 random samples, the expression of the mRNA of the proteins under investigation was also evaluated. For quantitative PCR the primers are prepared for TFEB, CARM1 and SIRT1; GAPDH is used as a housekeeping protein.

For all the proteins analyzed by immunohistochemistry, the coloration is evaluated in terms of H- score given by the product between percentage of positive cells and the intensity of the color evaluated as strong 3, moderate 2 and weak 1 (overall the result will be a value which ranges from 0 to 300).

**Results.** In our study, an increased TFEB expression is associated with lower survival in patients with invasive breast cancer and undergoing chemotherapy; it also emerges that the protein expression of SIRT1 is significantly correlated directly in proportion to TFEB and CARM1. A very low expression of SIRT1 associated with a low expression of TFEB and CARM1 also appears to be significantly correlated with greater survival. Furthermore, in the molecular type basal-like and Her2-enriched, TFEB and SIRT1 tend to have a lower H-score compared to luminal types.

Our data confirm a possible reduced expression of SIRT1 associated with the molecular basal-like type recently described in the literature [4]. This datum, together with a loss of the correlation between SIRT1 and TFEB elusively in the basal-like, effectively poses for a different role of SIRT1 in luminal types with respect to basal-like.

In the literature there is an article that relates an increased expression of TFEB in breast carcinoma to the initial states with a worse prognosis [5]. Also in our study we found that an increased immunohistochemical expression of TFEB correlates with a worse prognosis, but in our case the population affected by breast carcinoma had more advanced stages and had been treated with chemotherapy.

**Conclusions.** our data show that TFEB and SIRT1 have a potential prognostic impact, probably due to their role in regulating autophagy, in patients with breast cancer and undergoing chemotherapy and may therefore be promising therapeutic targets in combination with conventional therapies in breast carcinomas.

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## PLEOMORPHIC CARCINOMA WITH PARADOXICAL NEGATIVE STAINING FOR KERATONS AND OVEREXPRESSION OF HER2: A CASE REPORT.

C. Rossi, G. Cerrone, G. Senes, R. Murru, G. Faa, A. Ravarino

*Department of Surgical Sciences, Division of Pathology, University of Cagliari, Cagliari, Italy*

**Aim.** Pleomorphic carcinoma (PC) of the breast is a very rare high-grade invasive carcinoma. The World Health Organization (WHO) classification of tumors of the breast records PC as a morphological variant of invasive carcinoma of no special type (NST) characterized by a proliferation of pleomorphic and bizarre, sometimes multinucleated, tumour giant cells comprising > 50% of the tumour cells in a background of adenocarcinoma or adenocarcinoma

with metaplastic spindle and squamous differentiation [1]. In the literature, PC is described as an epithelial neoplasm with positive staining for keratins [2]. PC is generally triple-negative at immunohistochemistry: estrogen receptor (ER), progesteron receptor (PR) and HER2 negative. Nevertheless, a proportion of cases expresses hormon-receptor and/or overexpresses HER2 protein [2 3]. The aim of this report is to describe a case of PC with paradoxical negative staining for keratins AE1/AE3 (CKAE1/AE3) and overexpression of HER2 protein.

**Materials and methods.** A 40-year-old woman presented at physical examination, multiple firm, solid and painless masses between inner quadrants of the left breast. Mammography indicated a coarse opacity, with partly defined limits and internal calcifications, sospicious for malignancy (BI-RADS 4) with a maximum diameter of 10 cm. Ultrasound sonography showed multiple hypoechoic lesions, of the overall same mammographic dimension. Core biopsy under ultrasound guidance was performed. The patient underwent neoadjuvant chemotherapy. Afterwards, mammography and ultrasound sonography showed reduction of the maximum diameter from 10 cm to 7 cm. Eight months after carrying out the core biopsy, a total mastectomy with axillary lymphadenectomy of the left breast was performed.

**Results.** The core biopsy showed predominantly a diffuse proliferation of bizarre giant cells (about 80% of neoplastic cells), with hyperchromatic, pleomorphic, sometimes multinucleated, nuclei and relatively abundant eosinophilic or clear cytoplasm, arranged in a haphazard fashion. A minor component of the biopsy was composed of round cells with marked nuclear pleomorphism, numerous mitotic figures and a solid growth pattern. The bizarre giant cells did not show any immunoreactivity for CKAE1/AE3, ER and CD68, while were stained by PR antibody in 20% of cells, with a Ki-67 proliferation index of 30%. The round cells were immunostained by AE1/AE3 in 5% of cells and by PR in 3% of cells, with no reactivity for ER and CD68; they also showed a Ki-67 proliferation index of 70-80%. Bizarre giant cells and round cells, showed an intense and uniform circumferential staining in 100% of cells with HER2 (score 3+). Our diagnosis was invasive carcinoma NST, variant pleomorphic carcinoma. Eight months later, we received the breast with axillary lymph nodes. On gross examination, the breast showed confluent nodules measuring altogether 7×4×3 cm in size and grayish, yellowish or reddish in color on the cut surface, between inner quadrants of the left breast. Nineteen lymph nodes were found in the homolateral axillary fat tissue. Microscopically, the lesion was composed of large necrotic areas, sometimes haemorrhagic, and fibrotic areas. In the latter we found multiple aggregates of large, highly atypical cells, devoid of adhesive characteristics and with the same cytological features shown in the predominant component of the biopsy previously described (fig.1). Conversely, an invasive ductal carcinoma, like in the minor component of the biopsy, was not observed. Immunohistochemically, the bizarre giant cells were not stained for CKAE1/AE3 (fig.2), ER and CD68, while were stained for PR in 10% of cells, with a Ki-67 proliferation index of 30%. All the bizarre giant cells (100%) showed an intense and uniform circumferential staining with HER2 (score 3+) (fig. 3). None of the nineteen lymph nodes was affected by metastasis. Finally, after neoadjuvant chemotherapy, we diagnosed a multifocal invasive carcinoma NST, variant pleomorphic carcinoma.

**Conclusions.** PC of the breast is an uncommon high-grade invasive carcinoma. To the best of our knowledge, it has never been described a negative staining for CKAE1/AE3 in PC and it is generally reported as triple-negative at immunohistochemistry. Our case shows a paradoxical negative immunostaining for CKAE1/AE3, an immunoreactivity for PR and an overexpression of HER2 protein. This overexpression of HER2, despite the negative staining for CKAE1/AE3, was crucial for diagnosing PC. Moreover, only the overexpression of HER2 allowed us to better identify residual neoplastic cells after neoadjuvant chemotherapy.

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**Figure 1.** H&E. A) A fibrotic area with an aggregate of bizarre giant cells arranged in a haphazard fashion. Note a normal duct of the left. B) The bizarre giant cells show hyperchromatic, pleomorphic, sometimes multinucleated, nuclei and eosinophilic cytoplasm.

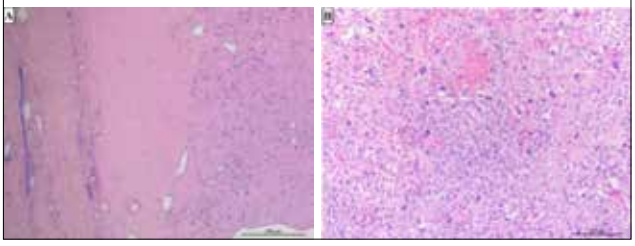


**Figure 2.** A, B) The bizarre giant cells do not show any immunoreactivity for CKAE1/AE3. Note the internal positive control of the normal duct in A.

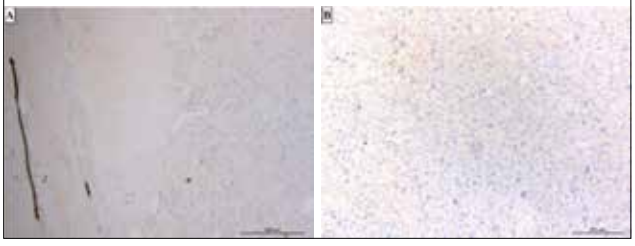
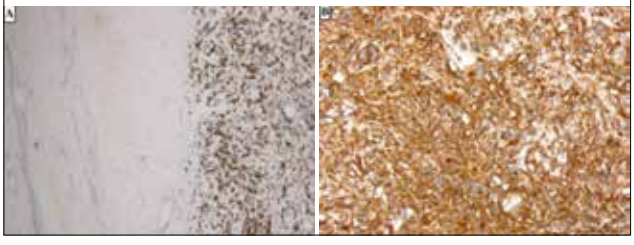


**Figure 1.** A) Low power view shows immunoreactivity for HER2 in the bizarre giant cells and the internal negative control of the normal duct on the left. B) High-power view highlights an intense and uniform circumferential staining with HER2.



## INTRATUMORAL AND STROMAL CD8+ T CELLS COULD HAVE A DIFFERENT IMPACT ON BREAST CANCER PATIENT SURVIVAL

C. Saponaro[1], I. Catacchio[1], L. Schirosi[1], F. Fanelli1, I. Trotti[2], D. Loisi[2], A. Lozupone[2], M.A. Botticella[2], A. Scattone[2], A. Mangia[1]

*[1] Functional Biomorphology Laboratory, [2] Pathology Department IRCCS-Istituto Tumori "Giovanni Paolo II", 70124 Bari, Italy*

**Background.** Tumor infiltrating lymphocytes (TILs) are widely considered a key sign of the immune interaction between host and tumor, and potentially prognostic biomarkers of good or bad outcome in many cancers, included breast cancer (BC) [1]. Subclassification of TILs is pivotal. However, results about the association between TIL typology, location and BC prognosis, are controversial [2 3].

The aim of our study was to evaluated the prognostic significance of TIL subtypes (CD4+, CD8+, FOXP3+ T cells) and their location (stromal "s" and intratumoral "i" CD4+ and CD8+) in invasive BC.

**Material and Methods.** CD4+, CD8+, FOXP3+ expression was examined by immunohistochemistry on tissue microarrays (TMAs) from 180 BC patients. Figure 1 shows some examples of immunohistochemical staining patterns in TMA cores. The expression of CD4+, CD8+ and FOXP3+ was evaluated in TILs and expressed as the number of positive cells counted in each TMA core at x400 magnification. Univariate Cox regression and Kaplan Meier analyses of disease free survival (DFS) were performed to evaluate the prognostic significance of marker expression (for OS, it could not be completed, due to the low number of deaths).

**Results.** The role of CD8+ in BC DFS depended on their location. Total CD8+ T cells were not significantly associated with DFS. Differently, patients with iCD8+ and sCD8+ overexpression showed a trend towards respectively a worse (p = 0.050) and a better 5-years DFS (p = 0.064) (Fig. 2a-b).

**Conclusions.** Our preliminary data show that iCD8+ T cells, but no sCD8+ T cells, identify a subgroup of patients with poor DFS. The molecular mechanisms that underlie the different impact of sCD8+ and iCD8+ T cells on BC survival are under investigation.

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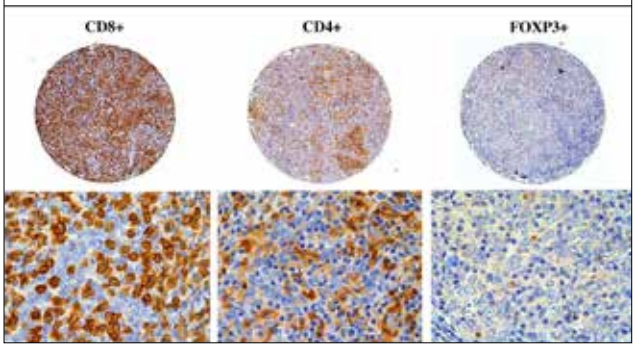
Figure 1. Immunoreactivity of TIL subtypes on breast cancer (BC) tissue microarrays (TMAs). Immunohistochemical expression of CD4+, CD8+ and FOXP3+ on the same TMA sample. Panoramic views of the TMA cores at original magnification 50X, and detail views at original magnification 630x.




