L’approccio metodologico per il corretto orientamento delle biopsie endoscopiche

Collocare i prelievi sul filtro e strappare

Immergere l’insieme filtro-biopsie in formalina

Processare e includere ruotando il tutto di 90

RISULTATI

CD3

PATENT PENDING

PATHOLOGICA

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

ORIGINAL ARTICLES

36 Lipomatous myofibroblastoma of the breast: case report with diagnostic and histogenetic considerations
S. Cinocca, F. Rossin, S. Avio, M. Di Vincenzo, D.C. Cucchi, G. Sagaglione, C.M. Betts, M.P. Foschini

41 Cytological features of nipple adenoma in scraping smears
S. Cinocca, F. Rossin, S. Avio, M. Del Vecchio, M.C. Cucchi, G. Sagaglione, C.M. Betts, M.P. Foschini

45 Cytologic features of solid pseudopapillary neoplasms of the pancreas: a single institutional experience based on evaluation of diagnostic utility of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA)
S.M. Gilani, R. Santamaria, M. Barawi, B. Al-Khadig

51 Neuroendocrine tumours of the pancreas: a clinicopathological study of nine cases including six insulinomas
F. Limone, N. Aref, E. Ben Hassen, A. Lahmar, S. Bourouzi, S. Mzabi-Regaya

BRIEF ORIGINAL ARTICLE

58 Pre-miR-146a expression in follicular carcinomas of the thyroid

CASE REPORTS

61 Oblitary neuroblastoma with focal ganglioneuroblastic differentiation
S. Squillaci

67 Papillary haemangiomatosis: a case report of multiple facial location
S. Squillaci, B. Fazio, W. Aoufi, L. Labbene, M. Kharfi, R. Zermani

70 Juvenile haemorrhagic fibromatosis: a case report
S. Mesri, N. Lafeche, N. Mame, A. Ayadi, M. Latifi, B. Sitra, H. Krifa, M. Mekni

73 Uterine tumour resembling ovarian sex cord tumours presenting as multiple endometrial and cervical uterine polyps: a case report
N. Abid, H. Mbir, M. Mekni, S. Chari, A. Khatir, S. Makni, F. Boudawara

82 “Pure” primary large cell neuroendocrine carcinoma of the urinary bladder
T. Pusiol, D. Morichetti, M.G. Zorzi

ATTI DI CONGRESSO

86 I Meeting Nazionale del Gruppo Italiano di Paleopatologia (GPaPal)

ISSN 0031-2983
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:

1. The manuscript must be submitted by e-mail to the address: pathologica@pacinieditore.it
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.
3. 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.
4. 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:
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.

**Specific instructions for the individual sections**

*Pathologica will give preference to the publication of original articles and reviews.*

**Editorials:** short general and/or practical papers on topical subjects invited by the Editor-in-Chief. Editorials are limited to 8000 characters including spaces with at least 10 references. They may include 1-2 figure or table. Should have no more than 2 authors. No abstract is requested.

**Updates:** They can be invited by the Editor-in-Chief. Papers should not exceed 20 printed pages, including tables, illustrations and references. No abstract is needed.

**Original articles:** specially written-up articles which present original observations, or observations deriving from a relevant experience (though not fully original) in a specific field. The text is to be composed in Abstract, Key Words, Introduction, Material and Methods, Results and Discussion. Text should not exceed 40,000 characters including spaces. Pages should be numbered, beginning with the title page without initiate every section on a separate page. All main text, with wide (2.5 cm) margins, must be double spaced throughout and typed in a 12 point font. Figures should be limited in Number of 5. The abstract (no longer than 300-400 word) should be clear and concise.

**Case reports:** will be considered, for publication only if they describe relevant cases (rare, of particular didactic interest, etc.). The clinical and pathologic data should be complete, using update methodology, and top-level images. The text should include a brief review of relevant references and a discussion on new data regarding the pathogenesis and/or the diagnostic role of pathology regarding the described case/disease. Text length: no less than 10000 and no more than 12000 characters including spaces with 10-15 references, and no more of 5 images (figures and/or tables). Should have no more than four authors.

**Special sections:** Guidelines and Check Lists, Cytopathology, Molecular Biology, Immunohistochemistry, Informatics, Organization and Management, Medical Education, Book Reviews and Society News.

*Letters to the Editor:* They should focus on particularly relevant and exciting topics in the field of pathology, already published articles or present original data. When referring to already published articles, the letter will be sent to the authors of the articles and their reply published in the same issue. They should not exceed 8000 characters including spaces, with one table or figure and 2-3 references.

**Reprints**

Reprints may be ordered at cost price when page proofs are returned. Pacini Editore will supply the corresponding author with one free copy of the relevant issue.

**Applications for advertisement space should be directed to:**

*Pathologica*

Pacini Editore S.p.A. - Via Gherardesca, 56121 Pisa, Italy
Tel. +39 050 3130217 - Fax +39 050 3130300
E-mail: mmori@pacinieditore.it

Applications for advertisement space should be directed to:

*Pathologica* - Pacini Editore S.p.A., Via Gherardesca, 56121 Pisa, Italy
Tel. +39 050 3130217 - Fax +39 050 3130300
E-mail: mmori@pacinieditore.it

**Subscription information**

*Pathologica* publishes four issues per year. The annual subscription rates for non-members are as follows:

- Italy € 105,00; all other countries € 115,00. This issue € 20,00
- for Italy, € 25,00 abroad.

Subscription orders and enquiries should be sent to: *Pathologica* - Pacini Editore S.p.A. - Via Gherardesca, 56121 Pisa, Italy
E-mail: abbonamenti@pacinieditore.it - On line subscriptions: www.pacinimedicina.it

Subscribers’ data are treated in accordance with the provisions of the Legislative Decree, 30 June 2003, n. 196 – by means of computers operated by personnel, specifically responsible. These data are used by the Publisher to mail this publication. In accordance with Article 7 of the Legislative Decree n. 196/2003, subscribers can, at any time, view, change or delete their personal data or withdraw their use by writing to Pacini Editore S.p.A. - Via A. Gherardesca 1, 56121 Pisa, Italy.

Photocopies, for personal use, are permitted within the limits of 15% of each publication by following payment to SIAE of the charge due, article 68, paragraphs 4 and 5 of the Law April 22, 1941, n. 633. Reproductions for professional or commercial use or for any other other purpose other than personal use can be made following a written request and specific authorization in writing from AIDRO, Corso di Porta Romana, 108, 20122 Milan, Italy (segreteria@aidro.org – www.aidro.org).

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

Journal printed with total chlorine free paper and water varnish.

Printed by Pacini Editore, Pisa, Italy - August 2014
CONTENTS

ORIGINAL ARTICLES

Lipomatous myofibroblastoma of the breast: case report with diagnostic and histogenetic considerations
G. Magro, F.R. Longo, L. Salvatorelli, E. Vasquez, G.M. Vecchio

We report rare case of myofibroblastoma (MFB) of the breast comprised predominantly of a mature fatty component, representing approximately 70% of the entire tumour area. This tumour, designated “lipomatous MFB”, should be interpreted as the morphological result of an unbalanced bidirectional differentiation of the precursor mammary-stromal cell, with the adipocytic component overwhelming the fibroblastic/myofibroblastic one. Lipomatous MFB is a rare variant of mammary MFB, which can mimic malignancy because of the close juxtaposition of fibroblasts/myo-fibroblasts with mature adipocytes, resulting in a finger-like infiltrative growth pattern of the former towards the latter. Histogenetic considerations and differential diagnostic problems with other bland-looking spindle cell tumours containing infiltrating fat are provided.

Cytological features of nipple adenoma in scraping smears
S. Cinocca, F. Rosini, S. Asioli, M. Del Vecchio, M.C. Cucchia, G. Saguatti, C.M. Betts, M.P. Foschini

Introduction. Nipple adenoma (NA) is a benign epithelial lesion of the breast that can clinically simulate Paget’s disease or invasive ductal carcinoma. Therefore, correct pre-operative diagnosis is important for appropriate management.

Methods. Cytological samples may be obtained by different methods such as fine needle aspiration, nipple discharge or nipple scraping. Herein, the cytological features of three cases of NA are described in which samples were derived from nipple scraping.

Results. In all three cases, patients were adult females presenting with a sub-areolar nodule, showing skin ulceration in 2 of 3 cases. The nipple scraping cytological smears were characterised by a bloody background with epithelial cells arranged in clusters or singularly, showing an irregular nuclear profile. These features could simulate a malignant process. However, at higher magnification, fine nuclear chromatin with inconspicuous nuclei and presence of myoepithelial cells were helpful to exclude malignancy.

Discussion. NA may present “worrisome” cytological features on smears derived from nipple scraping. Therefore, knowledge of the cytological spectrum of this lesion is important to avoid misdiagnosis.

Cytologic features of solid pseudopapillary neoplasms of the pancreas: a single institutional experience based on evaluation of diagnostic utility of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA)
S.M. Gilani, R. Tashjian, M. Barawi, B. Al-Khafaji

Background. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is an important modality for diagnosing solid and cystic pancreatic lesions. The objectives of this retrospective study are to review the cytologic criteria used to diagnose pancreatic solid pseudopapillary neoplasms (SPNs) and evaluate the utility of EUS-FNA by correlating cytologic and histologic samples.

Case reviews. Of the 924 pancreatic FNAs performed at our institution from January 2002 through February 2013, four histologically confirmed cases of SPN were identified; three had an initial cytologic diagnosis of SPN. All four cases lacked on-site evaluation. Cytologic smears were assessed by two reviewers for the presence of a cellular aspirate, fibrovascular stalks lined by neoplastic cells with pale to finely granular cytoplasm, and monotonous, oval nuclei containing delicate chromatin, inconspicuous nuclei, and grooves and inclusions.

Three cases were diagnosed as SPN on cytologic examination and confirmed histologically. The remaining case was deemed a pancreatic endocrine neoplasm on cytology, but SPN on final histology. The most consistent cytologic feature we encountered was the presence of a cellular aspirate containing fibrovascular stalks lined by monotonous neoplastic cells with oval nuclei and nuclear grooves.

Conclusion. We conclude that EUS-FNA is an effective diagnostic tool in the diagnosis of pancreatic SPNs.

Pre-miR146a expression in follicular carcinomas of the thyroid

Introduction. Micro-RNA, a new class of small, non-coding RNAs, have been shown to be deregulated in several human carcinomas. In particular, SNP rs2910164 in pre-miR146a appears to be correlated with papillary thyroid carcinoma and may be involved in its genetic predisposition. Since data on follicular thyroid carcinomas (FTC) are lacking, we evaluated the involvement of SNP rs2910164 in FTC.

Methods. Thirty-nine cases of FTC and 20 follicular adenomas, defined according to WHO criteria, were selected. DNA and RNA were extracted from formalin-fixed paraffin-embedded blocks of both neoplastic and non-neoplastic areas. The DNA region of pre-miR146a, containing SNP rs2910164, was sequenced. Total RNA including miRNAs was used for stem-loop RT reactions, and applying a standard TaqMan PCR kit protocol for real-time PCR. Wilcoxon signed-rank test and Friedman test were used for statistical analyses.

Results. In 31% of FTC, the G allele was observed in neoplastic tissues, compared with the non-neoplastic areas (p < 0.05), whereas the CC phenotype was completely absent in tumours. Moreover, the expression of pre-miR146a was found to be significantly down-regulated in neoplastic tissues from FTC cases (p = 0.043), although no significant differences were seen in follicular thyroid adenomas.

Discussion. The expression profile of pre-miR146a can be correlated with FTC tumourigenesis. The G allele in SNP rs2910164 appears to be correlated with the transition from normal to neoplastic tissue. The GG and GC alleles appear to be associated with an increased risk for FTC, while the CC allele seems to play a protective role.

Brief original article

Olfactory neuroblastoma with focal ganglioneuroblastic differentiation: a case report with literature review
S. Squillaci

Olfactory neuroblastoma (ONB) is a rare malignant neuroectodermal tumour, with clearly defined histologic and immunohistochemical
features, that typically arises in the superior nasal cavity. Although the classical clinicopathological features leave little room for misinterpretation, the wide variability in this tumour, including occasional divergent differentiation, may cause diagnostic difficulty. Herein, an unusual case of ONB with focal ganglioneuroblastic differentiation in an 81-year-old woman arising from the anterior ethmoid, filling the upper portion of the left nasal cavity and sparing the sinus cavities, is described. Histologically, the tumour was composed of atypical monotonous round cells that were positive for NSE, CD56, chromogranin, synaptophysin, neurofilament and calretinin and exhibited an irregular lobulated and nested growth pattern and sparse mitotic figures (3 to 4 mitoses per 10 HPF). Focally, the histology changed to ganglioneuroblastic differentiation consisting of large ganglion and spindle cells, positively staining for S-100, GFAP, CD99, neurofilament, calretinin, chromogranin and synaptophysin. Neuroblastomas, occurring in the nasal cavity, in analogy to other sites, tend to have an aggressive biologic behaviour and can histologically mimic other undifferentiated malignant neoplasms of the sinonasal tract. Differential diagnostic problems are discussed; a comprehensive review of the literature has also been performed with a focus on survival.

Papillary haemangiomma: a case report of multiple facial location S. Ramieh, B. Façaza, W. Ajouri, I. Labbene, M. Khafri, R. Zermani

Papillary haemangiommas were recently defined as morphologically distinct and benign cutaneous haemangiommas showing a predominantly intravascular capillary proliferation within dilated thin-walled dermal blood vessels. We describe the case of a 45-year-old woman who presented with multiple eruptive red-bluish raised papules and nodules distributed over the skin of the chin that were related to a papillary haemangiomma.

Juvenile hyaline fibromatosis: a case report S. Mestiri, N. Labaied, N. Mama, A. Ayadi, M. Ladib, B. Sritha, H. Krifa, M. Mokni

Juvenile hyaline fibromatosis is a rare, hereditary disease with distinct clinical and histopathological features. Clinically, it presents with gingival hypertrophy, papulonodular skin lesions and joint contractions. Bone involvement is usually an uncommon finding. We report a case of a 2-year-old patient, daughter of consanguineous parents, who presented since the age of 2 months with impairment of mental development, multiple joint contractures, motion limitation and nodules on the scalp. The calvarian lesions were surgically removed, and histopathological examination concluded to juvenile hyaline fibromatosis.


Background. Uterine tumours resembling ovarian sex-cord tumours (UTROSCT) are very rare, benign uterine tumours, composed solely of sex cord elements. These tumours have a polyphe- notypic immunophenotype that favours a derivation from uterine mesenchymal stem cells.

Case report. A 43-year-old female presented with recurrent vaginal bleeding. On hysteroscopy, she had multiple endometrial and cervical polyps that were removed endoscopically. Histologically, the specimen contained epithelioid cells arranged in tubules, trabeculae and anastomosing cords, without significant cellular atypia or mitotic activity. Immunohistochemical studies were performed. The tumour was found to be diffusely positive for vimentin, calretinin and desmin, focally positive for cytokeratin, CD99 and inhibin and negative for chromogranin and CD10. A subsequent total hysterectomy was performed and revealed neoplastic infiltration of the myometrium.

Conclusion. A polyphenotypic immunophenotype is a characteristic feature of UTROSCT, and may be helpful in diagnosis and in exclusion of other lesions. Familiarity with this tumour by gynaecologists and pathologists is essential to avoid misdiagnosis. correct diagnosis of this neoplasm is important in patient management.

Giant pedunculated polypoid submucosal lipoma of the splenic flexure of colon: case report and review of the literature MR. Zare-Khormizi, M. Moghim, F. Pourrajab

Lipomas of the colon are rare but clinically important conditions that require suitable evaluation for guiding appropriate therapy. The majority of lipomas arise from the submucosal layer in the ascending colon, especially near the ileocecal valve, which causes difficulties in diagnosis. Giant lipomas may be misinterpreted as a premalignant adenomatous polyp, particularly when arising in the left colon. A 38-year-old man presented with manifestations including hypogastric pain, constipation, loss of appetite and weight, accompanied by anaemia, nausea, vomiting and haematochezia. Colonoscopy revealed a large submucosal polyp about 5x4 cm, which was located at the splenic flexure of colonic. Surgery detected an oval polypoid tumour measuring 70x50x45 mm in size, having a pedunculated appearance and a stalk diameter of 20 mm. Histopathologic examination of the biopsy from the lesion confirmed diagnosis of a giant submucosal lipoma. In our experience, most giant colonic lipomas are found to be sessile and occur in the ascending colon in older patients. Herein, we report a pedunculated tumour in a 38-year-old male located in the splenic flexure of colon.

“Pure” primary large cell neuroendocrine carcinoma of the urinary bladder: case report, literature review and diagnostic criteria T. Pusiol, D. Morichetti, M.G. Zorzi

Introduction. Large cell neuroendocrine carcinoma (LCNC) is defined in the urinary bladder, as in other sites, as a high-grade neoplasm exhibiting neuroendocrine features at the H&E level, high mitotic activity and evidence of neuroendocrine differentiation by immunohistochemistry. We report a case of pure bladder LCNC with review of the literature.

Methods. A 68-year-old male presented with gross haematuria of two weeks’ duration in October 2011. Transurethral resection and subsequently radical cystoprostatectomy (CP) with bilateral lymphadenectomy (L) were performed in December 2012.

Results. Urinary cytology identified malignant cells. Histologically, the tumour showed organoid nesting, trabecular growth, rosettes and peribular palisading patterns, suggesting neuroendo- crine differentiation. Immunohistochemical staining showed intense positivity for CD56.

Discussion. We examined all published pure bladder LCNC (12 cases) excluding mixed neoplasms. Small cell carcinoma of the urinary bladder pure LCNC of the bladder is a very aggressive malignancy, unresponsive to therapy, presents in an advanced stage and has a propensity for early metastasis. Prior to the advent of immunohistochemistry, such cases would most likely have been categorised as poorly differentiated, high-grade urothelial carcinomas.
Lipomatous myofibroblastoma of the breast: case report with diagnostic and histogenetic considerations

G. MAGRO, F.R. LONGO, L. SALVATORELLI, E. VASQUEZ, G.M. VECCHIO
Department G.F. Ingrassia, Anatomic Pathology, University of Catania, Italy

Key words
Myofibroblastoma • Mammary-type • Breast • Lipomatous variant

Introduction
Myofibroblastoma (MFB) of the breast is a relatively rare benign spindle cell tumour first described by Wargotz et al. in 1987, which belongs to the family of the “benign tumours of the mammary stroma”. It is composed of stromal cells showing fibroblastic and myofibroblastic differentiation at the morphological, immunohistochemical and ultrastructural levels. Tumours that are similar if not identical to MFB of the breast have also been described with the term “mammary-type MFB” in soft tissues and in the vulvo-vaginal region. Although mammary MFB is typically a bland-looking spindle cell tumour, there is increasing evidence that it encompasses a wide morphologic spectrum. Several variants have been described, including infiltrating, cellular, collagenised/fibrous, atypical cell, epithelioid/deciduoid cell, lipomatous, fibromatosis-like, myxoid, palisaded/Schwannian variants. Only rarely do morphological features of two different variants coexist in the same tumour. Although some of these variants seem to be of academic interest, the recognition of others, such as epithelioid/deciduoid-like and lipomatous variants, may be crucial to prevent overdiagnosis of malignancy, especially when evaluating small incisional biopsies.