## HIGH-GRADE BREAST SPINDLE CELL NEOPLASMS: A REPORT OF 2 CASES IN ASL2 INSTITUTE OF SURGICAL PATHOLOGY

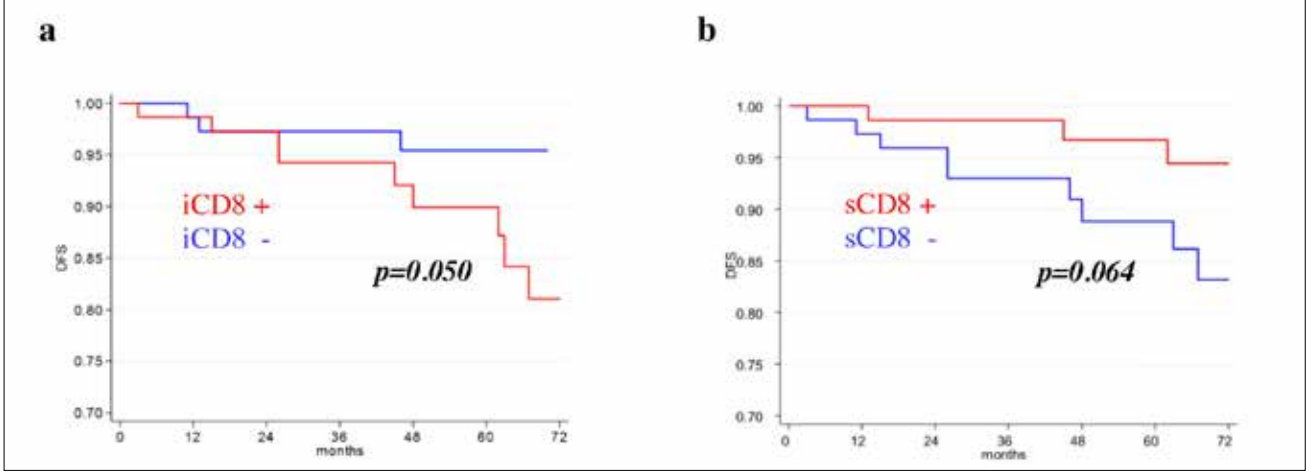
E. Venturino, Ve. Errigo, E. Pacella, S. Ardoino, S. Bruno, A. Pastorino

*Department of Anatomical pathology ASL2 Liguria*

**Aims.** high-grade breast spindle cell neoplasms are extremely rare (0.5-1% of all breast cancers) with a high rate of recurrence and distant metastases. Such neoplasms include malignant phyllodes tumor, metaplastic carcinoma variant with spindle cells, primitive sarcoma of the breast; these neoplasms, although presenting similarities, differ in some histological and management aspects.

Throughout this year at our Institute of Surgical Pathology (ASL2 Savona) we observed a case of malignant phyllodes

Figure 2. Kaplan-Meier survival curves. Disease free survival (DFS) according to iCD8 (a) and sCD8 (b) expressions in 160 breast cancer patients.



tumor with areas of liposarcoma and a case of spindle cell metaplastic carcinoma.

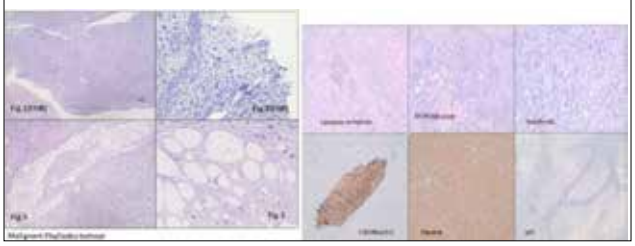
**Methods.** 1st clinical case: 46-year-old woman with 7 cm palpable mass at the QSE of the right breast, rapidly increased in volume, hypoechoic to ultrasound, polylobate, with internal vascularization to ECT and further 1.4 cm satellite nodule to the union of the ipsilateral QSS. Free left breast and bilateral lymph nodes. Previous mammograms were negative and reported dense fibroglandular tissue, without microcalcifications. FNB: the histological finding was of fibroepithelial lesion with a hypercellular stroma, atypical cells, often with mitosis (18/10 HPF) and slightly hyperplastic epithelial component. The histological diagnosis was phyllodes tumor with characters suspected of malignancy (LGE: B4) (Fig. 1). 1st surgery: quadrantectomy: three nodules of cm 7, cm 6.5 and 0.7 cm removed from the right QSE, as well as the 1.4 cm diameter nodule to the QSS; the three nodules were diagnosed with high-grade multifocal malignant phyllodes tumor (MPT), with malignant heterologous elements of liposarcoma accompanied by involved exeresis. The nodule to the QSS corresponded to intracanalicular FA. (Fig. 2-3). 2nd surgery: total mastectomy: MPT residue; margins of exeresis: free. The morphological aspects that lead to the conclusion of multifocal malignant phyllodes tumor were: high stromal cellularity, fused stromal elements, also plurinuclear, with markedly atypical and bizarre nuclei, high number of mitosis (> 9 x 10HPF). The particularity and rarity of this case lies in the multifocality and in the presence of a malignant liposarcomatosis component. The instrumental investigations do not allow to distinguish between benign and malignant phyllodes tumor , therefore a histological evaluation is indispensable. The main differential diagnoses include fibroadenoma, metaplastic carcinoma and primary breast sarcoma. Margin status is one of the principal prognostic factors; surgical treatment can vary from more to less conservative; sentinel node is not performed; control of local recurrence (23-30%) and distant metastasis.

2nd clinical case: 52-year-old woman with a previous left mastectomy, in February 2017 the ultrasound found a simple serous cyst at the QSE of the right breast with a diameter of 8x6 mm; onset of focal mastitis; in December 2017 the unchanged persistence of cystic formation with pseudo-solid and corpuscular content of 17x 8 mm led us to perform needle biopsy.

FNB: at biopsy the histological findings were of pleomorphic sarcoma with associated outbreaks of ductal carcinoma in situ G2 (B5b).Surgery (right mastectomy): macroscopically we observed nodular, solid, whitish lesion, with polycyclic outlines histologically diagnosed as spindle cell metaplastic carcinoma (SMC) characterized by pleomorphic nuclei, storiform pattern, high number of mitoses and Vimentina positivity; associated outbreaks of high-grade ductal carcinoma in situ (DCIS) with central "comedo" necrosis and focus of squamous metaplasia (CK5/6 positive); margins of exeresis free. Receptorial status: ER negative, Prg negative, Her2 negative, Ki67 70%.

Discussions and conclusions. MPT represents less than 1% of mammary neoplasms; it can rarely present itself as a multifocal lesion, as in the case presented here, or bilaterally. SMC is an extremely rare variant and includes a heterogeneous group of neoplasms characterized by differentiation of neoplastic epithelium into squamous and/or mesenchymal elements (including chondroid, rabdoid and bone cells). It represents between 0.2% and 5% of mammary neoplasms. Macroscopically it could be a well-circumscribed mass or with irregular edges; cystic degeneration is frequent. A high-grade breast spindle cell malignant neoplasms include different types of malignant mesenchymal neoplasia, for example MPT (Fig. 1-2), SMC (Fig. 4) and primary breast sarcoma (PBS). Malignant phyllodes tumor represents a specific subset of breast soft tissue tumors. Although there are some histological differences, the common features are the presence of atypical sarcomatous-like stromal component that, in the case of multifocal MPT, surrounds the hyperplastic epithelial component and, in this case, the stroma shows heterologous liposarcomatous differentiation (Fig. 3). In case of SMC, the tumor substitutes the mammary normal glandular structures but the adjacent presence of squamous metaplasia and high-grade ductal carcinoma in situ favours a diagnosis of metaplastic carcinoma. Long-term prognosis is unknown since there are only rare cases of long-term follow-up with high risk of local recurrence and distant metastases. For the treatment of SMC, MPT and PBS, the gold standard is simple mastectomy without axillary dissection, although there are major variations in the extent of local excision, ranging from wide local excision to radical mastectomy. The effect of adjuvant radiation or chemotherapy on PBS, KMP and MPT is still unclear due to the rarity of malignant breast neoplasms (Fig. 4. Spindle cell metaplastic breast carcinoma).

Figures 1-2-3-4.



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# PATOLOGIA MOLECOLARE

## DEVELOPMENT OF A FASTER AND REPRODUCIBLE ASSAY FOR MICROSATELLITE INSTABILITY ANALYSIS: FOCUS ON THE BETHESDA PANEL

M. Corsi[1], C. Caprera[1], G. Ramelli[2], M. Boergesen[3], R. K. Petersen[3], F. Perillo[2], P. Spina[2], L. Mazzucchelli[2], U. B. Christensen[3], S. Ascani[1], M. Frattini[2]

*[1] University of Perugia, Institute of Pathologic Anatomy "Santa-Maria", Terni, Italy; [2] Institute of Pathology, Locarno, Switzerland; [3] PentaBase Aps, Odense, Denmark*

**Background.** Microsatellites are non-coding DNA sequences and their instability indicates that the mismatch repair system is damaged. Microsatellite instability (MSI) is present in several human tumors, especially in colorectal cancers (CRC) and is used as molecular marker for assessing prognosis and treatment decisions in CRC patients. The Bethesda panel, the only test approved by the Food and Drug Administration, is the most diffused assay to establish MSI status and is based on the comparison between paired tumor and healthy tissues of five loci (2 mononucleotide and 3 dinucleotide repeats). Here we compare the reproducibility of a new assay for assessing the Bethesda panel, based on a ready-to-use mix approach, with that of the five loci analyzed in a separated manner.
**Materials and Methods.** After DNA extraction (QIAamp DNA FFPE Tissue Kit, Qiagen, Chatsworth, CA, USA), we characterized the MSI status of 184 consecutive patients affected by CRC. The samples were amplified with two methods: a multiplex, ready-to-use PCR mix (Pentiplex®, Pentabase, Odense, Denmark) approach, and a simplex PCR reactions (with a locus-by-locus approach) using the Simplex PCR mastermix (Qiagen). The PCR products were then subjected to capillary electrophoresis using a 3130 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA). The Cohen's kappa test (k) was used for statistical analysis.
**Results.** The results obtained with the ready-to-use mix showed that 31 patients were classified as MSI (16.8%) and 153 as microsatellite stable cases. Same results were obtained with the Simplex PCR mastermix and therefore, a perfect match between the two methodologies was observed in all the tested loci (k=1, Cohen's kappa test).
**Conclusions.** The ready-to-use mix is a faster and easy to use assay for a perfect analysis of MSI, enabling laboratories to decrease the work-on-time for such analysis. Moreover, the risk of contamination decreases significantly.

## LIQUID BIOPSY IN NON-SMALL CELL LUNG CANCER: A COMPARISON BETWEEN DIFFERENT METHODOLOGIES TO DETECT SOMATIC MUTATIONS

E. Germanà[1], U. Malapelle[2], A. Simone[1], R. Scarfì[1], A. Ieni[1], R. Smeraglio[2], M. Santarpia[1], G. Troncone[2], G. Tuccari[1], G. Giuffrè[2]

*[1] Department of Human Pathology in Adult and Developmental Age Gaetano Barresi, University of Messina, Messina, Italy; [2] Department of Public Health, University of Naples Federico II, Naples, Italy*

**Background.** In non-small cell lung cancer (NSCLC) somatic mutations in the epidermal growth factor receptor (EGFR) gene lead to constitutive activation of several downstream effectors and consequently dysregulation of cell proliferation, migration, differentiation and survival. Based on significant clinical benefits, EGFR tyrosine kinase inhibitors (TKIs) represent the standard first-line treatment of patients with advanced *EGFR*-mutant NSCLC. Nowadays, the so-called liquid biopsy is considered an effective method to analyse tumor DNA in NSCLC patients. In fact, the circulating tumor DNA (ctDNA) carries the same alterations as the tumor itself and it can be used to detect *EGFR* mutations, mainly when no biopsy is available for genetic analyses. Moreover, liquid biopsy has been also successfully used for patient monitoring as well as for early detection of acquired resistance to EGFR TKIs. In particular, the detection of T790M mutation in circulating free DNA (cfDNA) has been recommended in patients eligible for osimertinib treatment in order to avoid unnecessary rebiopsies. However, various methods such as real-time polymerase chain reaction (PCR) and next-generation sequencing (NGS) are available to detect genetic alterations in cfDNA with different limits in the detection of mutations.
**Objective.** The aim of the present study was to evaluate the sensitivity and specificity of two different commercial real-time PCR-based assays for detection of *EGFR* mutations in cfDNA of NSCLC patients and to compare results with those obtained utilizing a next-generation sequencing (NGS) methodology.
**Material and Methods.** Fifteen patients with advanced NSCLC already analyzed for *EGFR* mutations at the time of diagnosis were included in the study. In particular 13 patients (5 M, 8 F; mean age 69.6 yrs), previously treated with EGFR TKIs on the basis of *EGFR* mutation, were investigated for *EGFR* on cfDNA at the time of disease progression, while 2 patients (2 M; mean age 57 yrs), not showing *EGFR* mutations, were treated with chemotherapy and utilized as negative control. For each patient cfDNA was extracted separately from plasma and serum using the QIAamp MinElute ccfDNA Mini Kit (Qiagen) according to the manufacturer's instructions. The real-time PCR assays used to investigate *EGFR* mutations were the therascreen *EGFR* Plasma RGQ PCR Kit (Qiagen) and the ctDNA *EGFR* Mutation Detection Kit (EntroGen), while the NGS analysis was performed on Personal Genome Machine (PGM) instrument (Thermofisher), employing the SiRe (Genedin) gene panel able to detect mutations of *EGFR* as well as *KRAS*, *NRAS*, *BRAF*, *cKIT* and *PDGFR* genes. The analysis with therascreen *EGFR* Plasma RGQ PCR Kit and ctDNA *EGFR* Mutation Detection Kit had been performed on 12 and 14 patients respectively.
**Results.** By using the therascreen *EGFR* Plasma RGQ PCR Kit, 4/10 (40%) patients with *EGFR* mutations at diagnosis time showed the same *EGFR* mutations in plasma and/or serum samples following the manufacturer's instruction for ΔCt determination, while in another patient the ΔCt was out of range. No mutations were found in the plasma and serum of the 2 patients recruited as negative control. Repeating the run analysis setting the threshold of the fluorophore channel at the beginning of the exponential phase, the ΔCt values obtained did not fall in the positivity range for mutations.
Utilizing the ctDNA *EGFR* Mutation Detection Kit and setting the Ct value according to the manufacturer's instruction, 4/12 (33,3%) patients with *EGFR* mutations at diagnosis time showed the same *EGFR* mutations in plasma and/or serum samples; moreover, 1 (8,3%) of these patients also presented the T790M resistance mutation. Mutations with Ct values out of the positivity range were also detected in 2 other patients. No mutations were found in the plasma and serum of the 2 patients recruited as negative control. Repeating the run analysis setting the threshold of the fluorophore channel at the beginning of the exponential phase, 3 plasma

and 2 serum samples exhibited Ct values in the range of positivity for mutation.
By using the SiRe panel 11/13 (84,6%) patients with *EGFR* mutations at diagnosis time showed the same *EGFR* mutations in plasma and/or serum samples; moreover, 7 (53,8%) of these patients also presented the T790M resistance mutation. A discrepancy was found in the results obtained from plasma and serum samples from 4 patients; in particular, mutations were exclusively encountered in plasma samples. No mutations were found in the plasma and serum of the 2 patients recruited as negative control.
Comparing the results obtained from the SiRe panel with those achieved by the therascreen EGFR Plasma RGQ PCR Kit according to the manufacturer's instruction and excluding the 3 patients presenting mutations not detectable with this real-time PCR assay, the concordance between the two methodologies was 25%. In particular, the therascreen EGFR Plasma RGQ PCR Kit was able to identify the mutational status of *EGFR* with a sensitivity of 50% and a specificity of 100%. Moreover, using the threshold by us suggested, the concordance, sensitivity and specificity obtained did not undergo variations.
Comparing the results obtained from the SiRe panel with those achieved by the ctDNA *EGFR* Mutation Detection Kit according to the manufacturer's instruction and excluding the 4 patients presenting mutations not detectable with this real-time PCR assay, the concordance between the two methodologies was 40%. In particular, the ctDNA *EGFR* Mutation Detection Kit was able to identify the mutational status of *EGFR* with a sensitivity of 58.33% and a specificity of 100%. Moreover, using the threshold by us suggested, the concordance, sensitivity and specificity obtained did not undergo variations.
**Conclusions.** The results obtained with the therascreen *EGFR* Plasma RGQ PCR Kit and with the ctDNA *EGFR* Mutation Detection Kit, even if characterized by an excellent specificity, do not reach the level of sensitivity and the wide mutational spectrum of the SiRe panel. Therefore, the use of a narrow NGS panel is preferable in clinical practice for the identification of relevant mutations in purified cfDNA from plasma and serum samples of patients with NSCLC.

## ENDOPREDICT®, THE NEW GENERATION PROGNOSTIC MOLECULAR TEST FOR BREAST CANCER: THE CLINICAL EXPERIENCE OF ANATOMIC PATHOLOGY UNIT OF L. BONOMO

R. Nenna, T. Addati, V. Losito, G. Elicio, D. Di Clemente, A. De Lucia, C. D. Inchingolo Anatomic Pathology Unit, L. Bonomo

*ASL BT, Andria, Italy*

**Beckground.** Endopredict® is the test which has been specifically developed in ER+/HER2-/node negative/positive tumors – luminal type, St Gallen 2013 and 2015 [1 2] – 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. The molecular EP score combined with clinical-pathological parameters (tumor size, lymph node status) through a validated algorithm, generates an EPclin clinical score. In contrast with other commercially available multigene tests EP is suitable for decentralized testing instead of a single reference laboratory [2].
**Methods.** EndoPredict [2] test is available in our center from July 2017. Our study is based on two groups of patients. The first one is represented by a retrospective analysis of 5 cases (2014) of luminal breast cancers with a mean follow-up of 72 months and a complete therapeutic history available. The second group is constituted by 20 patients (September 2017 - April 2018) with a recent diagnosis of luminal breast carcinomas showing peculiar clinic-pathological features. The 25 samples were assessed for ER, PgR, Ki-67 and HER2 expression. Ten µm thick section with at least 30% of invasive cancer were obtained for each sample and used for RNA extraction and gene analysis was conducted according to the manufacturer's instructions (EndoPredict).
**Results.** EndoPredict analysis conducted on 5 retrospective cases revealed that 3/5 neoplasm had "High Risk" of recurrence while 2/5 had a "Low Risk" of relapse (Fig. 1), so on 20% of the patients (1/5) molecular test could change therapeutic approach, in fact in Case 2 chemotherapy could be omitted (Fig. 1). These 5 patients had a mean follow-up of 72 months and none of them had disease relapse, but in one case there was over treatment.
EndoPredict analysis conducted on 20 prospective cases revealed that 8/20 samples were High Risk and 12 were "Low

Figure 1. Data of the retrospective study, clinical-pathological parameters, therapeutic choice and EPclin Class.

| Cases | pT | N | G | ER | PGR | Ki67 | ChT | EPclin Class |
|---|---|---|---|---|---|---|---|---|
| Case 1 | pT1c 12mm | N1a (2/17) | G3 | 3+ (100%) | 3+ (30%) | 20% | yes | High Risk EPclin 4,1 |
| **Case 2** | **pT1c 12mm** | **N0(sn) 0/1sn** | **G2** | **3+ (100%)** | **3+ (20%)** | **20%** | **yes** | **Low risk EPclin 2,4** |
| Case 3 | pT2 25 mm | N0 (0/18) | G3 | 3+ (95%) | 3+ (35%) | 30% | yes | High Risk EPclin 4,4 |
| Case 4 | pT1c 15 mm | N1a 1/4(sn) | G2 | 3+ (100%) | 3+ (100%) | 15% | yes | High Risk EPclin 4,0 |
| Case 5 | pT1c 20mm | N0 0/21 | G2 | 3+ (100%) | negative | 10% | NO | Low risk EPclin 2,7 |

**Figure 2.** Data of the prospective study, clinical-pathological parameters and EPclin Class.

| Cases | Age | pT | pN | G | ER | PgR | Ki67 | Surrogate St Gallen 2013 | EPclin Class |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 43 | pT1c | N0 | G3 | 3+ (100%) | 3+ (100%) | 20% | Luminal B-like | High Risk |
| 2 | 68 | pT1c | N1a | G3 | 3+ (100%) | 3+ (100%) | 25% | Luminal B-like | High Risk |
| **3** | **61** | **pT1c** | **N0** | **G3** | **3+ (80%)** | **3+ (40%)** | **18%** | **Luminal A-like** | **High Risk** |
| 4 | 67 | pT1c | N0 | G2 | 3+ (100%) | negative | 15% | Luminal A-like | Low Risk |
| 5 | 82 | pT1c | N0 | G2 | 3+ (60%) | negative | 15% | Luminal A-like | Low Risk |
| 6 | 51 | pT1a/b | N0 | G2 | 3+ (95%) | 3+ (95%) | 14% | Luminal A-like | Low Risk |
| 7 | 38 | pT1c | N0 | G3 | 3+ (100%) | 3+ (15%) | 28% | Luminal B-like | High Risk |
| 8 | 41 | pT1c | N0 | G2 | 3+ (90%) | 3+ (80%) | 14% | Luminal A-like | Low Risk |
| 9 | 75 | pT1a/b | N0 | G2 | 3+ (100%) | negative | 14% | Luminal A-like | Low Risk |
| **10** | **70** | **pT1b** | **N1a** | **G2** | **3+ (70%)** | **3+ (<5%)** | **14%** | **Luminal A-like** | **High Risk** |
| 11 | 72 | pT1c | N0 | G2 | 3+ (100%) | 3+ (90%) | 15% | Luminal A-like | Low Risk |
| 12 | 44 | pT1c | N1a | G3 | 3+ (95%) | 3+ (75%) | 30% | Luminal B-like | High Risk |
| **13** | **44** | **pT1b(m) tricentric** | **N0** | **G3(2 nodules) G2 (1 nodule)** | **3+ (100%)** | **3+ (100%)** | **30%-18%(2 nodules) 10% (1 nodule )** | **Luminal B-like** | **Basso Rischio EPclin <3,33 (3 nodules)** |
| **14** | **52** | **pT1c(m) bicentric** | **N1a** | **G2** | **3+ (80%)** | **3+ (100%)** | **15% e 14%** | **Luminal A-like** | **High Risk (nodule >) Low Risk (nodule <)** |
| **15** | **50** | **pT1b** | **N0** | **G3** | **3+ (90%)** | **3+ (70%)** | **25%** | **Luminal B-like** | **Low Risk** |
| 16 | 52 | pT1b | N0 | G2 | 3+ (95%) | 3+ (65%) | 15% | Luminal A-like | Low Risk |
| 17 | 45 | pT1c | N1a | G2 | 3+ (90%) | 3+ (90%) | 12% | Luminal A-like | Low Risk |
| **18** | **70** | **pT1c** | **N0** | **G3** | **3+ (100%)** | **3+ (60%)** | **20%** | **Luminal B-like** | **Low Risk** |
| 19 | 35 | pT1c | N0 | G3 | 3+ (100%) | 3+ (90%) | 20% | Luminal B-like | High Risk |
| 20 | 54 | pT2 | N0 | G2 | 3+ (60%) | negative | 14% | Luminal A-like | Low Risk |

Risk" (Fig. 2).Our data showed that on the basis of pathological features case 3, 10 and 14 (Fig. 2) were Luminal A-like breast cancer [1-3] and they could not candidate to chemotherapy, but EndoPredict test underlined a "High risk" of relapse of these tumors, so clinical decision change, patients were candidate to chemotherapy. Conversely, case 13, 15 and 18, on the basis of pathological features, were Luminal B-like breast [1-3] cancer and they could candidate to chemotherapy, but EndoPredict test underlined a "Low risk" of relapse of these tumors, so clinical decision change, patients were not candidate to chemotherapy. Unfortunately, this is a prospective study so the data of the follow up will confirm, later, the effectiveness of the test and the consequent therapeutic choice.

**Conclusions.** Finally, prognostic parameters usually used for breast cancer do not identify the most intrinsic biological characteristics and do not predict accurately the evolution. Endopredict® has demonstrated analytical and clinical reliability, it represents a useful prognostic tool in cases with an ambiguous bio-pathological profile, allowing the oncologist a personalized therapeutic decision. In fact, our data show that in 30% (6/20) of the patients could change clinical approach and chemotherapy were administered only in patients with a high risk of disease relapse. So the follow- up was planned on the estimated individual real risk.

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## IMMUNOHISTOCHEMICAL AND BIOMOLECULAR ASPECTS IN CRANIOPHAR YNGIOMAS: IS THERE ANY SUGGESTION TO IMPROVE THEIR TREATMENT?

A. Ieni, C. Pizzimenti, L. La Rocca, G.Tuccari, G. Giuffrè

*Department of Human Pathology of Adult and Evolutive Age "Gaetano Barresi", Section of Pathological Anatomy and Cytopathology, University of Messina, Messina, Italy*

**Objective.** Craniopharyngiomas (CP) are rare benign epithelial tumors of the sellar region, with two identified histological subtypes represented by the adamantinomatous variant (ACP) and the rarer papillary variant (PCP). Generally, they are locally infiltrative and surgically challenging tumors with severe long term morbidity. Current data suggest that both variants are defined by specific genetic alterations and influenced by distinct molecular pathways. The aim of the present study is to analyze histopathological, immunohistochemical and biomolecular characteristics in a series of CPs.

**Materials and Methods.** We retrieved from our database 37 CPs, 34 of which were ACP and only 3 PCP. The patients were 21 female and 16 male (age range 5-75 years, mean age=43.48 yrs); CPs samples included 7 childhood patients. 4mμ thick silane-coated sections were stained using a Ventana BenchMark ultraimmunostainer (Ventana, Tuscon, AZ, USA) and the following antibodies: monoclonal mouse-anti-β-catenin (Cell Marque, clone 14), Ki 67 (Dako, clone MIB-1) and monoclonal antibody recognizing the BRAF V600E mutant epitope (BRAF V600E-specific clone VE1, Ventana, USA). The staining protocol included pretreatment with cell conditioner 1 (pH 8,4) for 64 min, incubation with antibody at 36 °C for 16 minutes, primary antibody detection using the ultraView Universal DAB Detection Kit (Ventana), followed by counterstaining with hematoxylin for 4 minutes.

In order to evaluate BRAF mutational status, four 10 μm thick H&E-stained sections were microdissected by scalpel using an inverted microscope in order to collect only regions with the highest MPHC representation. DNA extraction was performed by QIAamp DNA FFPE Tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations and DNA quantified by fluorometry with the Qubit® platform (Life Technologies, CA, USA). DNA samples were subjected to BRAF mutational analysis utilizing the BRAF Codon 600 Mutation Analysis Kit II (EntroGen, Inc, CA, USA) that allows to identify five BRAF somatic mutations in codons600 (V600D, V600E, V600K, V600M, V600R). The amplifications were carried out in a StepOnePlus™ Real-Time PCR system (Life Technologies) following the manufacturer's procedures and the recommendations of both the Italian Association of Medical Oncology (AIOM) and the Italian Society of Pathology and Cytology (SIAPEC).