It is widely known that mammary MFB may contain a variable amount, usually in the form of small islands, of adipose tissue as an integral tumour component, and not as the result of fat entrapment by neoplastic cells. The term “lipomatous myofibroblastoma (MFB)”, first coined by Magro et al. in 2000, has been used to designate those rare cases of mammary MFB containing a significant (> 50% of the entire tumour) mature fatty component. Since that original description, only a few cases reports have been reported in the literature. We herein report a new, intriguing case of mammary MFB with an extensive mature fatty component, which can mimic a lipomatous tumour, such as spindle cell lipoma, spindle cell liposarcoma or lipoma-like well-differentiated liposarcoma. Histogenetic and diagnostic considerations are provided.

Clinical history
A 60-year-old woman presented with a painless, solitary, 3-cm lump in her right breast that appeared firm and circumscribed on physical examination. Preoperative ultrasonography and mammography confirmed a well-circumscribed mass, without microcalcifications.

Correspondence
Gaetano Magro, Department G.F. Ingrassia, Azienda Ospedaliero-Universitaria, Policlinico Vittorio Emanuele, Anatomic Pathology, University of Catania, via S. Sofia 87, 95123 Catania, Italy - E-mail: g.mагro@unict.it
in the breast parenchyma. A complete surgical excision of the mass, including a rim of adjacent grossly normal parenchyma, was performed. No local recurrence has been observed at 1 year following surgery.

**Materials and methods**

The surgical specimen was submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques and embedded in paraffin; 5 μm sections were cut and stained with haematoxylin and eosin (H&E), Alcian blue at pH 2.5 and periodic acid-Schiff (PAS). Immunohistochemical studies were performed with the streptavidin-biotin peroxidase detection system using the ventana automated immunostainer (Ventana Medical Systems, Tucson, AZ). The antibodies tested were vimentin (dilution 1:100); α-SMA (dilution 1:200); desmin (dilution 1:100); myogenin (dilution 1:100); S-100 protein (dilution 1:500); CD99 (dilution 1:100); CD34 (dilution 1:50); B-cell lymphoma 2 (Bcl-2) protein (dilution 1:100); CD10 (dilution 1:200); CD117 (dilution 1:400); cytokeratins (AE1/AE3 clone; dilution 1:50); epithelial membrane antigen (EMA) (dilution 1:100); p63 (dilution 1:200); anti-human melanosome (HMB45) (dilution 1:300); estrogen receptor (ER) (dilution 1:100); progesterone receptor (PR) (dilution 1:100); all from Dako, Glostrup, Denmark. Appropriate positive and negative controls were included.

**Results**

Grossly, tumour consisted of a well-circumscribed, incompletely encapsulated lipomatous mass, 3 cm in diameter. On cut sections, a yellow tumour mass with some interspersed whitish areas was evident. Calcifications, haemorrhage and necrosis were absent. Histological examination revealed a well-circumscribed neoplasm composed predominantly (70%) of adipose tissue containing dispersed, vaguely nodular or irregularly shaped spindled cellular areas and fibrous septa (Fig. 1). At low magnification, the overall appearance was that of a fibrolipoma or spindle cell lipoma. The fatty component was represented by mature adipocytes, uniform in size and shape, and lacking nuclear pleomorphism (Fig. 2). Lipoblasts were not seen. The non-adipocytic component was represented by spindle-shaped cells arranged in short, haphazardly intersecting fascicles interrupted by keloid-like, highly eosinophilic collagen fibres (Figs. 2, 3). These spindled cells showed pale to eosinophilic cytoplasm, with ill-defined borders and an oval nucleus with occasional small nucleoli, and were closely reminiscent of conventional mammary MFB (Fig. 3). Nuclear pleomorphism was absent, and rare mitoses (< 1 mitosis x 10 HPF) were observed. Atypical mitoses were not observed. Neoplastic cells showing hybrid features between fibroblasts/myofibroblasts and adipocytes, namely spindled cells with varying degree of intracytoplasmic accumulation of lipids in the form of single large non-membrane-bound droplet or multiple small droplets, could not be identified, even after meticulous examination of the entire tumor. The adipocytic and the spindled components were variably admixed resulting in two distinct tissues with smooth interface or in a finger-like infiltrative growth pattern (Fig. 3). Mammary ducts or lobules were not trapped within the tumour. Immunocytochemically, the spindled cells were diffusely positive for vimentin, desmin (Fig. 4), and CD10, while heterogeneous immunoreactivity was obtained with α-smooth muscle actin, CD34, Bcl-2 protein and CD99. Interestingly, immunoreactivity for estrogen/progesterone receptors was observed in about 70% and 20% of neoplastic cells, respectively. No immunostaining was obtained with any other antibodies tested. Based on morphological and immunohistochemical findings, a diagnosis of “lipomatous MFB” was rendered.
Discussion

Mammary MFB is usually thought of as a bland-looking spindle cell tumour with both fibroblastic and myofibroblastic morphological, immunohistochemical and ultrastructural features. The differential diagnosis of this tumour includes a wide variety of tumour or tumour-like, bland-looking spindle cell lesions of the breast, such as inflammatory pseudotumor, leiomyoma, desmoid-type fibromatosis, cellular myxoma, and low-grade fibromatosis-like spindle cell metaplastic carcinoma. However, diagnosis of mammary MFB is usually possible if strict morphological and immunohistochemical criteria are applied, as discussed in detail. Unfortunately serious diagnostic problems may arise when the pathologist is dealing with unusual morphological variants, especially the “epithelioid cell”, “lipomatous”, and “pleomorphic lipoma-like” variants.

We herein report a rare case of benign spindle cell stromal tumour of the breast with a prominent (70%) mature fatty component. Due to this morphology, tumour was closely reminiscent of a lipomatous tumour, especially spindle cell lipoma or spindle cell liposarcoma. However, morphological and immunohistochemical findings were consistent with a fibroblastic/myofibroblastic tumour that fits within the spectrum of MFB of the breast, representing the uncommon morphological variant, for which the descriptive term “lipomatous MFB of the breast” seems to be most appropriate. The following morphological and immunohistochemical features, typically described in most cases of MFB of the breast, support this opinion: i) the tumour was unencapsulated, “pure” mesenchymal lesion lacking any epithelial component; ii) the non-lipomatous (minor) component was reminiscent, at least focally, of conventional mammary MFB; iii) neoplastic spindled cells had a fibroblastic/myofibroblastic profile at immunohistochemistry (immunoreactivity for vimentin, desmin, α-smooth muscle actin and CD34); iv) neoplastic cells were variably stained with markers, such as bcl-2, CD99, CD10, ER and PR; v) the presence of numerous eosinophilic keloid-like collagen fibres. The cellular mechanisms responsible of fat accumulation in mammary MFB are still unclear. It has been postulated that mammary MFB arises from a presumptive mammary stromal stem cell capable of multidirectional mesenchymal differentiation, including fibroblastic, myofibroblastic, leiomyomatous, osseous, cartilaginous and lipomatous differentiation. The evidence that mammary stroma may undergo similar morphological/immunohistochemical changes in other non-neoplastic conditions supports the hypothesis that mammary stromal cells can modulate their morphological profiles in response to genetic, microenvironmental and hormonal stimuli. According to this hypothesis, lipomatous MFB should be interpreted as a bimorphic tumour that reflects the plasticity of precursor mammary stromal cells to undergo dual myofibroblastic and lipomatous differentiation, with the former overwhelming the latter. We were not able to identify cells showing morphological features of transition between fibroblasts/myofibroblasts and mature adipocytes. This argues against the possibility that fat component is the result of a metaplastic process – intended as transdifferentiation from one fully mature cell type in another – from fibroblasts/myofibroblasts into adipocytes. Therefore, mature adipose tissue in lipomatous MFB seems to arise “ex novo” from precursor stromal cells.

Nevertheless, semantics aside, lipomatous MFB, owing to its bland cytology and low mitotic activity, needs to be distinguished from several bland-looking spindle cell lesions containing infiltrating fat. The predominant fatty component in our case may represent a potential diagnostic pitfall, leading to serious problems in differential diagnosis with adipocytic tumours (spindle cell lipoma, lipoma-like well-differentiated liposarcoma, spindle cell liposarcoma), fibromatosis (desmoid-type) and low-grade fibromatosis-like spindle cell metaplastic carcinoma. Tumours with morphological and immunohistochemical features similar to spindle cell lipoma of soft tissues have rarely been reported in the breast with
the terms “spindle cell lipoma” 36 or “benign spindle cell tumour with prominent adipocytic component” 37. These tumours are composed of mature adipocytes blended with CD34-positive, bland-looking spindled cells. Unlike spindle cell lipoma, the cells in lipomatous MFB are arranged in a short fascicular arrangement and exhibit significant expression of myogenic markers (desmin; α-smooth muscle actin). Although MFB and spindle cell lipoma are regarded as two distinct entities, there is increasing evidence that both belong to the same category of the benign mesenchymal tumours with deletion of the 13q14 38 39. Accordingly, the two tumours may be viewed as two distinct phenotypes of the same disease 39. Liposarcoma can rarely occur in the breast 40. Unlike our case, lipoma-like well-differentiated liposarcoma contains adipocytes with hyperchromatic and atypical nuclei, and atypical stromal cells in the fibrous septa, intersecting the adipocytic component 41. In addition, the detection of lipoblasts, which however are not always present, argues against a diagnosis of MFB. Spindle cell liposarcoma is a distinctive clinicopathological entity occurring in soft tissues 42 43. It is easily distinguishable from lipomatous MFB for the presence, even if only focally, of lipoblasts with cytological features that closely resemble the differentiation of human embryonic fat 43. Fibromatosis is a locally recurring lesion that rarely occurs in breast parenchyma 23-25. It is composed of long, sweeping cellular fascicles embedded in a fibrous stroma rather than of short fascicles separated by thick, keloid-like collagen fibres as seen in MFB 25. Unlike lipomatous MFB, fibromatosis exhibits infiltrating borders, entrapping both fat and glandular tissue of the breast parenchyma 23-25. Immunohistochemically, fibromatosis does not express CD34, while desmin is usually absent or only focally expressed 23-25. Low-grade fibromatosiform spindle cell metaphasic carcinoma may closely mimic lipomatous MFB, owing to the bland cyto-morphology of spindle cells which show only mild to moderate pleomorphism, low mitotic activity and a wavy fascicular growth pattern 27. Immunohistochemical analyses, showing variable expression of epithelial (cytokeratins, EMA) and myoepithelial markers 27 44, along with no staining for desmin and CD34, is mandatory for correct diagnosis. In conclusion, the case presented herein is unusual in that it was difficult to recognize as mammary MFB, owing to the large amount of lipomatous tumour component. Awareness by pathologist of the possibility that mammary MFB may exhibit a dominant fatty component is crucial to avoid confusion with other benign and malignant bland-looking tumours or tumour-like spindle cell lesions containing infiltrating fat.

References

21 Magro G, Mesiti M. Breast and pectoralis musculo-aponeurotic


Introduction

Nipple adenoma (NA) is a rare benign epithelial lesion of the breast that can clinically simulate Paget’s disease or invasive ductal carcinoma. Therefore, correct pre-operative diagnosis is important for appropriate management.

Methods

Cytological samples may be obtained by different methods such as fine needle aspiration, nipple discharge or nipple scraping. Herein, the cytological features of three cases of NA are described in which samples were derived from nipple scraping.

Results

In all three cases, patients were adult females presenting with a sub-areolar nodule, showing skin ulceration in 2 of 3 cases. The nipple scraping cytological smears were characterised by a bloody background with epithelial cells arranged in clusters or singularly, showing an irregular nuclei profile. These features could simulate a malignant process. However, at higher magnification, fine nuclear chromatin with inconspicuous nucleoli and presence of myoepithelial cells were helpful to exclude malignancy.

Discussion

NA may present “worrisome” cytological features on smears derived from nipple scraping. Therefore, knowledge of the cytological spectrum of this lesion is important to avoid misdiagnosis.
Tab. 1. Clinical data of patients with nipple adenoma.

<table>
<thead>
<tr>
<th>Data</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age</td>
<td>42</td>
<td>67</td>
<td>81</td>
</tr>
<tr>
<td>Site</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Skin ulceration</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Nipple retraction</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Palpable nodule below nipple</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nodule size</td>
<td>1.5 cm</td>
<td>0.5 cm</td>
<td>1.0 cm</td>
</tr>
</tbody>
</table>

Fig. 1. A) Cytological smears were characterised by plasmacytoid cells in a background of red blood cells; B) Cells showed a slightly irregular nucleus, with finely dispersed chromatin and minute nucleolus; C) Cells were sometimes arranged in clusters, showing elongated cells having the features of myoepithelial cells; D) Tubular structures that appeared in cases 2 and 3.
Results

**Cytological features**

At low magnification, all three cases presented numerous red blood cells, superficial keratinocytes from epidermis, neutrophilic granulocytes and keratin debris. At higher power, a moderate number of epithelial cells were present (Fig. 1A). These latter cells had plasmacytoid features with large, pale eosinophilic cytoplasm. Nuclei were slightly irregular in shape, however, chromatin was fine and nucleoli were inconspicuous (Fig. 1B). These latter cells presented both singularly or arranged in clusters. Even when the clusters appeared pluristratified or showed a three-dimensional appearance, some elongated myoepithelial cells were always identified (Fig. 1C). In Case 1, epithelial cells were mostly non-cohesive. In Cases 2 and 3, clusters had a tubular appearance (Fig. 1D). In Case 3, tubular epithelial structures were numerous. Occasional mitotic figures were observed. The presence of numerous red blood cells associated with epithelial cells, lead to the cytological diagnosis of “suspicious for malignancy - C4” in Cases 1 and 3. Case 2 was classified as “atypia in a probably benign lesion - C3”.

**Histological findings**

All lesions were surgically removed. Tissues were fixed in formalin and routinely embedded in paraffin. Immunohistochemistry was performed in an automated stainer (Ventana-Benchmark, Tucson, AZ) utilising the following antibodies: CK14, p63, HER-2, oestrogen and progesterone receptors (supplied pre-diluted by the same company).

At histology, all three cases exhibited the typical features of NA.

Case 1 showed a florid epithelial proliferation involving the lumen of the nipple ducts (Fig. 2A). Cases 2 and 3 showed a pseudo-infiltrative pattern of growth (Fig. 2B), characterised by tubular structures immersed in an oedematous stroma. In all cases, a myoepithelial cell layer was present that demonstrated at immunohistochemistry by p63 positivity. Epithelial cells were positive for oestrogen and progesterone receptors, while HER-2 was negative. Therefore, a final diagnosis of NA – epithelial hyperplasia – type was made in Case 1 and NA – sclerosing adenosis – type was made in Cases 2 and 3.

Discussion

Nipple adenoma is a rare benign epithelial proliferation of breast collecting ducts. It is mostly unilateral, occurring predominantly in females and only rarely in males. Patient age ranges from 20 to 87 years. The most frequent clinical features are mass or induration, erythema, erosion or retraction of the nipple, although sanguineous and serous nipple discharge may also be present. The recognition of NA is important as it can mimic Paget’s disease of the breast; moreover, several cases of NA misdiagnosed as carcinomas have been described. Therefore, correct pre-operative diagnosis is important. Cytological examination is a widely used pre-operative diagnostic tool in breast pathology. In cases of nipple lesions, smears may be obtained by fine needle aspiration (FNA) through spontaneous or provoked nipple discharge and/or by skin scraping. On FNA examination, NA samples are characterised by high cellularity. Naked oval nuclei are often seen in the background admixed with the epithelial cells. The latter are uniform in shape and dimension, and arranged both singularly and in monolayer clusters. The clusters are occasionally organised in a papillary architecture. Identification of myoepithelial cells supports the final diagnosis, as described by Kijima et al. In FNA smears, NA should be differentiated from fibroadenomas. Scraping smears are very easy to obtain, non-invasive and inexpensive, and can be performed by clinicians at the time of presentation. Unfortunately, the reliability of cytological samples obtained using this procedure is under debate. Evaluating rare lesions through this

Fig. 2. On histology, cases presented the typical features of NA: A) Case 1 was classified as NA epithelial hyperplasia type; B) Cases 2 and 3 were classified as NA sclerosing adenosis type.
method might become more problematic. To date, no cases of NA diagnosed on nipple scraping smears, have been reported.

All three cases presented herein were from adult women, showing a nodule localised immediately below the nipple, with skin ulceration in two cases. All these features were clinically suspicious for malignancy. Therefore, the clinician performed a gentle scraping of the lesion. The cytological smears were characterised by a bloody background with epithelial cells, showing slightly irregular nuclei, arranged in clusters or dispersed as single cells.

All these features could lead to diagnosis of a malignant lesion, and more specifically differential diagnoses include invasive carcinoma infiltrating the epidermis and Paget’s disease. Moreover, in the present cases of NA, the presence of myoepithelial cells was extremely helpful to exclude malignancy. In addition, at higher magnification, the delicate chromatin pattern and the small and inconspicuous nucleoli rendered a diagnosis of Paget’s disease extremely unlikely.

Conclusions

The purpose of the present report was to describe the cytological features of NA in smears derived from nipple scraping. These lesions may present features, such as bloody background and epithelial cell clusters, that could simulate malignancy. Nevertheless, absence of atypia and presence of myoepithelial cells can be helpful in order to reach correct pre-operative diagnosis. Pathologists should be aware of these features to avoid inappropriate management and to reduce distress in patients affected by NA.

References

Cytologic features of solid pseudopapillary neoplasms of the pancreas: a single institutional experience based on evaluation of diagnostic utility of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA)

S.M. GILANI¹, R. TASHJIAN², M. BARAWI², B. AL-KHAFAJI¹
¹ Department of Pathology, ² Department of Gastroenterology, St. John Hospital & Medical Center, Detroit, USA

Summary

Correspondence
Syed M. Gilani, Department of Pathology, St. John Hospital & Medical Center, 22101 Moross Road, Detroit MI 48236, USA - Tel. +1 313 343 3212 - Fax +1 313 881 4727 - E-mail: magilani@hotmail.com

Background. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is an important modality for diagnosing solid and cystic pancreatic lesions. The objectives of this retrospective study are to review the cytologic criteria used to diagnose pancreatic solid pseudopapillary neoplasms (SPNs) and to evaluate the utility of EUS-FNA by correlating cytologic and histologic samples.