**Results.** Twenty-five (73.52%) ACP cases showed β-catenin immunopositivity; interestingly, nine ACPs unreactive for β-catenin showed a Ki67 labelling index > 5%. Cytoplasmic immunopositivity for anti-VE1 antibody was observed only in all 3 PCPs, all of which also harboured *BRAF* V600E mutations. These latter cases exhibited a less favourable outcome, since 1 died for the disease and 2 presented recurrences.

**Conclusions.** The identification of hallmark immunohistochemical and biomolecular signatures in the two CPs histotypes may be utilized to identify alternate improved treatment modalities, taking also into consideration clinicopathological characteristics of these unusual brain tumors.

## EXPRESSION OF TRKB RECEPTOR AND ITS LIGAND BDNF IN BRAIN METASTASES OF LUNG ADENOCARCINOMA.

S. Scarpino[1], M. Lombardi[1], L. Ruco[1], C. Cippitelli[1], T. Adelizi[1], A. Salustri[2], E. Rathina[2], A. Ricci[2]

*[1] UOC Anatomia Patologica Morfologica e Molecolare, Azienda Ospedaliera Sant'Andrea, "La Sapienza" University, Rome; [2] UOC Pneumologia, Azienda Ospedaliera Sant'Andrea, "La Sapienza" University, Rome.*

**Introduction.** Tropomyosin-related kinase B (TrkB) is a receptor for brain-derived neurotrophic factor (BDNF) and is highly expressed in various neoplasms. It plays an important role in tumor progression and metastasis in various cancers, including lung adenocarcinoma. The main TrkB isoforms, full-length (TrkB.FL) and truncated (TrkB.T1), mainly play opposite roles. We examined the distribution of both TrkB protein and mRNA isoforms (TrkB.FL and TrkB.T1) in non- brain- metastatic versus brain- metastatic adenocarcinoma of the lung. Moreover, in this second group, we compared TrkB protein and BDNF mRNA expression between primitive cancerous versus brain-metastatic cells.

**Materials and Methods**. Paraffin tissue sections of 10 non brain-metastatic lung adenocarcinoma (A group, follow-up=12 months ) and of 16 samples from 8 patients with brain-metastatic lung adenocarcinoma (B group) were immunostained for TrkB. Moreover, expression of TrkB.FL and TrkB.T1 isoform mRNA were investigated by RT-PCR, in 9 patients from group A and in 5 from group B; furthermore, expression of BDNF mRNA, the ligand of TrkB, was evaluated in primary tumors and in metastatic samples.

**Results.** TrkB-protein was more expressed in metastatic tumors: 6 of 8 patients (75%, $p<0.05$) versus 3 of 10 patients (30%, $p<0.05$). Moreover, intensity of expression of TrkB-protein was 20% higher in brain metastasis vs primary samples. Finally expression of BDNF mRNA was 30% higher in brain-metastasis vs primitive samples ($p<0.05$).

**Conclusions**. Our findings provide preliminary evidence that the TrkB-receptor is more expressed in primary tumors of patients with metastatic lung adenocarcinoma. Interestingly, brain-metastasis express TrkB receptor and its ligand BDNF at higher levels than the corresponding primary tumors.

# PATOLOGIA NEFROLOGICA

## COMBINED PLASMATIC AND TISSUE APPROACH TO MEMBRANOUS NEPHROPATHY: PROPOSAL OF A DIAGNOSTIC ALGORITHM INCLUDING IMMUNOGOLD LABELING

V. L'Imperio[1], M. Nebuloni[2 3], A. Tosoni[2 3], A. Radice[4], F. Pagni[1 3]

*[1] Dept of Medicine and Surgery, Pathology, UNIMIB, San Gerardo Hospital, Italy; [2] Dept of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy; [3] Research Center for renal Immunopathology, University of Milan, Milan, Italy; [4] Microbiology and Virology Department, San Carlo Borromeo Hospital, Milan, Italy*

**Introduction and Aims**. The discovery of circulating autoantibodies against PLA2R and THSD7A, shed light on the pathogenesis of idiopathic forms of Membranous Nephropathy (MN) leading to the development of diagnostic and prognostic serum or tissue-based tests. However, only a comprehensive assessment can lead to the reduction of false negative cases, adopting useful immunohistochemistry (IHC) interpretation criteria for PLA2R/THSD7A and increasing sensitivity and specificity for the in situ detection of target antigens.

**Methods.** A retrospective series of 81 renal biopsies with diagnosis of MN was collected from different italian centers (Fig. 1). Cases were clinically classified as primary and secondary (Tomas, N Engl J Med. 2014). The agreement between serology (PLA2R and THSD7A HEK-293 transfected cell-based IIFT, Euroimmun) and IHC (PLA2R polyclonal rabbit Atlas Antibodies, Stockholm, Sweden, 0.4 mg/ml, 1:300; THSD7A Atlas Antibodies, 0.4 mg/ml, 1:400 on platform Dako Omnis, Glostrup, Denmark) have been evaluated (Fig. 2). For immunogold technique same primary antibodies used for IHC were employed.

**Results.** PLA2R assessment demonstrated a 86% of concordance between serum and tissue with 32 double negative cases (39%), three of which demonstrated positivity to THSD7A (Fig. 3). In doubtful cases the employment of immunogold has demonstrated to be useful for the detection of tiny deposits (Fig. 3).

**Conclusions.** The increased performance of a combined serum and tissue analysis for the assessment of MN cases highlights the weakness of a serum-based approach. Moreover, the diagnostic flow-chart of these cases can be further implemented with useful ancillary techniques, reducing the disagreement and enhancing the diagnostic accuracy (Fig. 4).

**Figure 1.** Clinical characteristics of the cohort (n=81). * Values are expressed in the form "average ± standard deviation"; ** Were considered to be affected by systemic hypertension those patients with at least one value above 140/90 mmHg.

| | | | |
|---|---|---|---|
| Age * | 62 | ± | 16 |
| Gender (M/F) * | 55 | / | 26 |
| Proteinuria (g/24h) * | 7,7 | ± | 6,3 |
| Serum creatinine (mg/dl) * | 1,7 | ± | 2,3 |
| eGFR (CKD-EPI formula) * | 68,1 | ± | 32 |
| Clinical etiology (P/S) | 68 | / | 13 |
| Systolic hypertension ** | 39 | | |

**Figure 2.** Comparison of the results of PLA2R IHC and serum anti-PLA2R testing with percentage of agreement/disagreement before and after multidisciplinary discussion. In parenthesis the cases positive to anti-THSD7A and/or THSD7A IHC after multidisciplinary discussion.

| | Before | | | After | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum anti-PLA2R | | | Serum anti-PLA2R | | | | | |
| IHC PLA2R | Positive | Negative | Total | Positive | Negative | Total | Before | Agreement | 75% |
| Positive | 39 | 12 | 51 | 47 | 9 | 56 | | Disagreement | 25% |
| Negative | 8 | 22 | 30 | 2 | 23 | 25 | After | Agreement | 86% |
| Total | 47 | 34 | 81 | 49 | 32 (3) | 81 | | Disagreement | 14% |

**Figure 3.** In **A)** negative case to THSD7A (internal control of the tubular brush border (black arrows). In **B)** granular and parietal staining of the capillary walls (bottom left magnification). In **C)** false positive PLA2R IHC for the presence of coarse granular and parietal deposits in a "dirty" background. Immunogold and serology were negative. The presence of anti-THSD7A was confirmed by serum and IHC. In **E)** IHC showed questionable granular positivity to PLA2R in absence of circulating antibodies. The employment of immunogold technique with anti-PLA2R antibody showed the presence of the target antigen in the context of the immune deposits – black circles, **D)** –.

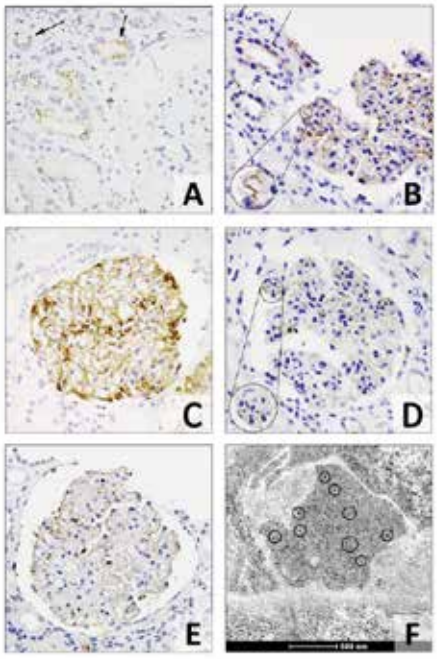
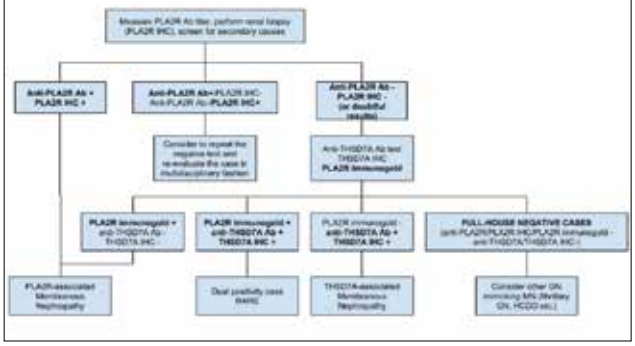



**Figure 4.** A proposed serum/histologic algorithm for the management of MN.



# PATOLOGIA PEDIATRICA

## THE RELATIONSHIP OF GENETIC POLYMORPHISMS WITH THE STATE OF NEWBORNS IN PREECLAMPSIA

E.A. Reshetnikov1, E.N. Krikun[2], M.I. Churnosov[1], R.F. Kapustin[3]

*[1] Department Biomedical Sciences, Belgorod National Research University, Belgorod, Russia; [2] Medical College, Belgorod National Research University, Belgorod, Russia; [3] Department of Morphology and Physiology, Belgorod State Agricultural University named after V.J. Gorin, Maiskii, Russia*

Preeclampsia is a pathological condition that is found in pregnant women and characterized by arterial hypertension, proteinuria, edema, as well as by considerable disorders of the circulatory system, immunity, hemostasis, by disorders of hemodynamics and microcirculation, the endothelial dysfunction that accompanies placental insufficiency, concomitant violation of the kidneys, liver, lungs [1-5]. Preeclampsia is the most severe complication of pregnancy leading to placental insufficiency, premature detachment of normally situated placenta, the occurrence of preterm birth. Disorders of blood flow in the system mother-placenta-foetus in developing preeclampsia lead to the development of fetal hypoxia. The purpose of this study was to examine the association of gene polymorphisms of the renin-angiotensin-aldosterone system with the state of newborns in preeclampsia. The study group included 132 pregnant women with preeclampsia. The average age of the surveyed women was 27.98±5.29 years. The indicators such as height, weight, and Apgar score was used to assess newborn condition. The typing of genetic polymorphisms of the renin-angiotensin-aldosterone system was carried out to all pregnant women: angiotenzinoguena (-6A/G AGT), angiotensin- converting enzyme (I/D ACE), angiotensin II receptor of the 1st type (-1166A/S ATIIR1). The median (Me) and interval scale (Q25-Q75), Mann-Whitney test were used for the description of indicators. In the analysis of associations of genetic markers of the renin-angiotensin system with the biomedical characteristics of infants in the group of patients who had in this pregnancy preeclampsia, found that women with genotype DD of ACE median birth weight was 3280 g. $ (Q25-Q75 = 2825-3645 g), which is statistically significantly lower than in pregnant women with II genotype of ACE (IU = 3475, Q25-Q75 = 3270-3660, p=0.01; pcor=0,03). Reliable associations of the polymorphism -6A/G and AGT-1166A/S ATIIR1c growth of the baby and Apgar score were not detected (p>0.05). Thus, the study established the association of ACE DD genotype with lower weight at birth in women with preeclampsia.

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# PATOLOGIA PLEUROPOLMONARE

## BEVACIZUMAB VASCULAR CHANGE IN NEOPLASTIC AND NON NEOPLASTIC LUNG

S. Aversa[1], L. Ricci[1], L. Spagnoli[1]; D. Spina[2]

*[1] Department of Medical Biotechnologies, University of Siena, Siena, Italy, Pathology Unit, "Azienda Ospedaliera Universitaria Senese", Siena, Italy; [2] Department of Medical, Surgical and Neuro Sciences,, Anatomic pathology Unit, University of Siena, Italy*

**Object.** Lung cancer is the second most common cancer in both men and women with the non- small cell lung cancer (NSCLC) accounting for approximately 85% of total cases and representing the most common cause of cancer-related deaths in Western countries. Although the complete surgical resection remains the most effective initial therapy for patients with early-stage NSCLC (stage I/II/selected IIIA), more than 50% of them present an advanced stage disease. Nowadays chemotherapy based on cisplatin with or without monoclonal antibody is the front-line therapy for advanced non squamous NSCLC. Bevacizumab is a monoclonal antibody that binds to vascular endothelial growth factor (VEGF) and prevents the binding of VEGF with its receptors, VEGFR-1 and VEGFR-2, which are found on the surface of endothelial cells. By blocking VEGF, bevacizumab stops the "leaking" of the vasculature, theoretically improving drug delivery to the tumor. Recent advances in chemotherapy have prolonged dramatically the survival of patients with advanced non small- cell lung cancer to >1 year. As a result, there is an emerging need in clinical practice to evaluate the impact of chemotherapy-induced vascular toxicity in patients with lung cancer. It is difficult to evaluate a change of vasculature directly in vivo, therefore alternative approaches are needed; the objective of this study is to assess the vascular modifications within the neoplasm and in distant healthy tissue induced by bevacizumab plus chemotherapy followed by surgery in patients with unresectable stage NSCLC and evaluating the structure of pulmonary vessels in the removed lung tissue.
**Material and Methods.** We selected nine patients with non resectable NSCLC who had to receive neoadjuvant therapy and subsequently underwent surgery. Patients were subdivided in two groups: the first one consisted of four patients treated with neoadjuvant chemotherapy (platinum based chemotherapy) plus bevacizumab; the second group consisted of five patients treated with chemotherapy only. In detail, two formalin-fixed paraffin-embedded tissue specimen respectively of neoplasia and distant normal parenchyma were collected from each case. Immunohistochemistry staining of CD31 on specimens was performed to define density and morphology of the vessels in tumor and in normal tissue. In particular, we examined vessels localized in the interlveolar septa, in the bronchial walls, into neoplasia and in neoplastic regression areas. In each field the perimeter of the vessels was designed assuming the number of vessels/area and the area of the vessels as parameters. Interlveolar septa and bronchial walls were selected excluding areas of inflammation, edema and fibrosis while vital neoplasia areas were identified excluding areas of fibrosis and necrosis. Fibrosis, coagulative necrosis, histiocytic and macrophage inflammation were considered and evaluated as signs of the neoplastic regression.
**Results.** After histological examination of the vascular pattern in the four specific areas, we noticed that there were some differences between the two groups. In particular, we observed a reduction in the number of vessels with a concomitant increase in their diameters in the group treated with chemoterapy plus bevacizumab, demonstrating the effects of bevacizumab on angiogenesis. Those effects were observed not only in the neoplastic and regression areas, but also in the alveolar septa and bronchial walls, suggesting that the anti-VEGF agents operated also on normal tissue.
**Conclusions.** Novel anti-angiogenic agents such as bevacizumab – which can directly affect tumor angiogenesis – are increasingly being used. However, the effects of these agents on normal vasculature in the lungs and in other organs are not well understood. This study demonstrated that variations in the number and diameters of vessels were present in patients who received chemotherapy plus bevacizumab, whereas these changes were not present in patients who received chemotherapy only. Consequently, basing ourselves on the important reduction in the number of vessels our research showed we can assume a reduced supply of the blood to the tumor, which is in contrast to others studies who presume that bevacizumab improves drug delivery. Furthermore, the increased risk of fatal pulmonary hemorrhages can be explained by the increase in diameters of vessels.
Further studies are warranted to confirm the relationship between bevacizumab and vascular changes during therapy and the possible increase of the risk of vascular toxicity in the lung and in other organs.

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## SUCCESS RATE OF PD-L1 TESTING BY IMMUNOHISTOCHEMISTRY USING SMALL SPECIMENS VS SURGICAL SAMPLES IN NSCLC: ONE YEAR'S EXPERIENCE

L. Caldarola, C. Cafiero, S. Magnasco, G. Napoli, P. Racugno, E. Rizzi, V. Maiarù, T. Montrone, M. Panarelli, M. Pirrelli

*Pathology Department, Ospedale SS Annunziata, Taranto*

**Objective.** Programmed death-ligand 1 (PD-L1) expression testing is recommended by guidelines for patients with advanced non-small cell lung cancer (NSCLC). Immunohistochemical assays for PD-L1 expression in NSCLC are required to predict response to therapy with immune checkpoint inhibitors. Only early NSCLC patients are suitable for surgical resection. In patients with advanced NSCLC (70%) materials obtained using non-surgical sampling techniques are only available. Small samples often limit the opportunity to perform these kind of assays either for diagnostic or prognostic/predictive purposes.
PD-L1 immunohistochemical assays have been clinically validated only on formalin-fixed paraffin-embedded (FFPE) tissue but predictive testing on metastatic NSCLC is often conducted on small specimens such as cytology cell blocks and small bronchoscopy biopsies.
In the present study we aimed to determine the feasibility of

Tab. I. NSCLC specimen categories and histotype.

| | | NSCLC histotype | | |
|---|---|---|---|---|
| Sample type | | ADC | SCC | Total |
| Surgical specimens | | 19 | 6 | 25 (32%) |
| Small tissue | bronchoscopy biopsies | 20 | 14 | 34 (45%) |
| | cytology cell blocks | 15 | 3 | 18 (23%) |
| Total | | 54 (70%) | 23 (30%) | 77 |

Tab. II. The rate of TPS ≥50% was observed to be statistically different in sample categories (P=0.07).

| | | PD-L1 expression | | | |
|---|---|---|---|---|---|
| Sample type | | Inadequate | Negative | Weak positive | Strong positive |
| Surgical specimens | | 0 | 17 | 6 | 2 |
| Small tissue | bronchoscopy biopsies | 2 | 17 | 6 | 9 |
| | cytology cell blocks | 1 | 10 | 2 | 5 |
| Total | | 3 | 44 | 14 | 16 |

performing PD-L1 testing by immunohistochemistry (IHC) using either "small NSCLC specimens" (including cytologic cell blocks and small biopsy) or surgical resection specimens.
**Methods.** A total of 77 NSCLC patients (median age 70 years) who had been diagnosed at Pathology Department, Ospedale SS Annunziata-Taranto, were included in the present study.
The adeno or squamous type of neoplasia and the surgical or small samples categories of specimens are summarized in Table I. PD-L1 expression was evaluated by automated immunohistochemical assay optimized for the VENTANA PD-L1 (SP263) Rabbit Monoclonal Antibody in the BenchMark XT (Ventana Medical Systems, Inc.).
PD-L1 expression was assessed as tumor proportional score (TPS) evaluating the percentage of PD- L1-staining tumor cells (TC). A total of 100 TC were required for sample adequacy. TPS was categorized as negative (<1% TC), weak positive (1%-49% TC) and strong positive (≥50% TC).
**Results.** Results were evaluable in 74 cases out of 77 total cases; 3 small samples (4%) were judged as inadequate for evaluation of PD-L1 expression due to the scarce amount of cells. No statistically significant difference was observed between small samples and surgical specimens in terms of cellular adequacy. The rate of TPS 1%-49% was observed in 8 (10%) small samples out of 77 versus 6 (8%) surgical specimens out of 77 and no significant difference was present between sample types. The rate of TPS ≥50% was observed to be statistically different in sample categories; it was evidenced in 14 small samples (18%) out of 77 cases versus 2 surgical specimens (3%) out of 77 (p=0.07) (Tab. II). No significant difference existed with regard to rates of PD-L1 positivity compared with histological type and the presence of ALK rearrangements.
**Conclusions.** These results based on our experience show that quantitative evaluation of PD-L1 expression is feasible on small specimens although in a small number of cases the sample was inadequate for analysis due to the low amount of cells. In the present study the rate of TPS ≥50% was observed to be statistically different in surgical specimens vs small samples. These results could be explained by different issues. Probably small samples are better fixed than surgical specimens so their antigens are better preserved. Moreover the heterogeneous expression of PD-L1 could have randomly favored small samples. Finally it could be hypothesized that PD-L1 expression could be time dependent so it can be evidenced in non resectable carcinomas. It would be interesting to investigate the results in a larger group of patients. Surgery specimens in advanced NSCLC are rarely available so small samples should be considered as a precious resource for PD-L1 testing in order to offer more therapeutic chances to NSCLC patients.

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## SOMATOSTATINE RECEPTORS SSTR2A AND SSTR5 IMMUNOHISTOCHEMICAL EXPRESSION IN LUNG NEUROENDOCRINE NEOPLASMS: CORRELATION WITH SCINTIGRAPY AND SOMATOSTATINE ANALOGS THERAPY

C. Della Ragione[1], V. Pellecchia[1], A.B. Acampora[1], C. Mocerino[2], M. Catalano[3], S. Campione[1]

*[1] AORN A Cardarelli of Naples, Department of advanced diagnostic therapeutic technologies and health utilities, sections of Anatomic Pathology; [2] AORN A Cardarelli of Naples, Department of onco-hematology and pneumo-hematology, section of Oncology; [3] AORN A Cardarelli of Naples. Department of advanced diagnostic therapeutic technologies and health utilities, section of Nuclear Medicine.*

**Objectives.** Neuroendocrine neoplasms (NEN), both well differentiated tumors (NET) and poorly differentiated carcinomas (NEC), can affect several parts of the human body, most frequently the gastro-entero pancreatic (GEP) and thoracic districts. NEN can show wide variability of biological behavior and are treated with several options, among the others, the use of somatostatin analogs that interact with the somatostatin receptors, expressed by tumor cells.
Somatostatin receptors (SSTRs) are divided into various subtypes, numbered from 1 to 5, and their expression by tumor cells is the condition for efficacy of biotherapy performed with the somatostatin analogue. In clinical practice the presence of somatostatin receptors is highlighted by scintigraphy with.
The expression of SSTR therefore allows to start a specific therapy and it can be also an indicator of favorable prognosis. Subtyping and cellular localization of SSTRs is possible in pathological anatomy by immunohistochemistry (IHC) [1].
Several authors have highlighted the possible prognostic significance of the IHC expression of some subtypes of SSTR, SSTR2 and SSTR5, revealing a correlation between IHC positivity, scintigraphy results and prognosis [2]. However, the majority of these studies highlight this correlation in the NETs of the gastro-entero-pancreatic district (GEP NET). Thoracic

NEN have been only studied by few authors and results are not equally satisfactory [3 4].
Here we present our experience on the NEN of thoracic district comparing the IHC expression of somatostatin receptor with the scintigraphy and the prognosis of patients.
**Materials and Methods.** Patients: fourteen cases of NET diagnosed and followed at our institution were selected from 2008 to 2016 according to the following criteria: i) available scintigraphy data with In- pentetreotide method; ii) tissue available for pathological review and immunohistochemistry; iii) follow-up availability of patients treated with somatostatin analogues.
The tissue of the 14 patients undergone to scintigraphy corresponds to 7 biopsy samples and 7 surgical samples. Samples include 10 cases of well differentiated NET, in the form of typical and atypical lung carcinoid and 4 cases of NEC (lung small and large cell NEC).
Five carcinoids and 1 NEC after scintigraphy were treated with somatostatin analogues (Octreotide or Lanreotide).
Immunohistochemistry: concentrated ABCam Primary monoclonal antibodies IgG type directed against the 2A receptor (UMB1) and against the receptor 5 (UMB4) of the somatostatin have been used. Work dilution 1:100. Platform employed was Ventana UltraView and UltraView Kit. HIER of 30 min with CC1 and an ab incubation of 60 min at room temperature was employed for the ab UMB1. HIER of 64 min with CC1 and an ab incubation of 120 min at room temperature followed by an enhancer system kit was employed for the ab UMB4.
Results were evaluated based on the following score: Score 0 absence of immunoreactivity. Score 1 pure cytoplasmic immunoreactivity, both focal and diffuse. Score 2 membranous reactivity in less than 50% of tumor cells, regardless of cytoplasmic staining. Score 3 circumferential membranous reactivity in more than 50% of tumor cells, independently of the presence of cytoplasmic staining. Score 3 or 2 were evaluated as positive cases.
**Results.** The antibody against UMB1 found 8 positive samples while 12 were identified by scintigraphy. One was IHC positive and negative at scintigraphy and 1 was at both negative. Five patients resulted negative by the IHC and positive by the scintigraphy. They were treated with Octreotide and showed to respond to the therapy and to be stable. Considering this data the sensibility and the specificity of the UMB1 versus scintigraphy resulted to be respectively 58% and 50%. Regarding the UMB4 antibody in IHC it achieved to identify 4 positive samples on 12 identified by scintigraphy. Negative patients to UMB4 and positive to scintigraphy, treated with Octreotide also resulted to respond and to be stable. Negative samples to UMB4 were also negative to scintigraphy. In this case sensibility and specificity of UMB4 versus scintigraphy resulted to be respectively 33% and 100%.
To consider together the two antibodies UMB1 UMB4 determinations versus the scintigraphy results, the sensibility come out to be 67% (8 positives on 12) and the specificity to be 100%.
In terms of success to the therapy the IHC with both UMB1 UMB4 achieved to have a sensibility of 70% and a specificity o 75%.
**Conclusions.** Data here presented are preliminary and low in numbers, reflecting difficulties on founding suitable cases for correlation with scintigraphy and somatostatine analogs therapy in thoracic district.
As found in other experiences, sensitivity of both UMB1 UMB4 antibodies seems to be lower then scintigraphy in determining the access to the therapy. However, their specificity appeared high, and it can suggest the combination of IHC, easy and cheaper, with scintigraphy to be sure that all eligible patients are included in biotherapy.
Correlation with prognosis prediction can be linked to the degree of differentiation of the neoplasm and was not substantial in our experience due to the low number of cases.