Case reviews. Of the 924 pancreatic FNAs performed at our institution from January 2002 through February 2013, four histologically confirmed cases of SPN were identified; three had an initial cytologic diagnosis of SPN. All four cases lacked on-site evaluation. Cytologic smears were assessed by two reviewers for the presence of a cellular aspirate, fibrovascular stalks lined by neoplastic cells with pale to finely granular cytoplasm, and monotonous, oval nuclei containing delicate chromatin, inconspicuous nucleoli, and grooves and inclusions.

Three cases were diagnosed as SPN on cytologic examination and confirmed histologically. The remaining case was deemed a pancreatic endocrine neoplasm on cytology, but SPN on final histology. The most consistent cytologic feature we encountered was the presence of a cellular aspirate containing fibrovascular stalks lined by monotonous neoplastic cells with oval nuclei and nuclear grooves.

Conclusion. We conclude that EUS-FNA is an effective diagnostic tool in the diagnosis of pancreatic SPNs.

Methods

We collected and reviewed data for all pancreatic FNA procedures performed in our institution from January 2002 through February 2013. A total of 942 FNAs were performed during that time period. Of these, four cases (three females, mean age 17 years; one male, age 61 years) were identified with a diagnosis of SPN initially rendered on cytologic material obtained by EUS-FNA. This procedure is usually performed before surgery at our facility. Each patient presented with a cystic mass involving the pancreatic parenchyma (Figs. 1, 2). A minimum of four passes were conducted in each instance to

Correlation between cytologic material and follow-up surgical resection specimens.

Key words

Solid pseudopapillary • Pancreas • EUS • FNA • Differential diagnosis • Prognosis
ensure adequate diagnostic capture. At least nine Pap- nicolau stained slides and one Diff-Quik stained slide were prepared for each case. No on-site evaluation was performed for any of the specimens. Cytologic smears for each case were then assessed by two independent reviewers for the presence of the following diagnostic features: 1) highly cellular aspirate; 2) presence of myxoid or hyalinised fibrovascular stalks or papillae lined by neoplastic cells with 3) pale to finely granular cytoplasm, 4) indistinct cell borders, 5) and monotonous round-to-oval nuclei containing 6) delicate granular nuclear chromatin, 7) inconspicuous nucleoli, 8) hyaline globules, and 9) nuclear grooves and inclusions; and 10) background consisting of foamy cells and necrotic debris.

**Results**

The first case is 16-year-old female who presented with a history of abdominal pain. The initial abdominal computed tomography (CT) scan was abnormal. The subsequent EUS-FNA revealed a 3.0 cm heterogeneous mass in the head of the pancreas (Figs. 1, 2). Cytologic material was obtained for 11 smears (10 Papanicolaou stained slides and one Diff-Quik stained slide) and one cell block preparation. Cytologic evaluation of the smears showed hyalinised fibrovascular stalks lined by monotonous neoplastic cells with round-to-oval nuclei. The cells were bland and uniform with nuclear grooves. A diagnosis of SPN of the pancreas was rendered. The subsequent histologic resection exhibited similar histologic features. A properly-controlled panel of immunohistochemical stains was performed on the surgical specimen. The neoplastic cells were negative for chromogranin and synaptophysin, confirming a diagnosis of SPN. The post-operative course was unremarkable.

The second case is that of a 17-year-old female who presented with abdominal pain. An 8.5 cm heterogeneous mass involving the head of the pancreas was seen on EUS-FNA. Cytologic material obtained during the EUS-FNA procedure produced 11 smears (10 Papanicolaou stained slides and one Diff-Quik stained slide) and one cell block preparation. Cytologic examination showed a cellular aspirate consisting of numerous neoplastic cells with delicate granular cytoplasm, indistinct cell borders, round-to-oval nuclei, and inconspicuous nucleoli. Scattered fragments containing hyalinised vascular stalks were identified. Morphologically, the neoplasm was consistent with SPN of the pancreas. The ensuing surgical resection specimen demonstrated features of pancreatic SPN. The neoplastic cells were bland and monomorphic with minimal cytologic atypia and no observed mitotic activity. There was a background of coagulative necrosis. A panel of properly-controlled immunohistochemical stains was performed on the neoplastic cells, which exhibited a positive staining pattern with α-1 antitrypsin, CD10, progesterone, and cyclin D1. The same cells were negative for cytokeratin AE1/AE3. The post-operative course was unremarkable.

The third case is that of a 19-year-old female who presented with abdominal pain, and the subsequent abdominal CT scan was abnormal. EUS-FNA revealed a mass in the pancreas. Cytologic material consisted of 14 smears (11 Papanicolaou stained slides and 3 Diff-Quik stained slides), evaluation of which revealed numerous individual cells and clusters of cells in a papillary arrangement. The neoplastic cells appeared monomorphic with nuclear grooves and a minimal amount of focally granular cytoplasm. A diagnosis of pancreatic SPN was rendered, which was later confirmed histologically. The patient was eventually lost to follow-up.

The final case is that of a 61-year-old male who presented with jaundice. The initial abdominal CT scan demonstrated a pancreatic mass. EUS-FNA revealed a 4.0 cm hypoechoic mass in the pancreatic body. Cytologic material obtained by EUS-FNA produced 10 smears (9 Papanicolaou stained slides and one Diff-Quik stained slide) and one cell block preparation. Cytologic examination showed a cellular aspirate with monotonous cells...
Solid PseudoPapillary neoPlasms of the Pancreas possess abundant cytoplasm. The cell block material showed scant cellularity. However, immunohistochemical stains were performed on the cell block, which revealed the neoplastic cells to be weakly positive for synaptophysin and negative for cytokeratin AE1/AE3 and S100 protein. A diagnosis of PEN was rendered. The ensuing distal pancreatectomy specimen was evaluated histologically, and it displayed uniform-appearing neoplastic cells and abundant hyalinised stroma. These cells were arranged in a solid sheet, micropapillae, and in a microcystic configuration. A properly-controlled panel of immunohistochemical stains was performed. The neoplastic cells were positive for α-1-antichymotrypsin, α-1 antitrypsin, CD10, neuron-specific enolase, and synaptophysin. They were negative for cytokeratin AE1/AE3 and chromogranin. The morphologic and immunohistochemical features were consistent with SPN of the pancreas. The post-operative course was uneventful.

Of the four cases presented above, three were diagnosed as SPNs on cytologic review. One was initially deemed an islet cell tumour on cytologic evaluation due to the presence of features resembling those of a pancreatic endocrine neoplasm (PEN), namely a rosette-like arrangement and finely granular, “salt and pepper”-type nuclear chromatin (Fig. 3). All three cases determined to be a SPN on cytology were confirmed histologically by review of available slides obtained from surgical resection specimens.

The most consistent and reliable cytologic features for diagnosis of SPN by EUS-FNA in our study was the presence of hyalinised fibrovascular stalks lined by monotonous neoplastic cells with round-to-oval nuclei, seen in three of the four cases (Figs. 4-6). Nuclear grooves were noted in two cases (Fig. 6). Each of the four cases exhibited a highly cellular aspirate with many cohesive clusters of neoplastic cells, a virtually universal but non-specific finding associated with SPNs. Nu-
clear inclusions and hyaline globules were not identified in any case.

Discussion

SPNs are uncommon pancreatic tumours of epithelial origin with a low overall malignant potential. The entity was originally termed as “papillary tumour of pancreas” in 1959 and since then it has been referred to by several different names, including “solid and cystic neoplasm” and “papillary and solid neoplasm.” In 1996, it was renamed as “solid pseudopapillary tumor” and was classified as an exocrine pancreatic tumour.

The majority of the cases of SPNs have been reported in young female patients, with a propensity to arise in the second and third decades of life. However, SPNs have also been observed both in males and in the paediatric age group. While asymptomatic presentation and incidental discovery of SPN is not uncommon, most patients present with a vague abdominal discomfort and distension. Symptoms such as non-bilious emesis may occur in cases of SPNs causing gastric outlet obstruction. Cystic SPNs may rupture spontaneously, resulting in an acute abdomen. No studies have found a link between SPNs and clinical defined syndromes.

Tumours vary in size and may exceed 10 cm in greatest dimension. The pancreatic tail and body are more commonly involved than are the head and the neck. CT scans may reveal an encapsulated cystic lesion with solid foci. Microcalcification, haemorrhage, and degenerative changes may be observed within the cystic areas. Magnetic resonance imaging (MRI) will demonstrate similar findings but is generally more diagnostically accurate than CT scans. Direct visualisation of SPNs and the opportunity to obtain cytologic material for microscopic evaluation may be accomplished during EUS-FNA. The advantage of performing EUS-FNA is that it allows for better patient management when a diagnosis may be rendered on cytology. Microscopic examination of cytologic material obtained through FNA typically demonstrates a cellular aspirate with cohesive, monotonous-appearing neoplastic cells arranged around a hyalinised or myxoid fibrovascular stalk. Their nuclei contain pale to finely granular nuclear chromatin. Nuclear grooves may be identified occasionally. Mitotic activity is minimal to none. In addition, rare eosinophilic cytoplasmic inclusions or hyaline globules may also be noted.

The neoplastic cells of SPNs are generally immunoreactive with CD 99, neuron-specific enolase (NSE), chromogranin-A, vimentin, CD10, α1-antitrypsin, α1-antichymotrypsin, and progesterone receptor, while they exhibit variable expression with pancytokeratin and synaptophysin. SPNs demonstrate nuclear accumulation of beta catenin and lack membranous staining with E-cadherin.

The differential diagnosis of SPNs is limited. In particular, these neoplasms must be distinguished from pancreatic endocrine/neuroendocrine neoplasms (PEN) and pancreatic acinar cell carcinomas. This is especially difficult because all three entities share overlapping cytomorphologic features. Other differential diagnostic considerations include papillary mucinous carcinomas, intraductal papillary mucinous neoplasms (IPMNs), and pancreaticoblastomas.

The neoplastic cells of PENs tend to be more discohesive and may be arranged in rosettes; papillary architecture is not a feature of these neoplasms. The nuclei are eccentrically-located, imparting a plasmacytoid appearance and contain finely granular, “salt and pepper”-type nuclear chromatin. Argyrophilic granules and periodic acid schiff (PAS) positive globules may be identified in the cytoplasm of these cells. Pancreatic acinar cell carcinomas are more common in elderly males and have a tendency to involve any portion of the pancreas. The majority of neoplastic cells are clustered in an acinar-like pattern, but scattered single cells or loosely cohesive groups may also be seen in the background. They vary in size and shape and contain a minimal to moderate amount of finely, granular amphophilic to eosinophilic cytoplasm, an enlarged, round-to-oval nucleus, and a single, centrally-located, prominent nucleolus. Mitotic activity is readily appreciated. Papillary mucinous carcinomas are generally large, unilocular, cystic masses. Columnar neoplastic cells are found in a mucinous background and often exhibit cytoplasmic vacuolation, nuclear atypia, and distinct nucleioli. Papillae lined by neoplastic, mucin-producing columnar cells may be seen in IMPNs, but the presence of a thick, vis-
cous mucinous background distinguishes these tumours from SPNs. Approximately two-thirds of pancreato-blastomas occur in children. Microscopically, these neoplasms show both a predominant acinar differentiation and the presence of squamous nests composed of whorls of plump spindle cells. Cells with endocrine differentiation are almost invariably seen. The acinar cells have granular cytoplasm, basally-oriented nuclei, and a single distinct nucleolus per nucleus.

Jhala et al. suggested that cytoplasmic vacuolisation is a characteristic feature of SPNs, and identification of this feature may aid in discriminating SPNs from PENs. Meriden et al. emphasised the importance of identifying hyaline globules in SPNs. Some researchers have placed significant importance on immunohistochemistry in differentiating SPNs from both PENs and pancreatic acinar cell carcinomas; positive nuclear staining with beta-catenin and lack of membranous staining by E-cadherin favours a diagnosis of SPN. Clear cell endocrine neoplasms of the pancreas, which are usually associated with von Hippel-Lindau disease, may also be excluded when encountering the clear cell variant of SPNs. The cytoplasm of the clear cell variant of SPNs is acidophilic or amphophilic, finely granular, and contains lipids, which imparts the clear cell appearance. Finally, metastatic clear cell renal cell carcinoma should also be considered. In these cases, the primary renal neoplasm will typically be immunoreactive with CD10 and lack staining with both beta-catenin and neuroendocrine markers.

SPNs are low grade malignant neoplasms with an overall favourable prognosis that rarely recur or metastasi. However, rare atypical cases of SPNs exhibiting aggressive features such as a high proliferative index (as measured by an increased Ki-67 labeling index), frequent mitoses, and cytologic atypia, have also been reported. The standard therapeutic approach for SPNs is complete surgical excision. Tipton et al. suggested complete surgical resection of both the primary neoplasm and any metastatic foci, if present, while Krug et al. demonstrated the utility of radiotherapy in locally aggressive recurrent cases.

**Conclusion**

We conclude that EUS-FNA is an effective, minimally-invasive diagnostic modality in obtaining adequate cytologic material for accurate characterisation and diagnosis of SPNs of the pancreas. However, SPNs should be differentiated from PENs and pancreatic acinar cell carcinomas, as well as other solid and cystic lesions of pancreas, by histologic review of subsequently resected surgical specimens and utilisation of immunohistochemical stains, if necessary. Reliance upon characteristic and reproducible cytologic features may help to establish a diagnosis of SPN in FNA material and improve overall diagnostic accuracy.
Neuroendocrine tumours of the pancreas:
a clinicopathological study of nine cases including six insulinomas

F. LIMAIEM1, N. ARFA2, E. BEN HASSEN2, A. LAHMAR1, S. BOURAOU1, S. MZABI-REGAYA1
Department of 1 Pathology and 2 Surgery, Mongi Slim Hospital, La Marsa, Université de Tunis El Manar, Faculté de Médecine de Tunis, Tunisia

Key words
Pancreas • Tumour • Neuroendocrine • Immunohistochemistry

Background. Pancreatic neuroendocrine tumours (pNET) are relatively uncommon, accounting for 1-2% of all pancreatic neoplasms. They are characterised by varying clinical presentation, tumour biology and prognosis.

Aim. To provide an updated overview on clinicopathological features, treatment and outcome of pNET.

Patients and methods. In our retrospective study, we reviewed 9 cases of pNET that were diagnosed at the Pathology Department of Mongi Slim Hospital over an 11-year period (2003-2013). Relevant clinical information and microscopic slides were available in all cases and were retrospectively reviewed. The latest WHO classification (2010) was adopted.

Results. Our study group included 3 men and 6 women (M/F ratio 0.5) with an age between 20 and 75 years (mean = 52 years). Pancreatic neuroendocrine tumours ranged in size from 0.5 to 10 cm (mean 4 cm). The sites of pNET were the head of the pancreas (n = 4), the body of the pancreas (n = 3) and the tail of the pancreas (n = 2). Enucleation of the tumour was performed in five cases. Three patients underwent distal pancreatectomy and splenectomy, whereas only one patient had central pancreatectomy. Histopathological examination of the surgical specimen coupled with immunohistochemical study established a diagnosis of pNET grade 1 (G1) in seven cases and grade 2 (G2) in two cases.

Conclusion. Pancreatic neuroendocrine tumours are a heterogeneous group of neoplasms with distinct tumour genetics, biology and clinicopathological features. Accurate clinical and pathologic diagnosis is an important first step in developing an appropriate management plan.

Introduction
Pancreatic neuroendocrine tumours (pNET) are relatively uncommon and account for 1-2% of all pancreatic neoplasms. Worldwide, the annual incidence of pNET is estimated to range from 0.2 to 0.4 per 100,000 1-3. Pancreatic neuroendocrine tumours originate from totipotential stem cells or differentiated mature endocrine cells within the exocrine gland, and are characterised by distinct tumour genetics, biology and clinicopathological features 4. The aim of the present report was to analyse epidemiological characteristics, clinical symptoms, radiological features, treatment and outcomes of 9 patients who were surgically treated at our institution.

Patients and methods
We undertook a retrospective study of 9 patients who were operated on for pNET at the General Surgery Department of Mongi Slim hospital of Tunis between April 2003 and May 2013. The cases were retrieved from the surgical registry files of the same hospital. Clinical records and microscopic slides of each patient were available for review in all cases. Clinical data, radiological investigations, treatment and outcome were retrospectively analysed. All patients underwent imaging evaluation during the preoperative period. All specimens were surgically obtained. Tissues were fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin and
sections were prepared for routine light microscopy after staining with haematoxylin and eosin. Immunohistochemical analysis was performed using the avidin-biotin complex technique with antibodies against chromogranin A, synaptophysin and MIB-1. Patient confidentiality was maintained.

Results

Clinical findings

Our group of patients comprised six insulinomas (cases 2, 3, 6, 7, 8, 9) and three nonfunctioning tumours (cases 1, 4, 5) (Tab. I). There were 3 male and 6 female patients (M/F 0.5) between 20 and 75 years of age (mean 52 years). The delay from onset of symptoms to diagnosis ranged between 15 days and four years. Past medical history of the patients was significant for diabetes (n = 3), hypertension (n = 1) and Sjögren’s syndrome (n = 1). Four patients had no significant prior medical history. The presenting clinical symptoms of insulinomas were dominated by hypoglycaemic collapse (n = 6), dizzy spells (n = 4), abdominal pain (n = 2) and weight gain (n=1). For non-functioning tumours, presenting clinical symptoms were abdominal pain (n = 2) and altered general health (n = 1). One case of non-functioning pNET was fortuitously discovered during check-up for Sjögren’s syndrome by routine ultrasonography.

Biological Findings

Concurrent measurement of fasting serum glucose, insulin and C-peptide levels were done in six patients. The ratio of insulin to plasma glucose was > 0.3 in all cases. During a monitored fast of six hours, a low blood glucose level (< 0.5 g/l) and high serum insulin level (> 3mUI/l) were detected in all six cases. C-peptide level was also elevated (> 200 pmol/l). The typical history of recurrent hypoglycaemic collapse and dizzy spells combined with biochemical evidence of fasting hyperinsulaemia allowed a diagnosis of insulinoma in six cases (cases 2, 3, 6, 7, 8, 9).

Radiological findings of pancreatic neuroendocrine tumours

Diagnostic imaging techniques included ultrasonography in nine cases, CT scan in nine, MRI in five and endoscopic ultrasound in five patients (Tab. II). Ultrasonography demonstrated a hyperechogenic lesion in three cases of pNET. In six cases of insulinoma, ultrasonography failed to reveal the tumour. On CT scan, pNET presented as an enhancing hypodense (n = 2), isodense (n = 1) and heterogeneous lesion (n = 1) (Fig. 1). In five cases of insulinomas, CT did not disclose a pancreatic lesion. In two cases, MRI demonstrated a pancreatic lesion that was hypointense on T1 and hyperintense on T2. In three cases of insulinoma, MRI failed to reveal the tumour. Endoscopic ultrasound failed to disclose the pancreatic lesion in five cases of insulinoma.