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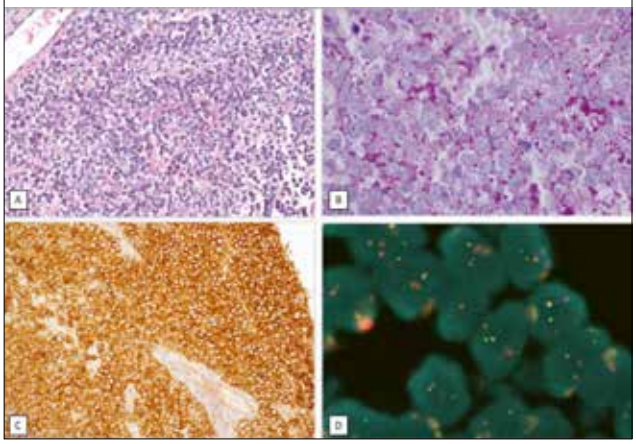
## A RARE CASE OF EXTRASKELETAL EWING'S SARCOMA OF THE LUNG IN AN ADULT MAN


M.A. Fedeli[1], A.M. Fara[1], V. Marras[1], G. Satta[1], M. Trombetta[1], G. Manzoni[2], V. Doneddu[1], F. Tanda[1]

*[1] Department of Medical, Surgical and Experimental Medicine, Unit of Pathology, University of Sassari, Sassari, Italy; [2] Department of Clinical and Experimental Medicine, Institute of Radiological Sciences, University of Sassari, Sassari, Italy*


**Objective.** Description of a rare tumour in an uncommon localization and unusual age of onset.
**Background.** Ewing's Sarcoma is a relatively uncommon neuroectodermal malignant neoplasm, accounting for 6- 8% of primary malignant bone tumours [1].
However, Ewing's Sarcoma is the second most common primary malignant bone tumour in children, adolescents and young adults [2] and usually involves long bones, pelvis and ribs [3].
Extraskeletal Ewing's Sarcomas are rare particularly in patients with advanced age and very few cases have been reported specially in the lung [4].
**Case report.** We present a case of a 53-years-old man having a rapid progression of the disease and lethal outcome. The early symptoms were chest pain, dyspnea and hyperthermia.
Thoracic CT-scan showed a big lung mass that measured 154 x 76 x 131 mm occupying the lower left hemithorax together with abundant pleural effusion.
**Materials and Methods.** Routine H&E and PAS sections were performed by formalin fixed and paraffin embedded blocks and immunohistochemical stainings were performed with the automated Ventana BenchMark Ultra with the UltraView Universal DAB detection Kit.
Molecular Cytogenetic analysis (FISH fluorescent in situ hybridization) were perfomed using Vysis LSI EWSR1 (22q12) Dual Color, Break Apart Rearrangement Probe (Abbott, EWSR1 #03N59-020).
**Results.** A needle biopsy was performed showing few hypercromic and atypical small cells with perivascular distribution. The exiguity of the material didn't allow to make a definite diagnosis.
A surgical biopsy was then performed showing abundant material that revealed a proliferation of uniform small round blue cells with scanty and clear cytoplasm mostly showing perivascular distribution and areas of necrosis. The tumour cells showed strong cytoplasmic PAS positivity.

Figure 1. Representative images of the tumour, highlighting the perivascular proliferation of small round blue cells **(A)** and the characteristic PAS **(B)** and CD99 **(C)** positivity. **(D)** Fluorescent in situ hybridization of EWSR1 gene; fusion signals: yellow or red/green double spot indicates intact EWSR1, separate green and red signals indicate translocation.



On immunohistochemical stainings the tumour cells showed strong positivity for vimentin and CD99 and negativity for cytokeratins, CD45, CD20, sinaptophysin, chromogranin, desmin, S100, HMB45 and WT1. On the basis of these findings the diagnosis of Ewing's Sarcoma was made confirmed by molecular genetic analysis of chromosome rearrangement t(11;22)(q24;q12).

The patient received chemotherapy with an early good response but his conditions worsened rapidly after radiotherapy and he died in few months after the diagnosis.

**Conclusions.** This case represents a very rare case of Extraskeletal Ewing's Sarcoma. The occurrence, very unusual in the lung, together with the unusual age of appearance make this lesion quite exceptional. However, Ewing's sarcoma should always be considered in the differential diagnosis of thoracic small cell tumours in spite of the localization and the age of the patient.

The diagnosis can be made on the usual histological and immunohistochemical technique since it consists of a characteristic proliferation of small round blue tumour cells mostly distributed in the perivascular areas and usually strongly positive for PAS, vimentin and CD99.

Nevertheless, the detection of EWSR1 translocation is the most reliable feature to confirm the diagnosis of Ewing Sarcoma.

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## NODULAR PLEUROPARENCHYMAL FIBROELASTOTIC LESION: REPORT OF A CASE

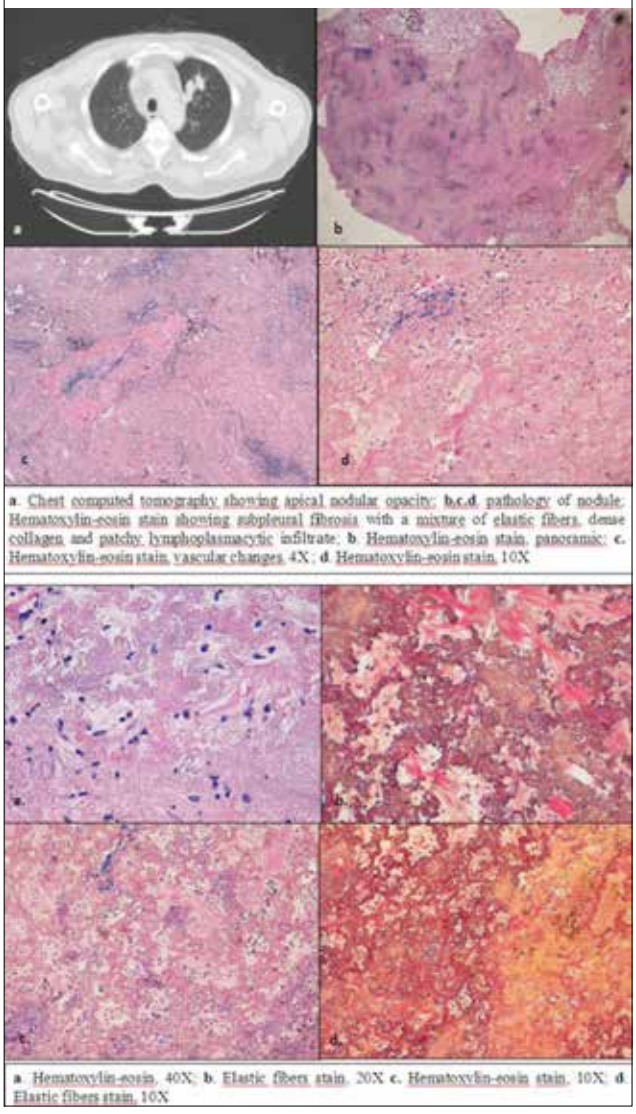

E. Venturino[1], E. Pacella[1], A. Dellachà[1], V. Errigo[1], R. Bianchi[2]

*[1] Department of Pathological Anatomy ASL2 Liguria; [2] Department of Pathological Anatomy Policlinico San Martino-IRCCS Genova*


**Aims.** The finding of a pulmonary fibroelastotic lesion in the upper lobe of an adult patient may lead to diagnostic difficulties for the pathologist, especially if it appears as a nodule and in the absence of accurate clinical-anamnestic information. The aim of this study was to describe a case of solitary fibroelastotic lesion in a 71-years-old male patient with a PET-positive nodule in the left upper lobe.

**Methods.** A 71-year-old man with arterial hypertension had an incidental finding of a lung nodule in the left upper lobe with a diameter of about 2.5 cm and retraction of the visceral pleura. Positron emission tomographic (PET) showed a lesion with medium-low metabolic index. A lobectomy and lymphadenectomy are performed with a request for urgent intraoperative examination for suspected malignancy. We performed Weigert's elastic fibers and Congo red histo-

Figure 1.



a. Chest computed tomography showing apical nodular opacity; b,c,d. pathology of nodule: Hematoxylin-eosin stain showing subpleural fibrosis with a mixture of elastic fibers, dense collagen and patchy lymphoplasmacytic infiltrate; b. Hematoxylin-eosin stain, panoramic; c. Hematoxylin-eosin stain, vascular changes, 4X; d. Hematoxylin-eosin stain, 10X

a. Hematoxylin-eosin, 40X; b. Elastic fibers stain, 20X c. Hematoxylin-eosin stain, 10X; d. Elastic fibers stain, 10X

chemical staining, immunohistochemistry for kappa, lambda chain and TTF1.

**Results.** The surgical resection sample included an area of pulmonary consolidation with atelectatic alveolar residues showing dense intra-alveolar fibrosis and prominent elastotic thickening of alveolar walls. In the central fibrotic area there was hemosiderin, anthracosis, and cholesterol clefts. The lesion was associated with thickening of pulmonary arteries, with severe stenosis and focal obliteration of the lumen suggesting the diagnosis of a vaso-occlusive disease. Focal nonspecific aggregates of chronic inflammatory cells were also found. Weigert's elastic fiber stain showed accumulation of disordered and disrupted elastic fibers highlighting the structure of pre-existing alveoli. Congo red was negative. In the lymph node was observed reactive hyperplasia. Overall, the data described above and the set of clinical and instrumental evidence suggested that the diagnosis of "apical cap" was more likely than "pleuropulmonary fibroelastosis".

**Conclusions.** The finding of a fibroelastotic lesion in the apical site of the superior lung lobe involves a complex differential diagnosis, in concert with clinicians and radiologists. The current case presents the peculiarity of being a PET positive nodular lesion, which led clinicians to misinterpret it as a pulmonary neoplasm. Differential diagnosis of such nodules includes neoplastic, infectious, inflammatory, vascular, traumatic, congenital and other benign conditions. Particularly the histological picture observed in this case imposes a differential diagnosis between pulmonary fibroelastosis (PPFE) that in some cases presents as a parenchymal nodule with positive absorption on the PET scan and the so called "Apical cap" fibrosis, histologically superimposable, but with very different clinical-prognostic implications. PPFE is a rare fibrotic pulmonary disease, affecting the visceral pleura and the subpleural parenchyma with a predilection of the upper lobe, and was included as a distinct clinicopathological entity in the last classification of idiopathic interstitial pneumonias. As such, it comes to clinical attention in a manner similar to other interstitial lung diseases, namely, prominent chest symptoms (such as dyspnea and cough), abnormalities on pulmonary function testing and evidence of diffuse parenchymal disease on chest imaging. As mentioned, the histologic findings in PPFE are strikingly similar to 'Apical cap' fibrosis and differ often only in extent. The 'Apical cap' fibrosis is relatively common, its prevalence increases with advancing age and is regarded as essentially asymptomatic; it is usually identified incidentally during chest imaging, where it may be mistaken for a neoplasm. The presence of occluded arterial vessels and the disappearance of the pre-existing parenchymal structure suggest an ischemic pathogenesis of the lesion in such case.

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# PATOLOGIA TESTA COLLO

## PAROTID GLAND MARGINAL ZONE LYMPHOMA OCCURRING IN THE CONTEXT OF A LYMPHOEPITHELIAL CYSTS IN A PATIENT WITH PERIPHERAL MONOCLONAL LYMPHOCYTOSIS

A. Ronchi[1], M. Montella[1], V. Tirino[2], G. Ferrara[1], F. Zito Marino[1], F. Collina[3], G. Colella[4], R. Franco[1], I. Cozzolino[1]

*[1] Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy; [2] Cytometric and Molecular Diagnosis, Dpt of Experimental Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy; [3] Pathology Unit, Istituto dei Tumori 'Fondazione G. Pascale', [4] Maxillofacial Unit, Department of of Medical, Surgical and Dental Specialties, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy*

**Objective.** Primary salivary gland lymphomas are unusual, accounting for 2,5% to 4,5% of salivary gland tumours. More than 90% are Non-Hodgkin lymphomas (NHL) and the parotid gland is the most common location. Salivary gland NHL is divided into two groups. The former develops in association with benign lympho-epithelial lesion, more frequent in HIV-positive patients or with Sjogren's syndrome. The latter occur in otherwise healthy gland. Monoclonal B-cell lymphocytosis (MBL) is defined as less than $5x10^9$/L monoclonal B cells in the blood of patients without any sign of lymphoma. This condition seems to be related to a higher risk to develop lymphoma or leukemia. Herein, we report a B-cell NHL occurring in the context of a HIV-unrelated benign lympho- epithelial cyst of the parotid gland, in a patient with MBL.

**Materials and Methods.** A 57-yrs-old woman presented with a 3-years history of peripheral monoclonal lymphocytosis CD5 and CD23 negative. During follow-up the CT showed the presence of nodular, pericentimetric areas, with different densities in the left parotid region. The patient underwent an ultrasound-guided FNC of the parotid region; FNC was performed on a hypoechoic, solid area and on an adjacent cystic anechoic area. Two passes were performed on both areas. The material taken from the solid area was dense, whitish; fluid, turbid material was taken from the cystic area. Direct smears fixed to air and in alcohol and coloured with Diff Quik and Papanicolaou respectively were set up from the material taken. Slides stained with Diff Quik were evaluated on-site and the orientation was, for both samples, of a lymphoid dispersed cell population, in a serum-proteinaceous background in the cystic area sampling. Based on the on-site orientation, the second pass was suspended in PBS for flow cytometry (FC) evaluation for both samples. Partial left parotidectomy was subsequently performed; histological evaluation associated with ancillary techniques (immunohistochemistry, ISH, FISH and PCR) were performed.

**Results.** Cytological evaluation of smears corresponding to the solid nodular area showed a dense cellularity represented by a relatively monomorphic lymphoid cell population. The cell population was predominantly composed of small cells with a monocitoid appearance. The nuclei were round with dispersed chromatin; sometimes a thin rhyme of cytoplasm was visible. In the smears corresponding to the cystic nodular area, the cell population was dispersed in a proteinaceous background. The cell population was more polymorphic than the previous sample and consists of small lymphocytes, monocitoid and plasmacytoid cells, plasma cells, few large transformed lymphocytes and histiocytes. FC evaluation showed, in the two samples, a cell population CD19+, CD10-, CD23-, CD5- with restriction for light chains kappa. Cytological diagnosis was of a B-cell lymphoproliferative process, clonal for kappa.

Macroscopically, the partial parotidectomy sample consisted of a 3x2.5x1.5 cm fragment which in section showed some solid nodule and some cystic nodules; the major nodule was 1.5 cm.

Histological evaluation showed cysts, lined by multi-layered squamous epithelium, which showed, in the context of the wall, lymphoid tissue containing lymphoid follicles with germinal centers. Lymphocytic exocytosis was also observed through the wall. These structures were in continuity with lobules of salivary gland mixed with adipose tissue. In the sample there was a small lymph node with a partially subverted structure; rare small reactive, germinal centres and inconstant subcapsular sinuses were recognized. The lymphoid cell population consists predominantly of small CD20 positive B-cell population. The cell population resulted positive for bcl2 and partially for CD43; negative for CD5, CD23, Cyclin D1, CD10, bcl6; some CD138 positive plasma cells were present. The immunocytochemical determination for CD23 showed the presence of irregular-shaped residual reticular of follicular dendritic cells, and images of centrophollicular "colonization" by small, bcl2- positive and CD2-negative lymphoid cells. ISH was performed on paraffin-embedded sections for the determination of the light chains that did not give a contribution. Furthermore, the FISH did not show IgH rearrangement. Finally, a molecular investigation was performed for the investigation of B-cell clonality by PCR on DNA extracted from the paraffined sample. The PCR showed positivity for the clonal rearrangement of genes coding for the heavy chain of immunoglobulins. A final diagnosis of marginal zone lymphoma occurred in the context of a lympho-epithelial cyst was rendered.

**Conclusions.** MBL is characterized by the presence of a monoclonal lymphocytic population in the peripheral blood (less than $5x10^9$/L) in otherwise healthy individuals. The immunophenotype of the monoclonal cells may be CLL-like or non-CLL-like. The latter seems to be correlated in some cases with the develop of marginal zone lymphoma. The occurrence of a marginal zone lymphoma in the setting of a salivary gland lympho-epithelial cyst is rare and the diagnosis can be exceeding difficult. A multidisciplinary approach is mandatory for a correct diagnosis, including morphology, immunohistochemistry, flow cytometry and molecular morphology (ISH, PCR).

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## EPITHELIOID HEMANGIOMA OF THE BUCCAL MUCOSA: A RARE ENTITY WITH PECULIAR MORPHOLOGICAL FEATURES

M. Salemme[1], E. Chirico[1], A. Cominotti[1], S. Negri[1], P. Fontana[1], M. Cottelli[2], L. Zanetti[2]

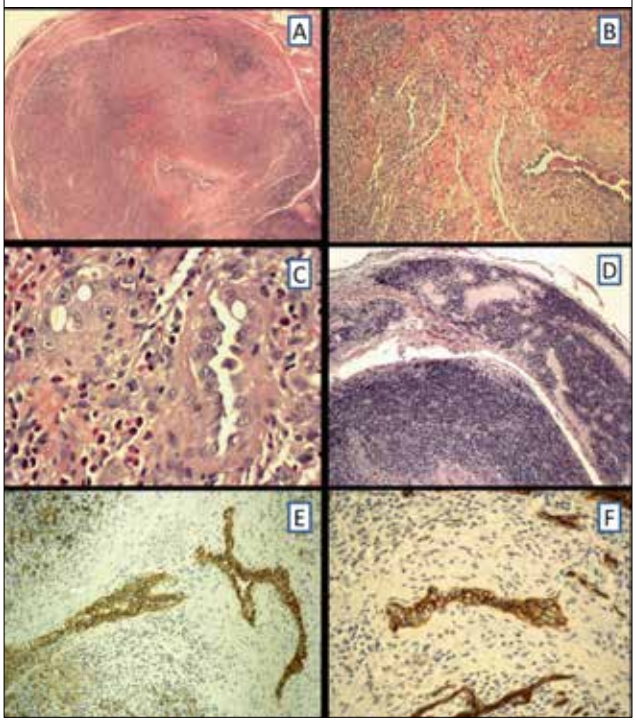
*[1] Department of Pathology, Manerbio Hospital, Brescia, Italy; [2] Department of Otorhinolaryngology, Manerbio Hospital, Brescia, Italy*

**Aims.** Epithelioid hemangioma (EH) is an uncommon benign vascular lesion first described by Wells and Whimser in 1969

Tab. I. The table summarizes the cases of oral EH involving the buccal mucosa reported to date in the literature.

| Authors | Year of publication | Sex and age of the patients |
|---|---|---|
| Kabani et al. | 1988 | Female, 42 y/o |
| Toeg et al. | 1993 | Male, 17 y/o |
| Toeg et al. | 1993 | Male, 12 y/o |
| Misselevich et al. | 1995 | Male, 59 y/o |
| Martin-Granizor et al. | 1997 | Female, 27 y/o |
| Tsuboi et al. | 2001 | Male, 60 y/o |
| Aggarwal and Keluskar | 2012 | Female, 25 y/o |
| Henriques et al. | 2016 | Male, 52 y/o |

Figure 1.




as angiolymphoid hyperplasia with eosinophilia. It commonly affects young adults appearing as red or brown small nodules in the intradermal or subcutaneous region of the head and neck. Histologically the lesion is composed of well developed vascular structures lined by epithelioid endothelial cells with abundant eosinophilic cytoplasm. These structures are associated with a prominent inflammatory infiltrate consisting of lymphocytes, plasma cells and eosinophils. Most lesions are well circumscribed and complete surgical excision usually leads to cure [1]. Extracutaneous lesions occurred in the oral mucosa are rarely reported [2]. Moreover, in this site there is a predominance of cases involving the lips, while only 8 cases affecting the buccal mucosa are reported to date in the literature (Tab. I). Clinical presentation of oral EH is nonspecific, usually characterized by a solitary asymptomatic nodule. Histopathological analysis, revealing the typical morphological features, is essential for the diagnosis. Here we report a case of EH arising in the buccal mucosa, to date the ninth case reported in the literature with a well- characterized histology.

**Materials and Methods**. Our patient is a 44-year-old woman who presented to department of otorhinolaryngology with the complaint of a lump in the right cheek. Intraoral clinical examination revealed a sessile painless nodule in the right buccal mucosa, tender to palpation and measuring 1,5 x 1 x 0,5 cm. The lesion was removed with excisional biopsy. Margins were well- delimited without adherence to deeper planes. Postoperative recovery was uneventful.

**Results.** Histopathological analysis revealed a delimited lesion with a lobular architecture (Figure 1A, hematoxylin-eosin, original magnification X2,5) composed of well-formed vascular structures lined with plump endothelial cells (Figure 1B, hematoxylin-eosin, original magnification X10) that contain abundant eosinophilic cytoplasm with large, round and vesicular nucleus (Figure 1C, hematoxylin-eosin, original magnification X40). The vessels were surrounded by a prominent inflammatory infiltrate which contains lymphocytes, plasma cells and abundant eosinophils (Figure 1C, hematoxylin-eosin, original magnification X40). A dense lymphoid infiltrate was identified at the periphery of the lesion (Figure 1D, hematoxylin-eosin, original magnification X10). Mitotic figures and cellular atypia were absent. Immunohistochemical staining revealed that epithelioid endothelial cells were positive for endothelial cell markers such as CD31 (Figure 1E, original magnification X10) and CD34 (Figure 1F, original magnification X20).

Taking together, the morphological features were consistent with oral EH.

**Conclusions.** We report a case of oral EH involving the buccal mucosa, to date the ninth case reported in the literature with a well-characterized histology. The rarity of the lesion and the clinical similarities with other diseases should prompt to perform a careful histological analysis which is therefore crucial in order to establish the diagnosis.

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# QUALITÀ E SICUREZZA

## GOING TO HPV TEST FOR CERVICAL CANCER SCREENING

M. Iacobellis

*UOSVD Servizio Centralizzato Aziendale di Citopatologia e Screening – P.O. "Di Venere", ASL Bari*

**Introduction.** According to the last Italian PNP (Piano Nazionale Prevenzione) 2014-18 we are going to substitute Pap test with HPV test, like a primary test, in cervical cancer screening. The aim of this work is to take stock of the situation of the Cytology Screening Unit of ASL Bari before this passage .

**Method.** Firstly we have took a look at the quantitative Unit performances of the last five years.Then we have reported the quality control on the Unit activity in the first six months of 2018.

**Material.** The activity of the Unit begun in 2013, all the activity data are collected in the two regional data base managed by Anatomic Pathology and Screening Laboratory Information System (SIRAP and SIRS).

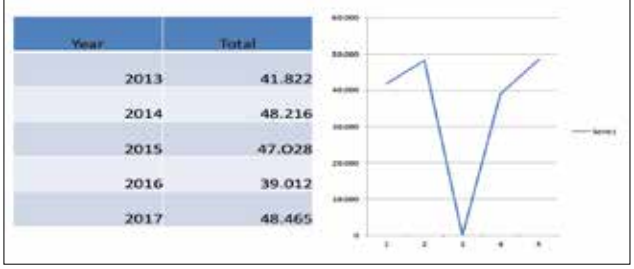
The screening procedure, in our Unit, are based on GISCI guideline and there is an internal quality control based on: monitoring of diagnostic categories, peer review,monitoring of cyto-histological correlation. Monitoring of positive predictive value (VPP) and detection rate (DR), periodic review of negative primary Pap-tests.

**Results.** (Fig. 1, Tabs. I, II, III).

**Conclusions.** During collection of data many problems are stand out. There is a big lack of uniformity in data recording. As a matter of fact, in five years, the Unit have had a great turnover of staff. Too much professionals have alternated, with very different point of view and cultural formation, then, many problems occurred in regional software management, so it is very difficult collecting reliable data.

Anyway, we can see we have some to improve in standardization of data recording and about diagnostic aspects of high-grade cytological lesion. Probably we have to program a continuous improvement of data recording. Without actual data, the effectiveness of the screening cannot be demonstrated.