Treatment

An exploratory laparotomy was performed in five cases to localize insulinomas that were not demonstrated by imaging studies and that were highly suspected based on clinical and biological findings. The tumours were identified by intra-operative ultrasound imaging with palpation. Five patients underwent tumour enucleation, three had distal pancreatectomy and splenectomy; central

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>Sex</th>
<th>Symptoms</th>
<th>Treatment</th>
<th>Size (cm)</th>
<th>Diagnosis</th>
<th>Location (pancreas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>F</td>
<td>Abdominal pain, altered general health</td>
<td>Distal pancreatectomy and splenectomy</td>
<td>2.5</td>
<td>NET G2</td>
<td>body</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>M</td>
<td>Hypoglycaemic collapse and dizzy spells</td>
<td>Enucleation</td>
<td>1.5</td>
<td>NET G1 (insulinoma)</td>
<td>head</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>M</td>
<td>Hypoglycaemic collapse and dizzy spells</td>
<td>Enucleation</td>
<td>1</td>
<td>NET G1 (insulinoma)</td>
<td>head</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>F</td>
<td>Incidental finding on ultrasonography</td>
<td>Distal pancreatectomy and splenectomy</td>
<td>10</td>
<td>NET G1</td>
<td>tail</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>F</td>
<td>Abdominal pain</td>
<td>Distal pancreatectomy and splenectomy</td>
<td>2.5</td>
<td>NET G2</td>
<td>body</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>M</td>
<td>Hypoglycaemic collapse and dizzy spells</td>
<td>Enucleation</td>
<td>1.2</td>
<td>NET G1 (insulinoma)</td>
<td>head</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>F</td>
<td>Hypoglycaemic collapse, dizzy spells and abdominal pain</td>
<td>Enucleation</td>
<td>2</td>
<td>NET G1 (insulinoma)</td>
<td>head</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>F</td>
<td>Hypoglycaemic collapse and weight gain</td>
<td>Enucleation</td>
<td>0.5</td>
<td>NET G1 (insulinoma)</td>
<td>tail</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>F</td>
<td>Hypoglycaemic collapse and abdominal pain</td>
<td>Central pancreatectomy</td>
<td>1.2</td>
<td>NET G1 (insulinoma)</td>
<td>body</td>
</tr>
</tbody>
</table>

F: female; G: grade; M: male; NET: neuroendocrine tumour.
Neuroendocrine tumours of the pancreas

Pancreatectomy was performed in only one case. Post-operatively, no patient had received adjuvant therapy.

Pathologic findings
Macroscopy: the sites of pNET were the head of the pancreas (n = 4), the body of the pancreas (n = 3) and the tail of the pancreas (n = 2). Neuroendocrine tumours ranged in size from 0.5 to 10 cm (mean 4 cm). Insulomas measured between 0.5 and 2 cm (mean 1.23 cm), whereas nonfunctioning tumours ranged in size from 2.5 to 10 cm in diameter (mean 5 cm). On cut sections, the tumour was well-delineated (n = 9) and firm in consistency (n = 9) in all cases (Fig. 2). The colour of the tumour was whitish in seven cases, brownish in one case and yellowish in one case.

Microscopy: histologically, pNET showed various “organoid” histological patterns (Fig. 3) characterised by nesting, trabecular, glandular and tubuloacinar arrangement of cells. The cells were relatively uniform showing finely granular eosinophilic cytoplasm and a centrally located round-to-oval nucleus. The chromatin pattern was characteristically coarsely clumped (“salt and pepper”). Mitotic figures were not detected in four cases. In three cases, the mitotic index was 1 mitosis/10 HPF and in two cases was 2 mitoses/10 HPF. Immunohistochemical study using the avidin-biotin complex technique with antibodies against chromogranin A, synaptophysin and MIB-1 was performed in all cases. Tumour cells showed strong and diffuse positive immunostaining with chromogranin A (Fig. 4b) and synaptophysin. The Ki67-

### Tab. II. Imaging studies performed in the nine cases.

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Tumours identified</th>
<th>Tumours not identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>NF pNET</td>
<td>I</td>
</tr>
<tr>
<td>Ultrasonography</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CT scan</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>MRI</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Endoscopic ultrasound</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* I: insulinomas; NF pNET: nonfunctioning pancreatic neuroendocrine tumour.

Fig. 1. CT scan demonstrating an enhancing heterogeneous well-delineated tumour of the pancreas measuring 10 cm in diameter (case 4).

Tab. II. Imaging studies performed in the nine cases.

Fig. 2. Macroscopic findings of an insulinoma (case 9): a well-delineated nodular whitish lesion of the pancreas.

Fig. 3. Microscopic findings of a grade 1 pNET. Tumour cells are relatively uniform and arranged in a trabecular pattern (haematoxylin and eosin, magnification × 200).
MIB1 labelling index was < 2% in seven cases (Fig. 4a). In two cases, it was estimated to be 4% and 6%. Grading was performed on the basis of morphological criteria and assessment of mitotic activity and proliferation fraction according to the ENETS scheme. Seven tumours were classified as grade 1 and two as grade 2. The classification of pNET of our series according to ENETS, WHO 2000 and WHO 2010 is summarised in Table III. Regional lymph node metastases were detected in two cases (Cases 1 and 5).

Operative morbidity and postoperative complications
One patient died on postoperative day 1 due to septic shock. There were no other postoperative complications.

Follow-up
Clinical data regarding follow-up was complete in all cases. The follow-up period ranged between 12 months and 8 years. One patient was lost to follow-up. There were no postoperative sequelae.

Discussion
Neuroendocrine tumours are a relatively rare subset of pancreatic neoplasms, although their incidence has increased during the last two decades [5]. Pancreatic neuroendocrine neoplasms are most frequently seen in adults, but may rarely occur in children. Patients are typically 30-60 years (mean of about 50 years). Men and women are equally affected, but high-grade (G3) carcinomas often occur in older males [5]. In our series, there were 3 male and 6 female patients (M/F 0.5) between 20 and 75 years of age (mean 52 years). Most cases of pNET occur sporadically, although approximately 10% of cases may be associated with multiple endocrine neoplasia type 1 (MEN1), von Hippel-Lindau disease, tuberous sclerosis and neurofibromatosis [6]. All cases of pNET of our series were sporadic. Pancreatic neuroendocrine neoplasms are broadly classified as functioning and non-functioning. Functioning pNET secrete biologically-active peptides such as insulin, gastrin, glucagon, somatostatin and vasoactive intestinal polypeptide (VIP), whereas nonfunctioning tumours express and secrete peptides that do not cause clinical symptoms [6]. Consequently, most non-functioning pNET are usually discovered incidentally or diagnosed at an advanced stage due to mass effect [6-7]. Insulinomas are the most common pNET, comprising 30-40% of these tumours. The symptoms and clinical features of patients with insulinomas are largely related to excessive release of insulin into the bloodstream from the tumour. Up to 85% of patients with insulinoma present with diplopia, blurred vision, palpitations, or weakness [7]. There is often a typical history of recurrent hypoglycaemic collapse and dizzy spells. Other symptoms include abnormal behaviour, confusion and amnesia. Grand mal seizures occur in 12% of patients. Hunger may be a prominent symptom and weight gain occurs in about 30% of cases [7]. In our series, the presenting clinical symptoms of insulinomas were dominated by hypoglycaemic collapse (n = 6), dizzy spells (n = 4), abdominal pain (n = 2) and weight gain (n = 1). For non-functioning tumours, the presenting clinical symptoms were abdominal pain (n = 2) and altered general health (n = 1). One case of non-functioning pNET was fortu-
itously discovered during assessment for Sjögren’s syndrome by routine ultrasonography. The presence of hypoglycaemia in the face of inappropriately elevated levels of insulin is key to diagnosis of an insulinoma. The calculation of an abnormal ratio of insulin to plasma glucose is diagnostic. During a monitored fast, a low blood glucose level and high serum insulin level enables diagnosis. Although conventional imaging which include CT or MRI scans are usually employed in the initial diagnostic workup, they detect less than 50% of most pNET that are less than 1 cm, and therefore small tumours, especially insulinomas, are often not detected. Imaging to localise insulinomas should only take place once a diagnosis has been confirmed biochemically. It must be remembered that the role of imaging is not to diagnose insulinoma, but to identify and localise the tumour. As tumours are usually less than 2 cm in size, they may be invisible on conventional imaging and specific dedicated sequences must be employed to detect small lesions. Two fundamental features of these tumours aid radiological detection. (A) most insulinomas are vascularised and are best visualised in arterial phase imaging. Tailoring CT and MRI sequences to optimise this factor is essential in maximising visualisation. (B) As insulinomas are functioning neuroendocrine tumours, this fact can be used by employing functional imaging with radiolabelled somatostatin analogues such as octreotide. Traditionally, intra-operative ultrasound imaging with manual palpation was the gold standard for localising an insulinoma in part, their larger size can be attributed to later detection. In our series, CT scan was performed in all cases but did not disclose a pancreatic lesion in five cases of insulinomas. MRI was performed in five cases of insulinomas and failed to reveal the tumour in three cases (60%). Several studies have revealed an impressive diagnostic capability of endoscopic ultrasound (EUS) paired with fine needle aspiration with a reported sensitivity between 80% and 90%. Endoscopic ultrasound is particularly helpful in localising insulinomas, which are small and frequently missed by conventional imaging. In our series, EUS failed to disclose the pancreatic lesion in five cases of insulinomas. The therapeutic plan of pNETs is based on histological classification and tumour stage. Surgery remains the cornerstone of treatment of early stage pNETs. Surgical resection of localised pNETs offers excellent prognosis and curative potential. Depending on the site and size, in the absence of distant metastases enucleation may be sufficient. This approach can easily be employed for many pNETs especially insulinomas, small nonfunctioning pNETs (< 2 cm) and small gastrinomas. Whipple pancreatectoduodenectomy, left pancreatectomy or total pancreatectomy can offer 5-year survival rates of 61-79% even in advanced. The role of surgery in patients with MEN1 syndrome is complicated and remains controversial because of the risk of additional neoplasms within the remaining pancreas and other sites. Systemic chemotherapy, including cytotoxic chemotherapy and biologic therapy with somatostatin analogues and interferon, is important in the management of patients with regional advanced and distal metastatic pNETs. Moreover, aggressive treatments such as hepatic artery embolisation and radiofrequency ablation are recommended as an option to reduce the tumour volume or to alleviate non-hormonally mediated symptoms in an effort to prolong patient survival. Recently, targeted therapies have become a valid and alternative consideration. For tumours with alterations of the mTOR pathway, everolimus has shown promise as a therapeutic agent and is currently in phase IV clinical trials. Cabozantinib, a tyrosine kinase inhibitor, and sunitinib, a vascular endothelial growth factor inhibitor, have also shown similar therapeutic. Grossly, most pNET are well-demarcated, solitary and white-yellow or pink-brown. They can be soft and fleshy or densely fibrotic. Areas of haemorrhage or necrosis can occur, usually in larger neoplasms. pNET are only rarely cystic. Among functioning neoplasms, insulinomas are usually smaller (< 2 cm in diameter) than glucagonomas or VIPomas, but the size of tumours is not related to the severity of the hormonally-induced symptoms. Nonfunctioning pNET are generally > 2 cm in diameter; in part, their larger size can be attributed to later detection. In our series, insulinomas measured between 0.5 and 2 cm (mean 1.23 cm), whereas nonfunctioning tumours ranged in size from 2.5 to 10 cm in diameter (mean 5 cm). Histologically, well-differentiated pNET show various organoid histological patterns characterised by a nesting, trabecular, glandular, gyriform, tubulo-acinar, or pseudorosette arrangements of cells. The cells are relatively uniform, show finely granular amorphophilic to eosinophilic cytoplasm and a centrally located round-to-oval nucleus that may display a distinct nucleolus. The chromatin pattern is characteristically coarsely clumped (salt and pepper). Occasionally, clear cells, vacuolated lipid-rich cells, oncocyttes, or rhabdoid features may be observed. By definition, pNET have < 20 mitoses per 10 HPF; most cases have < 10 per 10 HPF and in many cases mitoses are nearly undetectable. The mitotic rate is a critical component of grading. The amount of stroma and degree of fibrosis vary. Necrosis is usually limited and may be comedolike. In general, the histological pattern of a neoplasm does not indicate its functional state or type of hormone produced. There are two exceptions to this rule: amyloid deposits are more typical of insulinomas and glandular structures containing psammoma bodies are commonly observed in somatostatin producing tumours, usually not primary in the pancreas but rather in the peripancreatic duodenum. In our series, amyloid deposits were not detected in insulinomas. Pancreatic high-grade neuroendocrine carcinomas (NEC) commonly consist of tightly packed nests or diffuse irregular sheets of cells, often with extensive necrosis. NEC are classified as small cell or large cell NEC depending upon the size of the neoplastic cells, the prominence of nucleoli and the amount of cytoplasm. In the pancreas, large cell NEC are
more common than small cell NEC. By definition, mitoses are abundant (>20 per 10 HPF). Most cases have > 40-50 per 10 HPF and necrosis is frequent and often geographic. There were no cases of NEC in our series. Immunohistochemically, pNET can be identified by using antibodies against chromogranin A and synaptophysin, which are strongly expressed in the vast majority of cases. Protein gene product (PGP) 9.5 and neural cell adhesion molecule (NCAM1/CD56) are also expressed, but these are considered less specific. Pancreatic neuroendocrine tumours also contain keratins 8 and 18, while keratin 19 may also be expressed. Peptide hormones (insulin, glucagon) are generally detectable in the corresponding functioning pNET. Most pNET have a low proliferative rate, with a labelling index of 1−5%, but values of up to 20% are acceptable, although rarely observed. In our series, tumour cells showed strong and diffuse positive immunostaining with chromogranin A and synaptophysin. The Ki67-MIB1 labelling index was < 2% in seven cases. In two cases, it was estimated to 4−6% respectively. It is necessary to demonstrate immunolabelling for general neuroendocrine markers (chromogranin A and synaptophysin) to establish a diagnosis of large cell NEC of the pancreas. Classic small cell NEC may not express neuroendocrine markers, and following the definition used in the lung, this does not preclude diagnosis so long as alternative diagnostic considerations are excluded. As a rule, no reactivity for peptide hormones is found in pancreatic NEC. The Ki67-MIB1 labelling index is consistently > 20%. pNET are classified by WHO 2010 (Tab. IV) based on differentiation in order to assess their biological behaviour and potential for a malignant phenotype. Across all types of neuroendocrine neoplasms, prognosis is dependent on both histology and extent of disease. pNET are generally associated with less aggressive behaviour, and poorly differentiated pNET are characterized by extremely aggressive tumour biology and poor prognosis. Primary pancreatic neoplasms that must be distinguished from pNET include solid pseudopapillary neoplasm, acinar cell carcinoma and pancreatoblastoma, primitive neuroectodermal tumour and ductal adenocarcinoma. Due to the rarity of pNET, data regarding survival and prognostic factors exist mainly from small series of patients. Pancreatic neuroendocrine tumours carry a better prognosis than exocrine pancreatic tumours, but are often metastatic at diagnosis; the liver is the most common site of metastasis, although regional lymph node spread is also common. In our series, lymph node metastases were detected at diagnosis in two cases (case 1 and 5). Pancreatic neuroendocrine tumours have a 5-year survival that can range from 97% in benign insulinomas to as low as 30% in nonfunctioning metastatic pNET. In conclusion, pancreatic neuroendocrine tumours are a heterogeneous group of rare tumours with a wide range of biological activity, clinicopathological features and variable prognosis. Accurate clinical and histological diagnosis is an important first step in developing an appropriate management plan. Recently, considerable headway has been made in the realm of therapeutics. Therefore, it is imperative that oncologists have a heightened awareness of this disease entity in order to provide effective care for patients.

References


Tab. IV. WHO 2010 classification of neuroendocrine tumours.

<table>
<thead>
<tr>
<th>Differentiation</th>
<th>Grade</th>
<th>Mitotic rate</th>
<th>Ki-67</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-differentiated</td>
<td>Low-grade (G1)</td>
<td>&lt;2/10 HPF</td>
<td>&lt;3%</td>
<td>NET G1</td>
</tr>
<tr>
<td></td>
<td>Intermediate grade (G2)</td>
<td>2-20/10 HPF</td>
<td>3-20%</td>
<td>NET G2</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>High grade (G3)</td>
<td>&gt;20/10 HPF</td>
<td>&gt;20%</td>
<td>NEC G3 Small cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NEC G3 Large cell</td>
</tr>
</tbody>
</table>


Introduction. Micro-RNA, a new class of small, non-coding RNAs, have been shown to be deregulated in several human carcinomas. In particular, SNP rs2910164 in pre-miR146a appears to be correlated with papillary thyroid carcinoma and may be involved in its genetic predisposition. Since data on follicular thyroid carcinomas (FTC) are lacking, we evaluated the involvement of SNP rs2910164 in FTC.

Methods. Thirty-nine cases of FTC and 20 follicular adenomas, defined according to WHO criteria, were selected. DNA and RNA were extracted from formalin-fixed paraffin-embedded blocks of both neoplastic and non-neoplastic areas. The DNA region of pre-miR146a, containing SNP rs2910164, was sequenced. Total RNA including miRNAs was used for stem-loop RT reactions, and applying a standard TaqMan PCR kit protocol for real-time PCR. Wilcoxon signed-rank test and Friedman test were used for statistical analyses.

Results. In 31% of FTC, the G allele was observed in neoplastic tissues, compared with the non-neoplastic areas (p < 0.05), whereas the CC phenotype was completely absent in tumours. Moreover, the expression of pre-miR146a was found to be significantly down-regulated in neoplastic tissues from FTC cases (p = 0.043), although no significant differences were seen in follicular thyroid adenomas.

Discussion. The expression profile of pre-miR146a can be correlated with FTC tumourigenesis. The G allele in SNP rs2910164 appears to be correlated with the transition from normal to neoplastic tissue. The GG and GC alleles appear to be associated with an increased risk for FTC, while the CC allele seems to play a protective role.
morphisms (SNP) of miRNAs (isomiRNAs) have also been associated with tumourigenesis, and in particular for papillary carcinoma. More in detail, SNP rs2910164 in pre-miR146a appears to be correlated with papillary thyroid carcinoma and may be involved in genetic predisposition to this tumour.