**Figure 1.** Citology Unit Performances in last five years.




**Tab. I.** Performance from 1-1-18 to 30-6-18. Total pap test thin-prep (primary test) = 18.850.

| | Tot | % | Rif % |
|---|---|---|---|
| Positive | 635 | 3,6 | 2-7 |
| Inadeguate | 284 | 1,5 | 0,9-1,5 |
| ASC-US | 401 | 2,1 | 3-5 |
| ASC-US HPV + | 155 | 38,6 | 50% |
| ASC-H | 48 | 0,2 | 0,3-0,5 |
| L-SIL | 396 | 2,1 | 1,6-3 |
| H-SIL | 30 | 0,1 | 0,4-0,5 |
| CA SQU | 1 | 0,005 | 0,1 |
| AGC | 5 | 0,02 | 0,1-0,4 |

**Tab. II.** Performance from 1-1-18 to 30-6-18. N° cervical-vaginal biopsies: 268. N° conoid biopsy: 117. Agreement conoid/previous biopsy: 75/117 = 64%.

| Histology | N° |
|---|---|
| Coilocitosis | 20 |
| CIN 1 | 148 |
| CIN 2 | 72 |
| CIN 3 | 14 |
| Negative | 9 |
| No diagnostic | 2 |
| Squamous carcinoma | 0 |
| Adenocarcinoma | 2 |
| Other | 1 |

**Tab. III.** Performance from 1-1-18 to 30-6-18. N° cervical-vaginal biopsies: 268 N° conoid biopsy: 117. Agreement conoid/previous biopsy: 75/117 = 64%.

| | ASC-US | L-SIL | H-SIL | ASC-H | AGC | Altro |
|---|---|---|---|---|---|---|
| Coilocitosis | 8 | 10 | 1 | | | 1 |
| CIN 1 | 23 | 99 | 9 | 8 | | 9 neg |
| CIN 2 | 11 | 33 | 11 | 16 | | 1 |
| CIN 3 | 1 | 2 | 8 | 3 | | |
| Negative | 3 | 6 | | | | |
| No diagnostic | 1 | 1 | | | | |
| Squamous carcinoma | | | | | | |
| Adenocarcinoma | | 1 | 1 | | | |
| Other | | 1 | | | | |

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# UROPATOLOGIA

## NEXT GENERATION SEQUENCING IN ADVANCED CHROMOPHOBE RENAL CELL CARCINOMA

M. Brunelli[1], A. Caliò[1], F. Giunchi[2], M. Fiorentino[2], G. Martignoni[1]

*[1] Department of Diagnostics and Public Health, University of Verona; [2] Department of Experimental Medicine, University of Bologna*

Objective. Little is known about the cancerogenesis of advanced staged or metastatic chromophobe renal cell carcinoma by using deep sequencing.

**Methods.** Seven chromophobe renal cell carcinoma with sarcomatoid and/or tissue metastases have been provided for further analysis by using next-generation NGS 50 hot spot cancer gene study (ION Torrent platform).
Bio-informatic analysis was used to avoid false positive molecular findings using stringent cut-offs.
**Results.** 55 was the percentages of tumor cells analyzed and 8 the DNA concentration per case. Quality (>1000 coverage), frequency (30% vs 10%) of mutations were gated. Top gene alterations were grouped by decreasing frequency.
The NGS gene copy number analysis revealed multiple abnormalities (gains and losses). Hotspot mutations were found in cancer-critical genes (TP53, PIK3CA, APC, JAK). The most common deletions was the tumor-suppressor genes RB1. Abnormalities were not detected in both primary and metastatic tissue except for the RB-1 and PIK3CA genes.
**Conclusions.** Next generation sequencing on chromophobe renal cell carcinoma with aggressive behaviour shed light to biology of tumours and may be used as methods to select patients to targeted therapies in clinical trials.

## ASSOCIATION OF CENTROSOME AMPLIFICATION WITH LOSS OF BRCA1 EXPRESSION IN HUMAN UROTHELIAL CARCINOMA

F. Collina[1], S. Cipolletta[1], G. Aquino[1], V. Peluso[1], A. Ronchi[2], R. Tambaro[3], R. De Cecio[1], S. Pignata[3], R. Franco[2], G. Botti[1]

*[1] Pathology Unit, Istituto Nazionale Tumori- IRCCS Fondazione "G. Pascale", Naples, Italy; [2] Pathology Unit, Universita degli Studi della Campania Luigi Vanvitelli, Napoli, Italy; [3] Department of Uro-Gynaecological Oncology, Istituto Nazionale Tumori IRCCS Fondazione "G. Pascale", Naples, Italy*

**Background.** Urothelial carcinoma (UC) of the bladder is the most common solid tumor of the urinary tract. Approximately 30% of newly diagnosed patients present with muscle invasive bladder cancer (MIBC), of whom 50% will progress to distant metastasis, and 5% will initially present with metastatic disease [1 2]. UC is responsive to systemic chemotherapy of multidrug platinum combinations and in 2016 the FDA approved and granted breakthrough status as second-line immunotherapy for patients with locally advanced or metastatic disease that have progressed during or following platinum-containing chemotherapy. Unfortunately, only 20-30% of patients with metastatic UC will achieve a partial or complete response to checkpoint immunotherapy and there are currently no reliable methods to predict response [3]. Thus, further research to identify new strategies for treatment of UC remain a critical focus. The centrosome is a major microtubule-organizing center for the formation of bipolar mitotic spindles and plays an important role in accurate chromosome segregation to daughter cells. Accumulating evidence suggests that centrosome amplification (CA), which leads to the formation of multipolar spindles and unequal segregation of chromosomes, is both a common and major factor for CIN, notably in human malignancies. Previous studies have reported that CA is closely related to tumor grade, DNA ploidy, and CIN in urothelial carcinoma [4]. In UC the centrosome aberration was associated with loss of p53, Rb or ATM and with CCND1 and AURKAamplification. In 2004, Bentley et al. hypothesized that a deficiency in the repair of DSBs may generate high frequency of chromosomal instability observed in bladder cancer [5]. The TCGA analysis of bladder UC provided a possible explanation for these results in the presence of mutations in genes associated with the BRCAness phenotype and genes known to confer PARPi sensitivity (CHEK1/2, RAD51, BRCA1/2, ATM, ATR, MDC1, FANCF) [6]. Jian et al published encouraging data regarding PARPi in UC is in pre-clinical cell culture and xenograft models, but there are no clinical trials of PARPi in bladder UC [7].
**Aim.** The purpose of this study was to investigate the prognostic and predictive role of CA in urothelial carcinomas and to elucidate its relationship with the different centrosome regulators.
**Methods.** Centrosomes were evaluated by immunofluorescence staining using anti-$\gamma$-tubulin antibody and BRCA1, CCND1, p53, Rb, ATM and AURKA protein expressions by immunohistochemistry. Cyclin D1 and AURKA gene amplifications were evaluated by FISH. The relationship existing between the categorical variables included in the study was analyzed using SPSS software
**Results.** CA was detected in 21 (48.8%) of evaluable urothelial carcinomas and was associated with deep muscle invasion (pt2b) (p=0.05). Among all centrosomes regulators analyzed, centrosome amplification showed a statistically significant association only with the absence of BRCA1expression.
**Conclusions.** BRCA1, but not Cyclin D1, p53, Rb, ATM and AURKA, is significantly involved in the pathogenesis of centrosome aberration. In light of these data, BRCA1 could represent a new target in UC.

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## BILATERAL LEYDIG CELL TUMOR OF THE TESTIS AND ADRENAL MASS: TWO SEPARET ENTITIES OR SAME TUMOR?

I. Panarese, M. Montella, G. Roccuzzo, E. Lamantia, F. Zito

Marino, M. Nasti, R. Franco

*Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy*

**Objectives.** Leydig cell tumors (LCTs) are the most common pure testicular sex-cord stromal neoplasm and comprise 1-3% of all testicular neoplasm. Approximately 10% of LCTs are bilateral and 10% are malignant. Features of aggressive behavior are: older age presentation, tumors larger than 5 cm, infiltrative margins, necrosis, nuclear atypia and increased mitotic rate. However, the major criterion of malignancy is represented by distant metastasis.

**Materials and Methods.** We submit a case of a 28-year-old patient with mild cognitive delay, bilateral enlarged testis, obesity with gynecomastia and high serum testosterone levels. Scrotal ultrasonography was performed and detected hypoechoic bilateral testicular nodules that showed hypervascularization. The patient underwent bilateral radical orchiectomy. The gross examination of the right testis showed a well- circumscribed solid mass, with brown cut-surface measuring 6,3 cm in diameter. The microscopic examination of formalin-fixed and paraffin-embedded sections demonstrated a neoplastic proliferation with nodular growth-pattern, composed by solid sheet, separated by a delicate vascular network, of large polygonal cells with abundant eosinophilic cytoplasm and round nuclei with a single prominent nucleolus. Areas of clear and vacuolated cells and areas of large cells with nuclear pleomorphism and high mitotic rate (>10/10 HPF) have been reported. The epithelial and stromal component of rete testis was infiltrated by neoplastic cells. Necrosis was absent. The nodule in the left testis was 5,6 cm in diameter. Gross and microscopic features were similar to the tumor in the right testis. Immunohistochemically both tumors shown a positive stain for Inhibin, Melan-A and Calretinin. On the basis of morphological and immunohistochemical features the diagnosis of bilateral Leydig cells tumor with features of biological aggressive behavior was rendered. Total body CT scan was performed and highlighted a mass in left adrenal gland and bilateral enlargement. Surgical excision of left adrenal gland was performed and gross examination has showed nodular mass of golden yellow color measuring 4,5 cm in diameter and 65 g in weight. At microscopic examination the tumor was composed by solid sheets and trabeculae of small cells with granular and eosinophilic cytoplasm and large cells with abundant clear vacuolated cytoplasm. The immunohistochemical profile of this tumor was similar to the both tumor of testis (positive stain for Inhibin, Melan-A and Calretinin).

**Results and Conclusion.** We report a case of a patient with bilateral testicular mass and bilateral enlargement of adrenal glands. Our first diagnostic hypothesis was a malignant bilateral Leydig-cell tumor (LCT), with adrenal metastasis. Anyway due to similar morphological and immunohistochemical profile between normal adrenal gland and LCT we performed a genomic analysis of these 3 mass, using DNA extraction from paraffin-embedded samples performed on ION-TORRENT platform, that did not highlighted any molecular correlation between LCT and adrenal tissue. Afterwards, Endocrinologists hypothesized the possibility of a deficiency of 21-beta hydroxylase. Therefore, in our opinion, correlating clinical information to the morphologic data the more probable diagnostic hypothesis should be a testicular adrenal rest tumors (TARTs). On the basis of this, a molecular evaluation of the enzyme 21-beta hydroxylase mutation is underway.

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## INFLAMMATORY LEIOMYOSARCOMA, A CASE REPORT

E.Unti, I. Di Lorenzo, N. Scibetta

*UOC Anatomia Patologica, ARNAS Ospedale Civico Di Cristina Benfratelli, Palermo*

**Introduction and Objectives**. Inflammatory leiomyosarcoma is a rare, malignant mesenchymal neoplasm, whose clinical, morphological and genetic evidence depose for a myogenic sarcoma rather than for a myofibroblastic lesion. In fact, recent studies have shown that inflammatory leiomyosarcoma has a specific genetic profile, characterized by near-haploidization, and global gene expression profiling revealed prominent differential expression of genes involved in muscle development and function. Patients with inflammatory leiomyosarcoma have a lower average age (41 years) and fewer relapses than the classical leiomyosarcoma.

It predominantly afflicts males and arises in the deep soft tissues of the trunk and proximal limbs. We report a case of inflammatory leiomyosarcoma for its rarity, and to underline the difficulties encountered in the differential diagnosis.

**Materials, Methods and Results.** A 65-year-old man, was visited at our hospital, because he was suffering from a pain in the right iliac fossa for about three months.

The ultrasounds and the CT scan of the abdomen with contrast medium detected in the right external iliac site an oval mass, non-homogeneously hyperdense after contrast medium, of 6,2x5,5 cm, contiguous to a distal ileal loop and compressing the right sperm vessels with a consensual endovaginal effusion.

The laboratory tests were normal. The patient underwent surgery for mass removal and lymphadenectomy of right iliac lymph nodes was performed.

Macroscopically it was a mass of 5,5 cm in maximum dimension, nodular, solid and of greyish-white color.

Microscopically, a cellular population was observed composed partly by fused elements with cytological atypia and in part by large, pleomorphic, sometimes multinucleated cells, with prominent nucleoli, eosinophilic cytoplasm, vacuolized, of sternbergoid type, immersed in a lively inflammatory context, consisting of lymphocytes, plasma cells, histiocytes and eosinophil granulocytes. There were phenomena of sclerosis and necrotic areas. The immunophenotypic caracterization of the neoplasm showed a weak, focal positivity for CD30 and EMA in some of the sternbergoid cells, whereas CD20, CD3, CD1a, CD68, Cytokeratin AE1/AE3, S100 were negative. The spindle cells also showed weak and focal positivity for desmin, and were diffusely positive for smooth muscle actin (alpha-SMA), caldesmon, specific muscle actin (MSA) and calponin, while negative for myogenin, CD34, MDM2. In

view of the difficult interpretation of the immunomorphological picture, it was decided together with the patient to send the case to a reference center, where the diagnosis of inflammatory leiomyosarcoma was established. This diagnosis was confirmed by a further reference center to which the patient had sent the histological slides. No evidence of metastasis in the four iliac lymph nodes examined.

**Conclusions.** We report a case of inflammatory leiomyosarcoma for its rarity, and to underline the difficulties encountered in the differential diagnosis between this neoplasia and another malignant tumor such as Hodgkin's lymphoma, or a less aggressive sarcoma such as myxo-inflammatory fibroblastic sarcoma. All these neoplasms are characterized in fact by the presence of mono or plurinucleate histiocytoid cells, with prominent nuclei of sternbergoid type, and by the presence of a polymorphic inflammatory infiltrate and of ialinizing fibrosis phenomena. Immunohistochemistry plays a fundamental role in diagnosis because myxoinflammatory fibroblastic sarcoma has no specific markers, whereas inflammatory leiomyosarcoma is positive for myofilament markers, as in our case.

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