**Methods**

For the first time, our research group has evaluated the SNP rs2910164 genotypic frequency of pre-miR146a, as well as the molecular expression of pre-miR146a in tumour tissue from follicular carcinomas and follicular adenomas, comparing them with matched non-neoplastic thyroid tissue. Thirty-nine cases of follicular carcinomas (Fig. 1) and 20 follicular thyroid adenomas, defined according to WHO criteria and from patients of both sexes, aged between 18 and 85 years, were included in our study. DNA and RNA were extracted from formalin-fixed paraffin-embedded samples of both neoplastic and non-neoplastic areas. The DNA region of pre-miR146a, containing the SNP rs2910164, was sequenced using the Sanger method. Total RNA including miRNAs was used for stem-loop RT reactions, and applying a standard TaqMan PCR kit protocol for real-time PCR. Wilcoxon signed-rank and Friedman tests were used for statistical analyses.

**Results**

In 34% of cases, SNP rs2910164 undergoes changes during the transition from normal tissue to tumoural tissue. Moreover, in 31% of FTC the presence of the G allele was observed in neoplastic tissues (Fig. 2), compared to the non-neoplastic areas (p < 0.05): the CC phenotype was not present in tumours. Pre-miR146a expression was found to be significantly down-regulated in neoplastic tissues from FTC cases (p = 0.043), although no significant differences were observed in follicular thyroid adenomas (Fig. 3). However, in eight cases, pre-miR146a over-expression in neoplastic tissue compared to non-neoplastic areas was observed. Interestingly, among these 8 cases, there were five cases of metastatic follicular carcinoma, and in particular with lymph node involvement.

**Discussion**

The miRNA-146a was found to over-expressed in papillary and anaplastic thyroid carcinoma: in vitro, over-expression of miRNA-146a stimulates cell proliferation, while a decrease in its expression induces cell apoptosis. Moreover, the proteins IRAK1 and TRAF6 are important cytoplasmatic targets of miRNA-146a. It has also been suggested that the over-expression of some miRNAs could be at the basis of THR-β gene silencing. Interestingly, the 3’UTR of THR-β contains 14 binding sites for the seven miRNAs that were expressed at higher levels in papillary thyroid carcinoma,
four of which (miRNA-21, miRNA-146a, miRNA-181a, miRNA-221) are experimentally able to inhibit the translation of the THRB. From our results, it appears that the expression profile of pre-miR146a can be correlated with FTC tumourigenesis. Although it is possible that there is not always a direct correlation between the pre-miR146a and its mature form, it is interesting to consider that pre-miR146a overexpression in patients affected by FTC may predict metastatic evolution of the neoplasia. The G allele in SNP rs2910164 is present in the transition from healthy tissue to neoplastic tissue; in contrast, the frequency of the C allele decreases during the transition (Fig. 4). The GG and GC doublets appear to be associated with an increased risk for FTC, while homozygous CC seems to play a protective role.

References

Olfactory neuroblastoma with focal ganglioneuroblastic differentiation: a case report with literature review

S. SQUILLACI
Division of Anatomic Pathology, Hospital of Vallecamonica, Esine (BS), Italy

Key Words
Olfactory neuroblastoma • Ganglioneuroblastic differentiation • Immunohistochemistry

Summary
Olfactory neuroblastoma (ONB) is a rare malignant neuroectodermal tumour, with clearly defined histologic and immunohistochemical features, that typically arises in the superior nasal cavity. Although the classical clinicopathological features leave little room for misinterpretation, the wide variability in this tumour, including occasional divergent differentiation, may cause diagnostic difficulty. Herein, an unusual case of ONB with focal ganglioneuroblastic differentiation in an 81-year-old woman arising from the anterior ethmoid, filling the upper portion of the left nasal cavity and sparing the sinus cavities, is described. Histologically, the tumour was composed of atypical monotonous round cells that were positive for NSE, CD56, chromogranin, synaptophysin, neuropilament and calcitelin and exhibited an irregular lobulated and nested growth pattern and sparse mitotic figures (3 to 4 mitoses per 10 HPF). Focally, the histology changed to ganglioneuroblastic differentiation consisting of large ganglion and spindle cells, positively staining for S-100, GFAP, CD99, neuropilament, calcitelin, chromogranin and synaptophysin. Neuroblastomas, occurring in the nasal cavity, in analogy to other sites, tend to have an aggressive biologic behaviour and can histologically mimic other undifferentiated malignant neoplasms of the sinonasal tract. Differential diagnostic problems are discussed; a comprehensive review of the literature has also been performed with a focus on survival.

Introduction
Olfactory neuroblastomas (ONBs) are rare, site-specific malignant neuroectodermal tumours accounting for 3-5% of all intranasal neoplasms, with an incidence of 0.4 cases per million inhabitants. They occur at various ages, but have bimodal peaks in the second/third and sixth/seventh decades of life, and their rarity and highly heterogeneous nature have caused considerable diagnostic difficulties and have led to divergent opinions concerning their origin, growth and management. Complex tumours presenting a variety of additional histologic patterns, including craniopharyngioma and carcinoma, have also been published, albeit rarely. Examples of ONBs presenting as polypoid masses that histologically combined several tissue types, including epithelial, melanocytic, rhabdomyoblastic and ganglioneuromatous differentiation have also been reported. These uncommon pathologic features are probably underreported, may be encountered in the pretreatment or posttreatment samples and can change after chemoradiotherapy.

The purpose of the present study is to describe a case of olfactory neuroblastoma (ONB) associated with focal ganglioneuroblastic differentiation at first appearance; problems in the differential diagnosis raised by this tumour and a review of the pertinent literature are also briefly discussed.

Case report
A 81-year-old woman presented to the outpatient clinic with a 4-month history of bloody nasal discharge. The patient had episodes of nasal bleeding and felt as if her nose was always stuffed up. At admission, anterior rhinoscopy showed the presence of a greyish-pink, ill-defined polypoid mass covered with normal-appearing mucosa. Computed tomography (CT) and magnetic resonance imaging (MRI) of the head and neck were performed, revealing an extensive mass filling the upper portion of the left nasal cavity arising from anterior ethmoid and involving the middle meatus up to the introitus.
of the maxillary sinus. The maxillary, sphenoidal and frontal sinuses as well as the ethmoidal cells were regularly pneumatized. The patient underwent endoscopic excision of the lesion, and the specimen was sent for examination of intraoperative frozen sections. Resection of the nasal mass yielded numerous fragments of red-grey soft tissue. The sinonasal epithelial lining showed focal squamous metaplasia. Infiltrating beneath the intact or focally eroded respiratory epithelium and destroying some of the nasal structures was a cellular tumour composed of irregular circumscribed lobules or nests of cells separated by a vascularised fibrous stroma. The neoplastic cells were round, small to medium in size, with indistinct cell borders, high nuclear cytoplasmic ratio, inconspicuous nucleoli, small and uniform, but hyperchromatic nuclei with a “salt-and-pepper” nuclear chromatin distribution. The cells also demonstrated neurofibrillary matrix forming an evident intercellular background. They were focally arranged into annular structures with faintly eosinophilic material in the centre (Homer-Wright pseudorosettes); a ‘gland-like’ tight circumferential array of neoplastic cells around spaces lined by distinct cell membranes as seen in Flexner-Wintersteiner rosettes was never seen (Fig. 1A, B, C, D). Some pleomorphic elements were observed. No areas of necrosis were seen and the mitotic count was 3-4 per 10 high power fields. Vascular invasion and extracellular mucin accumulation were not identified. No cytoplasmic glycogen was observed in tumour cells. Numerous stromal calcifications were frequently observed. An additional interesting finding was detected, namely the presence, inside the neoplastic growth, of some areas showing large scattered immature ganglion-like cells with large round nuclei, prominent nucleoli, eosinophilic cytoplasm intermixed with spindle cells resembling Schwann cells within a sclerotic stroma (Fig. 2A, B). Immunohistochemically, lobules of tumour cells were diffusely positive for NSE, CD56, chromogranin, synaptophysin, neurofilament and calretinin (Fig. 3A, B, C, D) (Fig. 4B, C). They were focally surrounded by S-100, and GFAP positive sustentacular cells and ganglion elements, positively staining for S-100, GFAP, CD99, neurofilament, calretinin, chromogranin and synaptophysin, were visible (Fig. 3C)(Fig. 4B) (Fig. 5A, B)(Fig. 6A, B). Immunostaining for cytokeratins (AE1/AE3 and Cam 5.2), MART-1, WT-1, desmin, actin, myogenin and P63 were negative in tumour cells (Fig. 4A)(Fig. 5C). The histological and immunohistochemical features were consistent with a diagnosis of ONB. The patient was postoperatively treated with chemoradiotherapy. Nine months after tumour resection, her follow-up was negative for tumour recurrence or metastasis.

Discussion

In this report, an ONB is described occurring in the upper respiratory tract presenting as an endonasal polypoid mass. This uncommon
malignant entity is currently believed to originate from the specialised olfactory epithelium generally found in the upper third of the nasal septum, cribriform plate and superior-medial surface of the superior turbinate. The histological and clinical variability observed in these tumours could be a manifestation of the multipotential characteristics of their mucosal progenitor cells lying on the basal layer of the olfactory epithelium. Additionally, pathologists are prone to consider ONB and neuroendocrine carcinoma (NE) as belonging to a histogenetic spectrum on the base of their common origin. The current case had some classical histopathologic features of ONB at histologic grade II according to Hyams' grading system, thus resembling previously reported tumours of this type, but also exhibited focal ganglioneuroblastic differentiation. While neuroblastomas at other sites are known to have the ability to differentiate into ganglion cells, this morphologic divergence in ONB is an exceptionally rare change, firstly described by Telleschi in 1971. A literature search revealed only 16 reported cases of ONB with ganglioneuroblastic transformation up to 2014 (Tab. 1). From the published data, including the present case, the age at diagnosis ranged from 18 to 87 years (mean 56.7 years) with a median of 56 years and a clear predominance (69.2%) over the age of 50 years. There were 5 women and 8 men, and 4 patients (23.5%) whose age and gender were not specified. The female to male ratio was 1:1.6. Moreover, some also presented other unusual features as focal epithelial divergent differentiation both with typical morphologic appearance (in the form of glands, squamous malignant cells) and purely immunohistochemical and/or ultrastructural, as observed in 6 cases. In 14 patients (82.3%), the ganglioneuronal component showed focal changes in ONB, including the present case and another with more abundant ganglion cells in the postradiation surgical specimen. Three other cases with a complete ganglioneuroblastic transformation of recurrent ONBs after radiation therapy have been reported, sharing similar morphologic features in the relapses. Despite the shared morphology of these 3 recurrent neoplasms, patients had different outcomes: two with residual disease after incomplete salvage surgery were asymptomatic 18 and 36...
months later; the other was submitted to incomplete resection after chemotherapy, but developed a mediastinal metastasis and died for aspiration pneumonia 3 months after surgery. Follow-up information on other 6 patients, including the current case, was available. Death was attributed to tumour invasion in only two patients, both 24 months after surgical resection because of recurrences and multiple metastases. The others were free of disease and followed-up for periods of time varying between 9 and 287 months (mean 139.75 months). Areas of ganglioneuroblastic differentiation exclusively arising in a metastatic lymph node of the neck were reported in one patient.

Therefore, by analogy to other sites, olfactory neuroblastoma (ONB), including pure or combined forms, tend to have aggressive biologic behaviour mainly involving adjacent structures (orbit and cranial cavity). Local recurrence and distant metastasis can occur years after initial diagnosis. From 30-70% of patients will experience local recurrence, 20-40% will develop cervical lymph node metastasis, and approximately 10% of patients will have distant metastasis. The most frequent sites of metastatic disease are the lungs and bones.

Because of the architecture and cellular features of ONB, differential diagnosis, particularly in high grade neoplasms, is broad and includes other undifferentiated malignant neoplasms of the sinonasal tract such as melanoma (MM), non-Hodgkin lymphoma, sinonasal undifferentiated carcinoma (SNUC), NUT midline carcinoma, Ewing sarcoma/primitive neuroectodermal tumour (EWS/PNET), rhabdomyosarcoma (RMS), high-grade neuroendocrine carcinoma (NC) and undifferentiated nasopharyngeal carcinoma (NPC).

The constellation of histologic and immunohistochemical features, such as the lobular growth pattern, lack of cytokeratin expression, moderate to strong pattern of staining for calretinin, and the presence of S-100 positive sustentacular cells can assist in confirming a diagnosis of ONB and differentiating it from NC, NPC and SNUC. Moreover, ganglioneuronal differentiation, rare in ONBs, has never been reported in NC or SNUC. SNUC is a rare and highly aggressive sinonasal neoplasm of uncertain histogenesis without evidence of squamous or glandular differentiation, with or without neuroendocrine marker expression that needs to be differentiated from ONB, as the latter tumour has a better 5-year median survival rate. The situation may be further complicated by the occurrence of rare morphologic variants of SNUC with differentiated foci and NUT rearrangements. The lack of a carcinomatous component and of immunohistochemical, molecular and/or cytogenetic features, such as a t(15;19) balanced translocation and BRD4-NUT fusion oncogene, argue against the diagnosis of NUT midline carcinoma. Among rare tumours in the sinonasal tract, the most important one to be considered in young adults is EWS/PNET because of a similar morphologic appearance, but which differs from ONB in lacking a fibrillary matrix, and CD99 expression, whereas CK and S-100 cannot be detected. If necessary, FISH or PCR for the typical t(11;22) translocation of EWS/PNET can be helpful in dis-
Olfactory neuroblastoma with focal ganglioneuroblastic differentiation

...tivating these entities.\textsuperscript{7,15} By the same token, sinonasal MMs may occasionally show a small cell morphology, simulating ONB, but in addition to their specific cytologic features including vesicular nuclei and prominent nucleoli, they usually show immunohistochemical features including diffuse positivity for MART-1 and S-100, which are not present in ONB.\textsuperscript{1,2,13} Furthermore, pleomorphism, anaplasia, rhabdomyoblastic differentiation and immunostaining for desmin and myogenin are distinct features of adult RMS of alveolar type, but not observed in ONB. Finally, currently, the possibility of non-Hodgkin lymphoma must be explored for those small cell neoplastic proliferations displaying a prevalently diffuse pattern of growth, not generally seen in ONB, and completely negative for keratins, S-100, muscle and neuroendocrine markers and CD99.\textsuperscript{13}

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Author/Year</th>
<th>Age/sex</th>
<th>Size (cm)</th>
<th>Site</th>
<th>Stage</th>
<th>Hyams' grade</th>
<th>Divergent component</th>
<th>Post-treatment appearance and other features</th>
<th>Recurrence/metastasis</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tellecschi (25 in Ref.6) 1971</td>
<td>72/M</td>
<td>NA</td>
<td>Right nasal cavity</td>
<td>NA</td>
<td>NA</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>Silva et al. 11 1982</td>
<td>NK</td>
<td>NK</td>
<td>Sinonasal cavities</td>
<td>B</td>
<td>III-IV</td>
<td>Ganglioneuronal and epithelial</td>
<td>Areas of ganglioneuroblastoma in a metastatic LN of neck</td>
<td>+/- (neck LN)</td>
<td>DOD 24 months later</td>
</tr>
<tr>
<td>3</td>
<td>Miller et al. 4 1984</td>
<td>77/M</td>
<td>3x1</td>
<td>Right nasal cavity, eroding the upper septum with extension to both maxillary and both ethmoid sinuses, and protruding into the nasopharynx</td>
<td>C</td>
<td>IV</td>
<td>Ganglioneuronal and adenocarcinomatous</td>
<td>No</td>
<td>+/-</td>
<td>ANED 17 months later</td>
</tr>
<tr>
<td>4</td>
<td>Mills et al. 8 1985</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>5</td>
<td>Mills et al. 8 1985</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>6</td>
<td>Mills et al. 8 1985</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>Ganglioneuronal</td>
<td>Postradiation specimen contained more abundant ganglion cells</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>7</td>
<td>Chatel et al. 8 1988</td>
<td>51/M</td>
<td>2x1.5</td>
<td>Nasal septum</td>
<td>A</td>
<td>NK</td>
<td>Ganglioneuronal</td>
<td>Yes</td>
<td>+/-</td>
<td>AWED 36 months later</td>
</tr>
<tr>
<td>8</td>
<td>Hirose et al. 12 1995</td>
<td>18/F</td>
<td>NK</td>
<td>Right middle turbinate and ethmoid</td>
<td>NK</td>
<td>I-II</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>+/-</td>
<td>ANED 287 months later</td>
</tr>
<tr>
<td>9</td>
<td>Hirose et al. 12 1995</td>
<td>41/M</td>
<td>NK</td>
<td>Left middle turbinate, ethmoid and cribriform plate</td>
<td>NK</td>
<td>I-II</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>+/-</td>
<td>ANED 246 months later</td>
</tr>
<tr>
<td>10</td>
<td>Hirose et al. 12 1995</td>
<td>55/M</td>
<td>NK</td>
<td>Right nasal cavity; ethmoid and antrum</td>
<td>NK</td>
<td>NK</td>
<td>Ganglioneuronal and epithelial differentiation</td>
<td>No</td>
<td>+/- (neck, face)</td>
<td>DOD 24 months later</td>
</tr>
<tr>
<td>11</td>
<td>Argani et al. 7 1998</td>
<td>58/M</td>
<td>NK</td>
<td>Ethmoid</td>
<td>NK</td>
<td>II</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>12</td>
<td>Iura et al. 10 2001</td>
<td>56/M</td>
<td>NK</td>
<td>Right ethmoidal sinus with extension to the right sphenoidal sinus and both maxillary sinuses reaching the anterior cranial fossa</td>
<td>C</td>
<td>III</td>
<td>Ganglioneuronal and epithelial differentiation</td>
<td>Yes</td>
<td>+/- (mediastinum)</td>
<td>DOD 3 months later</td>
</tr>
<tr>
<td>13</td>
<td>Seethala et al. 5 2007</td>
<td>46/F</td>
<td>NK</td>
<td>NK</td>
<td>B</td>
<td>I-II</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>14</td>
<td>Seethala et al. 5 2007</td>
<td>87/F</td>
<td>NK</td>
<td>NK</td>
<td>A</td>
<td>I-II</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>15</td>
<td>Seethala et al. 5 2007</td>
<td>36/M</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>III-IV</td>
<td>Rare ganglion cells, adenosquamous</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>16</td>
<td>Babeset et al. 8 2012</td>
<td>60/F</td>
<td>NK</td>
<td>Posterior roof of the left nasal cavity with extension through the cribriform plate into the inter-hemispheric fissure</td>
<td>C</td>
<td>IV</td>
<td>Epithelial in primary tumor and ganglioneuronal in recurrence</td>
<td>Yes</td>
<td>+/-</td>
<td>AWED 18 months later</td>
</tr>
<tr>
<td>17</td>
<td>Present case 2014</td>
<td>81/F</td>
<td>2.6x1.2</td>
<td>Left nasal cavity, ethmoid</td>
<td>A</td>
<td>II</td>
<td>Ganglioneuronal</td>
<td>No</td>
<td>+/-</td>
<td>ANED 9 months later</td>
</tr>
</tbody>
</table>

Ref., reference; F, female; M, male; NK, not known; NA, not available; LN, lymph node; AWED, alive with evidence of disease; ANED, alive with no evidence of disease; DOD, died of disease.
An accurate literature search retrieved only a small number of cases that could be confidently ascribed to ganglioneuroblastic differentiation in ONB. Most of the cases have been documented in small clinicopathological series with often insufficient follow-up data. Therefore, information on the prognosis of ganglioneuronal differentiation in ONB remains limited. Nevertheless, the findings of the current review lend further support to the prognostic distinction between ONBs with ganglioneuronal and epithelial divergence proposed by a recent clinicopathologic study. ONBs with focal or prominent ganglioneuronal differentiation would seem to be associated with a better prognosis than mixed ganglioneuronal-epithelial differentiated tumours. Larger studies addressing the clinical behaviour of ONBs with divergent phenotypes are needed to confirm this trend.

Acknowledgements
This work was presented in abstract form (Poster presentation) at the Annual Italian SIAPEC-IAP Congress of Anatomic Pathology, Rome, Italy, 26-30 October, 2013.

References
**Case report**

**Papillary haemangioma:** a case report of multiple facial location

S. RAMMEH\(^1\), B. FAZAA\(^2\), W. AJOULI\(^1\), I. LABBENE\(^2\), M. KHARFI\(^2\), R. ZERMANI\(^1\)

\(^1\) Department of Pathology; \(^2\) Department of Dermatology, Charles Nicolle Hospital, Tunis, Tunisia

**Key words**

Cutaneous • Hemangiomas • Papillary • Benign

**Summary**

Papillary haemangiomas were recently defined as morphologically distinct and benign cutaneous haemangiomas showing a predominantly intravascular capillary proliferation within dilated thin-walled dermal blood vessels. We describe the case of a 45-year-old woman who presented with multiple eruptive red-blush raised papules and nodules distributed over the skin of the chin that were related to a papillary haemangioma.

**Introduction**

Papillary haemangiomas (PHs) were recently defined as morphologically distinct and benign cutaneous haemangiomas showing a predominantly intravascular capillary proliferation within dilated thin-walled dermal blood vessels. Herein, we report a case of a young adult with multiple PHs.

**Case report**

A 45-year-old otherwise healthy woman presented with multiple eruptive red-blush raised papules and nodules (0.3-1.0 cm) distributed over the skin of the chin (Fig. 1). The lesions had been stable in size for 5 years and there was no other skin lesion with similar appearance. Cutaneous biopsy showed histological findings consistent with a PH. The epidermis was normal. Through the dermis, many dilated vascular spaces contained a capillary vascular proliferation with branching papillary architecture that appeared to invaginate and protrude into the lumen of ecstatic thin-walled dermal blood vessels (Fig. 2a, Fig. 2b). The endothelial cells covering the papillary structures showed a bland cytomorphology and contained numerous eosinophilic hyaline globules of variable size (Fig. 2c). Endothelial cells showed a variable immunopositivity for factor VIII, CD31 and CD34 (Fig. 3). Smooth muscle actin was expressed in beneath the endothelium lining the ecstatic vessels and the outer surface of the papillae. Endothelial cells containing hyaline globules showed cytoplasmic immunoreactivity with \(\alpha\)-1-antitrypsin.

Our patient was followed-up by the Department of Dermatology; multiple laboratory and radiological studies were performed to rule out POEMS syndrome. All examinations were normal and failed to detect the presence of polyneuropathy, organomegaly, endocrinopathy and monoclonal protein. After 12 months of follow-up, the patient remained well with no features of POEMS syndrome. This case, as the 13 other reported previously, has localizations on the face, but to our knowledge, this is the first case reported of PH that was multiple and eruptive.

**Discussion**

The microscopic appearance of PHs shares a number of features with the rare entity originally described in 1990 by Chan et al. \(^2\) as glomeruloid haemangioma (GH). GHs are glomeruli-like capillary tufts lined by endothelial cells that contain periodic acid-Schiff (PAS) positive eosinophilic globules (EGs). PHs appear as well-
circumscribed capillary vascular proliferations with typical branching papillary structures that invaginate and protrude into the lumen of ecstatic thin-walled dermal blood vessels. The cytoplasm of the endothelial cells covering papillae contains numerous intracytoplasmic hyaline globules staining positively with PAS. The nature of the EGs in GHs and in PHs remains to be elucidated. It has been suggested that the EGs seen in GHs possibly represent the deposition of immunoglobulins and some other proteinaceous material from the circulation that might act as angiogenic factors for the dermal capillaries. Other more recent immunohistochemical and ultrastructural studies have suggested that EGs in both GHs and PHs are giant lysosomes. Although GHs and PHs share overlapping morphological details, it seems important for practical reason to distinguish PH from GH, the latest is a hallmark of POEMS syndrome (acronym for polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) and/or multicentric Castleman’s disease, whereas the former clinically presents as solitary cutaneous haemangiomas of the head and neck region in otherwise healthy individuals. Experience with both vascular lesions is very limited. To our knowledge only 30 cases of GH have been reported. After the original 11 PH from 10 patients reported in 2007 by Suurmeijer and Fletcher, only one other case has been described by Fumio Ide et al. The case described by Velez et al. as a solitary GH without POEMS syndrome was reviewed Suurmeijer who replaced the initial histological diagnosis of glomeruloid hemangioma by a diagnosis of papillary hemangioma. Our case represents the 14th case of PH.
References


3 Suurmeijer AJH, Fletcher CDM. Papillary haemangioma. A distinctive cutaneous haemangioma of the head and neck area containing eosinophilic hyaline globules. Histopathology 2007;51:638.


Juvenile hyaline fibromatosis: a case report

S. MESTIRI1, N. LABAIED1, N. MAMA2, A. AYADI1, M. LADIB1, B. SRIHA1, H. KRIFA4, M. MOKNI1

1 Department of Pathology, F. Hached Hospital, Sousse, Tunisia; 2 Department of Radiology, Sahloul Hospital, Sousse, Tunisia; 3 Department of Paediatrics, Tahar Sfar Hospital, Mahdia, Tunisia; 4 Department of Neurosurgery, Sahloul Hospital, Sousse, Tunisia

Key-words
Juvenile • Hyaline • Fibromatosis • Skull • Child

Summary

Correspondence
Mestiri Sarra, Department of Pathology, Farhat Hached hospital, Sousse, Tunisia - E-mail: mestirisarra75@yahoo.fr

Juvenile hyaline fibromatosis is a rare, hereditary disease with distinct clinical and histopathological features. Clinically, it presents with gingival hypertrophy, papulonodular skin lesions and joint contractures. Bone involvement is usually an uncommon finding. We report a case of a 2-year-old patient, daughter of consanguineous parents, who presented since the age of 2 months with impairment of mental development, multiple joint contractures, motion limitation and nodules on the scalp. The calvarian lesions were surgically removed, and histopathological examination concluded to juvenile hyaline fibromatosis.

Introduction

Juvenile hyaline fibromatosis (JHF) is a rare autosomal recessive genodermatosis characterized by a triad of cephalic fibrous outgrowths, gingival hypertrophy and flexion contractures. The authors present a case report of juvenile hyaline fibromatosis in a 2-year-old patient.

Observations

S.M., a 2-year-old girl, was the first-born child from a consanguineous marriage with a normal weight at birth (2.8 kg). At 2 months, she was admitted at the orthopædic department for multiple joint contractures and motion limitation. At 15 months, the parents noticed multiple painless lesions in both occipital and parietal regions. At first evaluation, the girl had evident delayed psychomotor development, and impaired flexion of both the knee and elbow. Cranio-facial examination found: hypertelorism, gingival hypertrophy and three soft, mobile, painless cranial masses: The largest one was occipital, measuring 5x5 cm; The other two, temporal and parietal, of smaller size, measured respectively, 5 and 4 cm in diameter. The rest of physical examination and routine laboratory investigation were within normal limits. Doppler echography and caryotype were both normal. At MRI at 1.5 T, the three lesions showed a hypointense signal on T1-weighed images and a hyperintense signal on T2-weighed images (Figs. 1 and 2). Heterogeneous and massive enhancement was noticed after gadolinium administration. There were no bony lytic features. All lesions were surgically removed. At histopathological examination, the lesions were composed of abundant deposits in the dermis of an amorphous and eosinophilic hyaline material with a relative paucity of cellular elements (Fig. 3). Numerous spindle-shaped, fibroblast-like cells were seen in the vicinity of the deposits (Fig. 4). The deposits appeared pink on periodic acid-schiff (PAS) staining (Fig. 5). Congo red-negative and Alcian blue staining were negative. Histopathological diagnosis of JHF was retained.

Discussion

JHF is a rare, autosomal recessive, hereditary disease with distinct clinical and histopathological features occurring in early life. A survey of the medical literature revealed fewer than 70 cases reported worldwide. JHF is a disease that belongs to the group of hyalinoses 1. The latter are rare autosomal recessive disorders in which there is accumulation of amorphous hyaline material in the skin and other organs. There is no sex predilection. Clinically, JHF usually associates with papulonodular skin lesions, gingival hyperplasia, joint contractures and osteolytic bone lesions 2.
Juvenile hyaline fibromatosis: a case report

Infants with JHF are normal at birth, but abnormalities develop progressively during the first two years of life. Gingival hypertrophy is the most common symptom that often develops in the first year of life, and can cause severe feeding and mastication problems.

Papules are distributed around the nose, behind the ears, in the genital area and on the thighs. Joint contractures often develop. Nodules and tumours develop between 2 and 5 years of age, commonly on the scalp and the neck. Infection often results from ulceration of lesions. Joint contractures and retractions are due to pseudo-tumoural infiltration of the joints. Bone lesions consist generally in osteolysis of the distal phalanges and cortical defects of the long bones.

According to De Rosa and al., there are two clinical forms. The first form is localised with limited cutaneous involvement, and small very slow-growing tumours. The second form is diffuse with extensive cutaneous involvement, and large rapidly growing tumours.

Our patient had gingival hyperplasia without alimentation difficulties, large scalp lesions and severe mental retardation. There are few cases in the literature with a diffuse form of JHF.

At imaging, MRI shows white matter lesions. Low or intermediate signal is found with all pulse sequences, with a band of low signal intensity that represents highly collagenised tissue. In lesions with a higher degree of cellularity and less collagen, high signal intensity is frequent. Histopathological examination shows the deposition of an amorphous, eosinophilic hyaline material in the extracellular spaces of the dermis around blood vessels. The deposits are PAS-positive and Alcian blue-negative.

Several genetic studies suggest that the disease is due to mutations of the capillary morphogenesis gene 2 (CMG2), located on the long arm of chromosome 4 (4q21). This genetic abnormality induces either perturbation of the glycosaminoglycan metabolism by fibroblasts or dysregulation of collagen metabolism, causing abnormal deposits of a hyaline ground substance. In our case, no mutation was found in CMG2.

Other fibrous proliferations of infancy and childhood should be considered in the differential diagnosis of JHF including the following. 1) Infantile systemic hyalinosis is a disorder clinically similar to juvenile hyaline fibromatosis, but with far more severe joint involvement, joint contractures and thickened skin. Infants are affected within the first few weeks or months of life. Death occurs secondary to sepsis with renal, respiratory and heart failure, usually by the age of two years. 2) Congenital generalised fibromatosis (dermic nodules since birth with involvement of internal organs). 3) Winchester syndrome (low stature and opacity of the cornea). 4) Farber lipogranulomatosis (laryngeal abnormalities, delayed mental development and opacity of the cornea). The prognosis is affected when gingival hyperplasia causes malnutrition with recurrent infections. Functional prognosis is also poor because of joint and bone lesions that leave patients with deformities and joint contractures. There is no specific treatment for JHF. Early surgical excision in JHF is recommended by some authors to prevent the appearance of new lesions, although excision may be followed by recurrences. Intraleosional steroids may reduce the size of early lesions. Excision is indicated only for lesions that either present a significant cosmetic problem or produce some degree of functional impairment. Head lesions can be removed without a risk of recurrence. Capsulotomy of joints may show some temporary benefits; radiotherapy is ineffective. Gingival hyperplasia may be treated with partial gingivectomy.
Conclusion

JHF is a very rare genetic disease. Physicians should consider a diagnosis of JHF when a young patient presents with multiple maculopapular skin lesions, gingival hypertrophy and joint contracture. Early diagnosis and surgical intervention, including the proper supportive care, may be the best strategy for managing this rare disease.

References


**Uterine tumour resembling ovarian sex cord tumours presenting as multiple endometrial and cervical uterine polyps: a case report**

N. ABID, H. MNIF, M. MELLOULLI, S. CHARFI, A. KHABIR, S. MAKNI, T. BOUDAWARA  
Department of pathology, Habib Bourguiba University Hospital of Sfax, Tunisia

**Key words**  
Uterine • Tumour • Sex cord • UTROSCT • Gynaecologic

**Summary**

**Background.** Uterine tumours resembling ovarian sex-cord tumours (UTROSCT) are very rare, benign uterine tumours, composed solely of sex cord elements. These tumours have a polyphenotypic immunophenotype that favours a derivation from uterine mesenchymal stem cells.

**Case report.** A 43-year-old female presented with recurrent vaginal bleeding. On hysteroscopy, she had multiple endometrial and cervical polyps that were removed endoscopically. Histologically, the specimen contained epithelioid cells arranged in tubules, trabeculae and anastomosing cords, without significant cellular atypia or mitotic activity.

Immunohistochemical studies were performed. The tumour was found to be diffusely positive for vimentin, calretinin and desmin, focally positive for cytokeratin, CD99 and inhibin and negative for chromogranin and CD10. A subsequent total hysterectomy was performed and revealed neoplastic infiltration of the myometrium.

**Conclusion.** A polyphenotypic immunophenotype is a characteristic feature of UTROSCT, and may be helpful in diagnosis and in exclusion of other lesions. Familiarity with this tumour by gynaecologists and pathologists is essential to avoid misdiagnosis; correct diagnosis of this neoplasm is important in patient management.

---

**Correspondence**

Najla Abid, Department of Pathology, Habib Bourguiba Hospital, Sfax, 3029 Tunisia - Tel. +216 74 240 341 - Fax +216 74 243 427 - E-mail: najlamtibaa@gmail.com
indistinct nucleoli. The mitotic count was low with < 1 mitotic figure per 10 high power fields (HPFs). The stoma contained a minor component of smooth muscle cells that simulated entrapped myometrium (Fig. 1). Immunohistochemical stains showed diffuse positivity for vimentin, calretinin, desmin and focal positivity for cytokeratin, alpha-inhibin and CD99. Chromogranin A and CD10 were negative (Fig. 2). A diagnosis of uterine neoplasm resembling an ovarian sex cord tumour was made, and subsequent total abdominal hysterectomy with bilateral salpingoophorectomy was performed. On gross examination, the uterine cavity was enlarged presenting an endometrial polyp of 0.8 cm, the wall was 4 cm in thickness and harboured multiple leiomyomatous nodules. Among these leiomyomatous nodules, an ill-defined, yellow-coloured, 1.5 cm, intra-myometrial mass was noted in the isthmus (Fig. 3A). Microscopic examination of the intra-myometrial yellowish nodule was consistent with a pure UTROSCT that exhibited minor irregularity of the edge with infiltration and entrapment of the surrounding myometrial smooth muscle without vascular invasion (Fig. 3B). Small foci of ischaemic necrosis were also noted. Gross and histological examination of adnexae was unremarkable. The post-operative course was uneventful after 1 year of follow-up.

Discussion

Since the initial description of UTROSCTs in 1976, there have been numerous efforts to further characterize this unusual group of uterine neoplasms. To date, histogenesis of UTROSCTs remains controversial, and it is still unclear whether these tumours represent a variant within the spectrum of endometrial stromal tumours (ESTs), which may rarely exhibit areas of sex cord-like differentiation, or whether they form distinct uterine neoplasms unrelated to ESTs. In the most recent WHO classification, these tumours have been separated from the main group of endometrial stromal and related tumours and placed in the category of "miscellaneous tu-
UTERINE TUMOUR RESEMBLING OVARIAN SEX CORD TUMOURS PRESENTING AS MULTIPLE ENDOMETRIAL AND CERVICAL UTERINE POLyps

mours” where they are termed “sex cord-like tumours” and defined by the absence of otherwise classical endometrial stromal or smooth muscle tumours 6.

These tumours may be seen in either reproductive or post menopausal-age patients. They frequently present with abnormal vaginal bleeding or uterine enlargement, but patients can be totally asymptomatic. Macroscopically, UTROSCTs are solid, round, well-circumscribed intra-myometrial masses. Occasionally, tumours are submucosal or subserosal and may be polypoid. Rare tumours are predominantly cystic. The cut surfaces are yellow, grey or tan, soft and fleshy, without the whorled pattern of leiomyomas 7.

Histologically, tumours are generally well circumscribed, but foci of irregularity, and infiltration of the surrounding myometrium may be present. The sex cords may take several forms and appear as anastomosing trabeculae, cords, retiform areas, small nests and sometimes well-formed tubules with lumens. The neoplastic epithelioid cells range from small and round with scanty cytoplasm to large cells with abundant eosinophilic, clear, or foamy cytoplasm. The small uniform nuclei display rare or absent nuclear grooves and indistinct nucleoli. Mitotic figures are rare and necrosis is usually absent. UTROSCTs lack legitimate Call-Exner bodies, which further distinguishes them from true adult granulosa tumours. The stroma is usually fibrous, but may contain a prominent component of smooth muscle that has the appearance of incorporated myometrium 8. Importantly, no neoplastic endometrial stroma should be identified within the tumour 1.

Several studies have attempted to phenotype the sex cord-like cells with variable evidence supporting myoid, epithelial, and true sex cord differentiation; the polyphenotypic immunophenotype of UTROSCTs suggests that these tumours are most likely derived from an uncommitted cell with the capacity to differentiate along several lines and express epithelial, myoid and sex cord markers 6,13. The results of the present case confirm the polyphenotypic immunophenotype of UTROSCT. CD10, a widely used marker of endometrial stromal neoplasms, was negative in our case; this is a further argument against an endometrial stromal origin of UTROSCTs.

The differential diagnosis of UTROSCT includes low-grade stromal sarcomas with extensive areas of sex cord differentiation, mixed Müllerian tumours, epithelioid smooth muscle tumours, perivascular epithelial tumours (PEComas), metastatic ovarian sex cord stromal tumours, carcinosarcomas, and primary and metastatic epithelial neoplasms, especially endometrioid adenocarcinoma with sex cord-like features.

The histological features and immunoreactivity for epithelial, myoid, and sex cord–like markers usually lead to the recognition of UTROSCT. Therefore, an immunohistochemical panel including at least 3 markers of steroid, epithelial and myoid differentiation will aid in differential diagnosis. In addition, negative immunostaining for CD10 and HMB45 can help to rule out low-grade stromal sarcoma and PEComas, respectively, whereas careful clinical examination and radiological investigations can be of help in differentiating metastatic ovarian sex cord stromal tumours 9,10. Abdullazad et al. reported a case of synchronous UTROSCT and an ovarian sex cord tumour (granulosa cell tumour) 10, and found that both ovarian and uterine masses had benign morphological and cytological criteria with a small size and a lack of pleomorphism, mitotic activity and necrosis. Immunohistochemical study showed that both tumours were positive for inhibin and calretinin, revealing sex cord differentiation of both tumours. The endometrial tumour showed weak positivity for CD56, strong positivity for the two other markers. The bland cytology of both tumours and their dissimilar staining pattern led to their diagnosis as synchronous benign tumours instead of an occurrence of metastasis 10.

UTROSCTs are generally considered to be of uncertain but low malignant potential. Although favourable histologic features including well-circumscribed borders
and the absence of vascular invasion are usually present within UTROSCTs, these tumours may occasionally show infiltrative borders, as seen in our case, and focal vascular invasion. Few cases of recurrence and distant metastasis have been reported in the literature, but a review of these cases, showed that all these tumours presented a stromal component intermixed with the true sex cord component. Given the relatively ill-circumscribed nature in our case, an uncertain behaviour of the tumour might be expected. Hurrel et al. consider that uncertain behaviour is an additional argument for not classifying these as variants of endometrial stromal tumour since the behaviour of the latter group of neoplasms is well established based on the circumscription of the lesions.

Conclusion

Uterine tumours resembling ovarian sex-cord tumours are rare. A polyphenotypic immunophenotype is a characteristic feature of UTROSCT and may be helpful in diagnosis and in exclusion of other lesions. Familiarity with this tumour by gynaecologists and pathologists is essential to avoid misdiagnosis, since the correct diagnosis of this neoplasm is important in patient management.

References

Introduction

Gastrointestinal (GI) lipomas are benign well-differentiated tumours that predominantly arise from adipose tissue in the bowel wall. Colon is the most common site for occurrence of GI lipomas. Although lipoma is a rare intramucosal lesion of the colon, it is the most common non-epithelial tumour in the GI tract \(^1\). Colonic lipomas are most commonly found in the right colon, and tend to occur on or near the ileocecval valve \(^2\). They affect men and women equally, and occur in children as well as in adults. Although GI lipomas are usually solitary, some patients develop multiple lesions \(^3\). Most lipomas are compressible polypoid submucosal tumours covered by an intact mucosa. Rare lesions develop in the subserosal region. On cut surface, lipomas appear bright yellow, round, greasy and encapsulated unless they have become infarcted \(^2\). Colonic giant lipomas may be misinterpreted as a premalignant adenomatous polyp, especially when they arise in the left colon \(^3\).

In contrast to small lipomas which usually have no symptoms, large lesions often become symptomatic with abdominal pain, intestinal obstruction, intussusception and bleeding which is accompanied by iron deficiency and anaemia. Gastric lipomas whose diameter exceeds 3 cm tend to ulcerate and cause peptic ulcer-like symptoms \(^2\). Despite the contribution of new imaging modalities (CT and MRI), definite diagnosis of these lesions is based on histopathological examination on biopsy of the resected specimen.

Management strategies are dependent on the size and sessility of the lesions. For successful removal of tumours with diameters less than 2 cm, colonoscopy is considered, while for larger and symptomatic lesions, surgery with a wide range of operative techniques is suggested. In case of giant lipomas, open surgical resection is recommended to remove the risk of malignancy and relieve symptoms \(^4,5\).

To our knowledge, the majority of giant colonic lipomas are found to have sessile appearance. Herein, we report a pedunculated tumour in a 38-year-old male located in the splenic flexure of colon.
our experience with a case of giant submucosal lipoma at the splenic flexure of colon, and discuss clinical features, diagnosis and treatment, in addition to reviewing the relevant literature.

Case report

A 38-year-old male referred to the emergency department of our hospital in Yazd, Iran in January 2012. The patient had a history of continuous ambiguous hypogastic pain and alternating constipation, lasting for approximately 2 months. His pain was worsening after meals or during defecation. There was also history of nausea, vomiting and haematochezia. He complained about loss of appetite and weight with a continuous constipation in the past 2 weeks. He denied consumption of alcohol or non-steroidal anti-inflammatory drugs. Upon physical examination, he had slight tachycardia (pulse rate 104 per minute) with normal blood pressure, and his abdomen was soft. On deep palpation, he had some tenderness in the left lower quadrant. Auscultation showed an increase in bowel sounds.

Blood examination demonstrated the following: white blood cell count 11,200/mm^3^, haemoglobin 118 g/L, and MCV 79.7 fL; all other routine laboratory tests were within normal limits. At colonoscopy (Olympus PCF-160AL video colonoscope, Japan), a submucosal polyp about 5×4 cm was seen in the left colon at the splenic flexure.

Endoscopic biopsy revealed considerable necrotic fat tissue. Computed tomography (CT) of the abdomen exhibited a pedunculated ovoid mass, about 8 cm in diameter, with sharp margins and soft tissue density, protruding into the splenic flexure of colon. Finally, based on the patient’s symptoms and according to the features attained by the imaging modalities, our diagnosis was colonic giant lipoma, and a wide segmental resection with primary anastomosis was considered.

By excision of the colonic segment, an ovoidal pedunculated polypoid tumour measuring 70×50×45 mm in size with a stalk of 20 mm in diameter was found (Fig. 1). The lesion was located in the submucosal layer of intestinal wall. Cut surface of the resected specimen was lobular and yellowish with areas of fat necrosis. Further microscopic study of this mass revealed that the lobules were composed of mature adipose tissue. Further microscopic study of this mass revealed that the lobules were composed of mature adipose tissue separated by fibrous septa. Submucosal proliferation of fibrous tissue was observed, but mitotic figures and atypical cells were not seen. There was ulceration in the tumour along with extensive fat necrosis (Fig. 2). The final diagnosis was giant colonic lipoma originating from the splenic flexure.

In this case, due to the large size of the lipoma which was located in the splenic flexure, and due to the uncertainty about the depth of involvement of colonic wall, the tumour was excised through wide segmental resection, and a primary anastomosis was performed. The patient was discharged from the hospital about one week after the operation without any unusual complication. There was no recurrence during a post-operative follow-up period of 7 months. Seven months after the operation, the patient was well and free of symptoms.

Discussion

Colonic lipomas are rare benign tumours with an estimated prevalence of 0.035 to 4.4% of the general population. There is no sex preference for this tumour, and the mean age at diagnosis ranges from 50 to 70 years, similar to that of individuals with colon carcinoma. In the colon, lipomas occur mostly in the right colon, mainly near or on the ileocecal valve, whereas the giant lipoma of our 38-year-old case was located in the left colon at the splenic flexure. Lipomas are generally situated submucosally. However, they can be located in the subserosal region. In the majority of reports they have been isolated, but in some cases (~6.1%) there are multiple lipomas.
Small-sized lipomas are usually asymptomatic and detected incidentally during colonoscopy, laparotomy, or autopsy. Although the majority of lipomas remain asymptomatic, colonic lesions more than 2 cm in size may present with symptoms (25%) such as pain, diarrhoea, bowel obstruction and bleeding, and can be the lead point for intussusceptions. Symptoms of lipomas are non-specific, and may be of long duration. The most common signs and symptoms consist of bleeding with anaemia (54.5%), abdominal pain (42.4%), alteration in bowel habits (24.2%), and diarrhoea or constipation. Occasionally, lipomas of the colon are complicated with obstruction, intussusception, prolapse, perforation, or very rarely with massive haemorrhage. Giant lipomas often cause symptoms, and therefore their removal should be considered. Clinical manifestations of giant lipomas depend on the size of the tumour, but not on the involved section of the large bowel. Features of giant colonic lipomas are summarized in Table 1. As mentioned earlier, our patient presented with a solitary giant submucosal lipoma resulting in continuous ambiguous hypogastric pain and constipation accompanied by anaemia.

Notably, giant lipomas of the colon may be misinterpreted as a premalignant adenomatous polyp, particularly when they arise from the left colon. Though imaging findings are not specific and shared with other gastrointestinal diseases, they still contribute to preoperative diagnosis.

Initially, diagnosis of a benign lesion of the colon is based on its demonstration by barium enema displaying a well-defined, smooth, intraluminal filling defect, with changes in its form under peristalsis. Barium enema can discover lipomas, but it is not specific and the lesion can be mistaken for another type of neoplasm, especially when the tumour is extensively ulcerated. The chance of reaching definite diagnosis in this way is about 20% of cases. A water enema with low kilo voltage technique

<table>
<thead>
<tr>
<th>Ref</th>
<th>Sex</th>
<th>Age</th>
<th>Site</th>
<th>Figure</th>
<th>Signs and symptoms</th>
<th>GD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author</td>
<td>Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahadursingh</td>
<td>2003</td>
<td>M</td>
<td>67</td>
<td>SC</td>
<td>N</td>
<td>Yellow</td>
</tr>
<tr>
<td>ÜSTÜNSOY</td>
<td>2003</td>
<td>M</td>
<td>51</td>
<td>AC</td>
<td>-</td>
<td>Yellowish-brown</td>
</tr>
<tr>
<td>Ghidirim</td>
<td>2005</td>
<td>F</td>
<td>51</td>
<td>C</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td>Jovanovic</td>
<td>2007</td>
<td>M</td>
<td>67</td>
<td>SC</td>
<td>N</td>
<td>Yellow</td>
</tr>
<tr>
<td>Mai</td>
<td>2007</td>
<td>F</td>
<td>34</td>
<td>AC</td>
<td>-</td>
<td>Red</td>
</tr>
<tr>
<td>Jiang</td>
<td>2007</td>
<td>M</td>
<td>42</td>
<td>DC</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td>Mnif</td>
<td>2009</td>
<td>F</td>
<td>67</td>
<td>TC</td>
<td>N</td>
<td>Yellow</td>
</tr>
<tr>
<td>Barchetti</td>
<td>2010</td>
<td>M</td>
<td>31</td>
<td>DC</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td>Haei</td>
<td>2010</td>
<td>M</td>
<td>33</td>
<td>SC</td>
<td>Y</td>
<td>Green</td>
</tr>
<tr>
<td>Seob Lee</td>
<td>2012</td>
<td>F</td>
<td>68</td>
<td>AC</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td>Current case</td>
<td>2014</td>
<td>M</td>
<td>38</td>
<td>SF</td>
<td>Y(2 cm)</td>
<td>Dark green</td>
</tr>
</tbody>
</table>

M, male; F, female; AP, Abdominal pain; CIBH, Change in bowel habits; WL, Weight loss; RB, Rectal bleeding; GD, Grater diameter; SC, Sigmoid colon; AC, Ascending colon; C, Cecum; DC, Descending colon; TC, Transverse colon; SF, Splenic flexure; Y, Yes; N, No
may take advantage of the different absorption coefficients of fat and water: fat-containing lesions will appear relatively radiolucent. The shape of the mass may be observed to change during fluoroscopic examination as a consequence of peristalsis or manual pressure, which is the so-called “squeeze sign” \(^{17}\).

CT scan is another imaging modality that is more beneficial for conclusive definition of colonic lipomas and permits positive diagnosis of such lesions by indicating a well-defined, homogeneous, round, intraluminal lesion which changes in size and form during peristalsis, with a fat density between -80 and -120 UH \(^{1,16}\). Thus, CT can provide definitive preoperative diagnosis of colonic lipomas, especially in those with a large size. The precise CT characteristics of lipomas are round, ovoid or pear-shaped with sharp margins, and densitometry values related to fatty tissue \(^{17,18}\). CT also provides the exact topographical information about lipomas, and can display some related complications such as intussusception, infarction and necrosis. On the other hand, certain features of lipomas are described by endoscopy, including the cushion sign (identification of the lipoma with pressure from a biopsy forceps), the “tenting sign” (elevation of the overlying mucosa with the biopsy forceps) and the “naked fat” sign, which occurs when the fat is grossly extruded after biopsy.

The submucosal lesions found at colonoscopy screening may attain substantial sizes of up to several cm in diameter. Their size may vary from 3.5 mm to 10 cm, with an average size of 4 cm at the time of detection \(^5\). Colonoscopy of our patient defined a submucosal polyp measuring 5×4 cm in diameter. In the most cases, giant lipomas are found to have a sessile appearance (Tab. I), while in the present case, there was a truly pedunculated polyoid mass with a stalk.

As mentioned, the greatest clinical importance of giant lipomas lies in their potential to be confused with aggressive pathologies. Thus, diagnosis should be confirmed by colonoscopic findings, where the polyp will have the specific appearance of not involving the mucosa of the colon \(^3\).

Endoscopic removal of colonic lipomas is associated with increased morbidity compared to excision of adenomatous polyps, and can lead to increased heat production and damage to the adjacent bowel wall with potential perforation \(^{19}\). This approach is preferably reserved for symptomatic patients and in lesions that are somewhat pedunculated. Endoscopic treatment is more suitable for colonic lipomas < 2 cm in diameter \(^3\). Nowadays, with the availability of colonoscopy and CT, and because of confusion with a malignant process, the requirement for resection can be considered a rare occurrence. Once diagnosis has been established and carcinoma has been ruled out, the patient needs only be reassured, although in the symptomatic patient a limited resection or colectomy and lipomectomy will be advised \(^{17}\).

Traditionally, surgical treatment has been the therapy of choice for symptomatic large colon lipomas. It is commonly accepted that the difficulty of achieving a preoperative diagnosis influences the type of surgical treatment undertaken. Laparoscopic removal has also been reported. Meanwhile, surgeons should be careful since colonoscopic biopsies do not have the same value as histopathological examination of open biopsies, as the lesion is under the normal mucosa. For precise diagnosis, histopathologic assessment of a large specimen should remain the gold standard \(^2,17\).

Histological characteristics of giant colonic lipomas consist of sharply-circumscribed submucosal masses of mature adipose tissue with an overlying intact or eroded mucosa. Usually, a thick fibrous capsule surrounds the tumour. Nuclear enlargement, hyperchromasia, fat necrosis, fatty cysts and foamy macrophages may be present \(^{20}\).

Finally, in our case, gross examination of the specimen showed that the encapsulated, bright yellow, soft mass measuring 70×50×45 mm in size was a giant lipoma. Mature adipose tissue was confirmed by histological examination; neither increased mitotic activity nor lipoblast was found. The patient underwent a wide segmental resection to relieve symptoms, and had an uneventful postoperative course with dramatic improvement after surgery.

**ACKNOWLEDGMENTS**

We would like to greatly thank all of the authorities and staff of the Shahid Sadoughi University of Medical Sciences in Yazd, Iran for their kind cooperation and support. We are also very thankful to Dr Seyed Hossein Hekmati Moghadam for his kind help.

**References**

"Pure" primary large cell neuroendocrine carcinoma of the urinary bladder: case report, literature review and diagnostic criteria

T. PUSIOL, D. MORICHETTI, M.G. ZORZI
Institute of Anatomic Pathology, Rovereto Hospital, Rovereto (Trento), Italy

Key words
Bladder neuroendocrine tumours • Bladder large cell neuroendocrine carcinoma • Bladder carcinoma • Genitourinary cancer • Malignancy • Immunohistochemistry • Urinary cytology • Carcinoma

Introduction. Large cell neuroendocrine carcinoma (LCNC) is defined in the urinary bladder, as in other sites, as a high-grade neoplasm exhibiting neuroendocrine features at the H&E level, high mitotic activity and evidence of neuroendocrine differentiation by immunohistochemistry. We report a case of pure bladder LCNC with review of the literature.

Methods. A 68-year-old male presented with gross haematuria of two weeks’ duration in October 2011. Transurethral resection and subsequently radical cystoprostatectomy (CP) with bilateral lymphadenectomy (L) were performed in December 2012.

Results. Urinary cytology identified malignant cells. Histologically, the tumour showed organoid nesting, trabecular growth, rosettes and perilobular palisading patterns, suggesting neuroendocrine differentiation. Immunohistochemical staining showed intense positivity for CD56.

Discussion. We examined all published pure bladder LCNC (12 cases) excluding mixed neoplasms. Small cell carcinoma of the urinary bladder pure LCNC of the bladder is a very aggressive malignancy, unresponsive to therapy, presents in an advanced stage and has a propensity for early metastasis. Prior to the advent of immunohistochemistry, such cases would most likely have been categorised as poorly differentiated, high-grade urothelial carcinomas.

Case report
A 68-year-old male presented with gross haematuria of two weeks’ duration in October 2011. No significant clinical history was found. Urinary specimens were examined. Chest radiography computerized tomography (CT) of the abdomen and pelvis, a contrast total body CT were performed. Transurethral resection (TR) and subsequently radical cystoprostatectomy (CP) with bilateral lymph-
adénecomy were performed in December 2012. The PET CT total body was made in April 2013. Radiotherapy (R) and adjuvant chemotherapy (Ch) were administered.

Chest radiography and CT of the abdomen and pelvis showed no evidence of other primary tumours, but contrast total body CT revealed a 3x4 cm mass in the dome of urinary bladder. All the fragments of the TR were examined. One block for each cm of surgical specimen was made. Six histological sections for each block were examined. The macroscopic examination of the CP revealed a 2.8x2.2 cm mass situated on the dome (Fig. 1) with infiltration of the muscularis propria. Urine examination identified round or polygonal malignant cells with nuclear moulding and rosette arrangements (Fig. 2A).

Histologically the tumour showed organoid nesting, trabecular growth, rosettes and perilobular palisading patterns, suggesting a neuroendocrine differentiation. The tumour cells where large, with moderate cytoplasm. Nucleoli were frequent and prominent. Mitotic counts were 12 per 1 mm². Large zones of necrosis were found (Fig. 2B). The tumour invaded the deep muscularis propria. The 56 lymph nodes examined were free of disease (pT2bN0). Immunohistochemical analysis showed that the tumour neuroendocrine component was positive for cytokeratin 7 and for neuroendocrine markers such as neuron specific enolase (NSE) and CD56 (Fig. 2C). After discolouration of urinary cytological slide containing typical neoplastic cells, immunostaining for CD56 was performed which showed focal positivity (Fig. 1; insert). Total body PET CT revealed diffuse liver and bone metastases.

Discussion

Primitive bladder neuroendocrine carcinomas are extremely rare, accounting for less than 1% of bladder malignancies; most are small cell carcinoma (SCC). In recent years, LCNC have been rarely reported. In previous literature reviews, the authors considered both pure and mixed LCNC cases. In fact, LCNC may be a component of mixed bladder malignancies including urothelial carcinomas (UC), SCC, adenocarcinoma or sarcomatous neoplasms. We examined all published “pure bladder LCNC” cases to establish the clinical-pathological, immunohistochemical, prognostic features of the disease, excluding mixed neoplasms.2-21 (Tab. I). Thirteen cases have been reported, but the sampling procedures of the histological material were not explained in any report.
### Conclusion

Considering SCC of the urinary bladder as a very aggressive malignancy, and unresponsive to therapy, that presents in an advanced stage and has a propensity for early metastasis. Prior to the advent of immunohistochemistry, these cases would most likely have been categorised as poorly differentiated, high-grade UC. A retrospective review of archival poorly differentiated, high-grade UCs using neuroendocrine im-

<table>
<thead>
<tr>
<th>Source</th>
<th>Age/Sex</th>
<th>Symptoms</th>
<th>Clinical history</th>
<th>Location</th>
<th>Tumour size (cm) / T/N/M</th>
<th>Immunostaining</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hallemarian et al. 1998</td>
<td>75 / M</td>
<td>H</td>
<td>R for prostatic cancer (clinical stage T2N0M0), diagnosed on needle biopsy. Insulin-dependent diabetes mellitus. Kidney transplant 2 years previously.</td>
<td>Dorsal wall</td>
<td>4 / T3N0M0</td>
<td>NSE+, CK+, Cg+, Syp+, LCA-, PSA-, PP-, VIP-, In-, Glu-, So-, SP-, Se-</td>
<td>Radical CP and bilateral pelvic L</td>
<td>DOD 2 months after disseminated metastases</td>
</tr>
<tr>
<td>Lee KI et al. 2006</td>
<td>32 / M</td>
<td>H</td>
<td>Unremarkable</td>
<td>Dome and anterior wall</td>
<td>3 / T3N0M0</td>
<td>NSE+, CK+, Cg+, Syp+, LMA+, CD56+, S100, LCA-, PSA-, VIM-</td>
<td>Partial C</td>
<td>Multiple metastases in the lung and liver.</td>
</tr>
<tr>
<td>Aljoo SF et al. 2007</td>
<td>40 / M</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NSE+, TTF1+, Leu7+, PSA+, **Cg+ 1 de 2,**Syp+ 1 de 2</td>
<td>TR, radical CP, Ch</td>
<td>AWD 13 months after treatment</td>
</tr>
<tr>
<td>Bertacchi A et al. 2008</td>
<td>57 / NR</td>
<td>H</td>
<td>History of cigarette smoking.</td>
<td>Posterior wall</td>
<td>2.5 / T3N2M0</td>
<td>NSE+, CK+, Cg+, Syp+</td>
<td>CP, Ch.</td>
<td>AWD 22 months after surgery.</td>
</tr>
<tr>
<td>Oshiro H et al. 2008</td>
<td>74 / F</td>
<td>H</td>
<td>Initial history of TR for BUC situated on the left posterior side and 8 courses of intravesical bacillus Calmette-Guérin immunotherapy.</td>
<td>Left lateral wall</td>
<td>1 / T2N0M0</td>
<td>CK+, Syp+, Cg+, CD56+, CD57+, TTF1-</td>
<td>TR, radical C, bilateral pelvic L</td>
<td>Healthy and free from recurrence of cancer for 48 mo after C.</td>
</tr>
<tr>
<td>Lee W et al. 2009</td>
<td>20 / M</td>
<td>Metastatic cutaneous nodule on the scalp</td>
<td>Partial C and Ch 1 year before of cutaneous metastasis.</td>
<td>NR</td>
<td>NR / NR</td>
<td>NSE+, CK+, Syp+, TTF1+, CD56+</td>
<td>Partial C and Ch.</td>
<td>Lung, retroperitoneal nodal metastases, 12 months after surgery</td>
</tr>
<tr>
<td>Martin Li et al. 2011</td>
<td>69 / M</td>
<td>Absent</td>
<td>Uterine adhesions and amigdalectomy.</td>
<td>Anterior wall</td>
<td>5 / T2N0M0</td>
<td>NSE+, CK-, Cg-, Syp+, S100-, HMBS-</td>
<td>TR, radical C and L</td>
<td>AWD 12 months after surgery</td>
</tr>
<tr>
<td>Tsugu A et al. 2011</td>
<td>74 / M</td>
<td>Brain metastasis</td>
<td>No clinical evidence of bladder cancer before brain metastasis diagnosis.</td>
<td>Left lateral wall</td>
<td>NR / NR</td>
<td>CD56+, Cg+, Syp+, TTF1+.</td>
<td>Ch, R</td>
<td>DOD 5 months after brain metastasis rejection for pulmonary embolism.</td>
</tr>
<tr>
<td>Colorossi C et al. 2013</td>
<td>55 / F</td>
<td>H</td>
<td>NR</td>
<td>Posterior wall</td>
<td>4 / PT3B1N2M1</td>
<td>NSE+, CD56+, Syp+, Cg focally+, TTF1+, CK-</td>
<td>C, hynoeranessiectomy, L and Ch.</td>
<td>DOD 7 months after initial diagnosis.</td>
</tr>
<tr>
<td>Macak J et al. 2015</td>
<td>66 / M</td>
<td>H</td>
<td>NR</td>
<td>NR</td>
<td>NR / NR</td>
<td>CK-, CD56+, Syp+, Cg+, NSE+, C-, C-, PP-, VIP-, So-, Glu-, Se-, TTF1-</td>
<td>Ch</td>
<td>Liver, adrenal, nodal, spleen metastasis.</td>
</tr>
<tr>
<td>Sani A et al. 2015</td>
<td>67 / M</td>
<td>H</td>
<td>Chronic obstructive lung disease and congestive heart failure for 15 years</td>
<td>Right half</td>
<td>6 / PT3B1N1M0</td>
<td>Syp+, Cg+, CD56+, TTF1-+, PSA-+, CK7+, high molecular weight CK-, PSA-, PSAP-</td>
<td>TR,</td>
<td>DOD 15 days later.</td>
</tr>
<tr>
<td>Our case</td>
<td>58 / M</td>
<td>H</td>
<td>Unremarkable</td>
<td>Dome</td>
<td>2.8 / pt2B8N0</td>
<td>CD56+, NSE+, TTF1+, CK7+, Syp-Cg-</td>
<td>CP, PR and Ch</td>
<td>Liver and bone metastases. 16 months after surgery.</td>
</tr>
</tbody>
</table>

**Comment:**

In our review we have excluded the cases reported by:

1. Abenosa et al. 13: the case is a mixed adenocarcinoma – LCNC of probable urachal origin.
2. Evans et al. 14: the case is a primary mixed adenocarcinoma - LCNC with adenocarcinoma component less than 5% of the total tumour volume.
3. Dundr et al. 15: the case is neuroendocrine carcinoma with lymphoepithelioma-like features and high grade papillary UC.
4. Li V et al. 16: the tumour is a mixed malignancy composed by LCNC and mesenchymal components.
5. Quek et al. 17: the 25 neuroendocrine tumours including five cases with secondary UC and the clinical-pathological and prognostic features has been reported.
6. Trimeche et al. 18: the tumour is a mixed neoplasm composed by LCNC and high grade invasive papillary UC.
7. Akamatsu et al. 19: the LCNC showed areas of UC and UC.
8. Enges et al. 20: the tumour is mixed malignancy composed by high grade UC, SCC and LCNC.
9. Hata and Tasaki 21: the tumour is LCNC localised on the anterior wall of the bladder associated with UC. 
munohistochemical markers might better define their true incidence, clinical behaviour and optimal management. A diagnosis of LCNC may be made only after complete and accurate histological examination of surgical specimens to exclude small foci of other neoplastic patterns.

References

I MEETING NAZIONALE DEL GRUPPO ITALIANO DI PALEOPATOLOGIA (GIPaleo)

L'AQUILA, SABATO 22 MARZO 2014
ORE 9:00
INGRESSO LIBERO

PALAZZETTO DEI NOBILI
PIAZZA SANTA MARGHERITA

Segreteria Scientifica:
Prof. Gino Fornaciari (Pisa)
Dott. Luca Ventura (L'Aquila)
Dott.ssa Nadia Rucci (L'Aquila)
Dott.ssa Valentina Giuffra (Pisa)

Segreteria Organizzativa:
Dott. Luca Ventura
Dott.ssa Cinzia Mercurio
Dott.ssa Daniela Rullo

U.O. Anatomia Patologica,
Ospedale San Salvatore,
Coppito 67100 L'Aquila
Tel.:0862 368766
E-mail: luca.ventura@tin.it

Banca popolare
dell'Emilia Romagna
GRUPPO BPER
The exploration of the tomb of Giovanni dalle Bande Nere (1498-1526)

G. FORNACIARI

Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy

The study of the skeleton of Giovanni revealed that he was a vigorous man, 1.78 m tall, with an athletic body, estimated skeletal age of 25-30 years, medium-sized skull, narrow nose and great skull capacity (1494 cc). His well-developed upper limbs muscular insertions (deltoid, great pectoral, great dorsal, biceps, forearm muscles) and thigh muscles confirmed his great physical strength and robusticity. Strong hypertrophy of rotator cuff, great dorsal, teres minor and anconeous insertions were all present, as well as gluteal insertions to the femur, confirming he was a highly skilled horsemen. The presence of numerous Schmorl’s hernias and a wedge partial collapse, with right spondylolysis, of the fifth lumbar vertebra, revealed that Giovanni had carried heavy loads since adolescence due to horse-riding and body armor. Diffuse bilateral entheseopathies were found at the clavicular insertions of deltoid and pectoralis major, as well as at the small trochanter (psosas muscle). Skeletal markers left by habitual horseback riding were all present: exostoses and ovalization of acetabula, hypertrophy of femoral rectum muscle, strong hypertrophy of the femoral biceps, great adductor, small and great gluteus, Poirier’s facet. Paleopathological investigation showed the aftermaths of several injuries: fractures of nasal septum and proximal third of the left humerus, injury from blade affecting right ulna and radius and swelling of the posterior surface of the right tibia, with underlying osteomyelitic focus in reparative phase, as well-documented on CT. The amputation level was exactly assessed: the tibia was sawn immediately below the proximal half of diaphysis and only the lateral portion was surgically treated with an horizontal cut. Only oblique splitting was found at the medial site of the tibia. At stereoscopic microscope, surgical section revealed a marked proliferation of endosteal callus, due to a previous harquebus shot injury occurred about one year before the death. Distal extremity of fibular fragment showed an oblique splitting and a horizontal cut, with no sign of reparative process in the medullar canal. Considering the morphological aspect of the tibial and fibular injury, it was probably due to a cannonball from a falconet of caliber 6-7 cm, as written by Benedetto Agnello in the same day of injuring. The limb had been severely damaged by a traumatic hemi-amputation when surgeon Abramo performed the intervention, consisting in a simple completion of the amputation and regularization of proximal fragments. In conclusion, paleopathological investigations lead to exclude the hypothesis of an amputation above the knee, since the surgeon Abraham performed the procedure as better as he could in conformity with surgical knowledge of that period.

Acknowledgements

The Italian Society of Orthopedic and Traumatology sponsored the project of exhumation and study. The field studies were performed by Angelica Vitiello, Valentina Giuffra, Simona Minozzi, Antonio Fornaciari, Francesco Coschino, Raffaele Gaeta from the Division of Paleopathology, University of Pisa and Luca Ventura from the Unit of Pathology, S. Salvatore Hospital of L’Aquila.

The mummy of Borgo San Dalmazzo. Paleopathological approach in modern forensic medicine

M. ABRATE1, R. BOANO2, F. GRILLO3, E. FULCHERI1,4

1 Anatomia Patologica ASL CN1, Ospedale di SS Annunziata di Savigliano (CN), Italy; 2 Dip. Scienze della Vita e Biologia dei Sistemi, Laboratorio di Antropologia, Università di Torino, Italy; 3 Anatomia Patologica, Dip. DISC Università di Genova, Italy; 4 Istituto Giannina Gaslini, Genova, Italy

In November of 2013, in an apartment in Borgo San Dalmazzo (CN, Italy), the mummified body of an old, obese woman which had gone missing for several years was found. External examination revealed no visible signs of trauma thus excluding a violent death. Skin elasticity and the absence of visible surface infesting agents led to the assumption that a process of embalming had been performed. The collection of some insect exuviae allowed us to trace infestation to at least the previous spring, but more likely to a few years before. The case, clearly of forensic relevance, also presented the necessity of an anthropological and paleopathological approach. The histological examination carried out using paleopathological techniques on mummified tissues clearly demonstrated that the embalming process was performed using an application of a mixture of salt and fatty substances still traceable on the dermal surface. Histology, moreover, confirmed relatively good preservation of histological details thus permitting a pathological analysis of the body. A complete autopsy was therefore performed and multiple tissue samples were taken. Putting together various data from the histological study of tissue samples obtained from the body (showing the transformation phenomena), entomological examination of the insects and the environmental context as well as the analysis of circumstantial evidence, placed the timing of death in the spring of 1996. Some pathological lesions strongly suggestive of cause of death were found however these are still pending the end of the official inquiry.

In this case, we wish to highlight the importance of a multidisciplinary approach in solving an unusual, complex and intriguing forensic case.
Applications of microcomputed tomography (µCT) for conservative analyses in paleopathology

M. CAPULLI, N. RUCCI, S. GEMINI PIPERNI, A. MAURIZI, A. TETI
Department of Applied and Biotechnological Clinical Sciences, University of L’Aquila

The micro-computed tomography (µCT) or high-resolution x-ray tomography is an imaging technique in which x-rays are used to create virtual cross-sections of a sample. These sections are then merged together to generate a virtual 3D model (without destroying the original sample). The prefix micro- (symbol: µ) indicates the pixel size and consequently the resolution of the device. µCT has applications in different fields, such as medical imaging, basic research and industries. Recently, it has been shown that this device could be used also to analyze the micro structure of samples with paleopathological relevance. The first application of µCT in paleopathology was described in 2011 by Martinón-Torres et al. 1, followed in 2011 and 2012 by the works of Versiani et al. and Nicklisch et al., respectively 2,3. In all these works it is clear the great contribution given by the conservative µCT analysis.

In our laboratory, we used the µCT to trace a detailed 3D map of the external surface and of the internal part of: fragments of mummified tissues found in Egyptian canopic jars, renal stones found in the mummy of Pandolfo III Malatesta, Lord of Fano (1370-1427) and in the mummy of an anonymous nobleman from Popoli (XVIII century). In all the samples, the µCT gave useful information on their internal and external morphology and composition. In conclusion, the use of µCT could be of great relevance for the paleopathology since the samples are always rare, precious and often calcified, and this technique is conservative, accurate and well established for the analysis of calcified samples.

References

The mummies of Borgo Cerreto (XVIII-XIX century): anthropological and paleopathological study

A. LUNARDINI1, L. COSTANTINI2, L. COSTANTINI BIASIMI3, G. FORNACIARI1

1 Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy; 2 Bioarchaeological Research Center, National Museum of Oriental Art “Giuseppe Tucci”, Roma, Italy

A group of mummified bodies were found in 1969, in the northern crypt of the church of “The Saints Jesus and Mary” of Borgo Cerreto, Perugia. Twelve individuals were found in anthropoid coffins, while eleven bodies were pilled in the middle of the crypt. All the bodies were still dressed with their original or funerary clothes; the study of these elements made possible to date back the burials between the first half of the XVII century and the middle of the XIX century.

The preservation of the human remains was not uniform: eight bodies were mumified, seven partially mumified and eight completely skeletonized. The mumification was the natural result of the microclimate of the burial chamber. The crypt was the vault of the physician and surgeon, Baronio Vincenzi, who lived and worked in Borgo Cerreto between the end of the XVI century and the middle of XVII century. Initial researches have been carried out to obtain information about life-style and medical activity of Baronio Vincenzi.

Since 2001, an interdisciplinary study was performed, including funerary archaeology, anthropology and paleopathology. The deposit, composed by nine men, six women, six subadults and two infants, for a total of twenty-three individuals, represents a very important opportunity of study, because the complex of human bodies was not decontextualized. The paleopathological study articulated in a series of specialized surveys including: macroscopic, radiological and histological examination of the human remains useful to determine the incidence of the several pathological clinical and sub-clinical pictures relative to the rural population of Valnerina in the Modern Age. The scientific analysis of the bodies, at present, has evidenced: a case of mortal gunshot wound of the right thigh in a soldier of the first half of the XVIII century, aged 25-30 years, a case of giant bladder stone in a mature woman, over 55 years old, and a case of congenital syphilis and tuberculosis in a young female aged 15-18 years. Evidence of surgical activity, trepanation and anatomical studies was also found.
Falciparum malaria has been endemic in Sardinia since ancient times. It has been speculated that the infection was introduced in the island in 502 BC by north-African infected workers after the Carthaginian conquest. Its widespread distribution is attested by written accounts from the Roman Period onwards.

In order to trace back the origin of malaria in Sardinia, paleo-climatological and bioarcheological investigations have been initiated in 2013. The skeletal remains of 34 individuals exhumed from the three different burial sites dating to Nuragic (14th-12th centuries), Phoenician (8th centuries), Carthaginian Periods (7th century) and to the Modern Age (second half of 16th century), have been investigated.

Plasmodium falciparum highly specific HRP-II protein was targeted. Since before malaria’s complete eradication, its foci overlapped with those of canine leishmaniasis (a protozoan disease supposed to have been highly endemic in agricultural ancient populations), a differential diagnosis was carried out. The antibody response anti-rk39 antigen specific to Leishmania infantum was also targeted.

The preliminary results of our investigation apparently confirm the absence of both malarial and leishmanial infections in the Nuragic samples. Conversely, infections by leishmaniasis and malaria have been detected, respectively, in samples dating to the Phoenician and Carthaginian Periods. No cases of malaria were identified in Modern Age samples.

Our initial findings confirm of malaria back to the Carthaginian Period. The first evidence for zoonotic leishmaniasis dates back to the Phoenician Period, instead. For earlier periods the absence of evidence is not an evidence of absence. On this ground, analyses on both ancient proteins and pathogens DNA are on going in order to chronologically fix the origin of the above diseases into ancient Sardinian populations.

In the Museum of Anthropology and Ethnology of Florence are conserved several pre-Columbian mummies. These bodies were brought from South America to Italy in the second half of the XIX century.

We performed a histopathological study on mummified tissue specimens from seven pre-Columbian mummies, with hematoxylin-eosin and immunohistochemical stains. The results confirm that the modern techniques of pathological anatomy are successfully applied on mummified tissues.

Among the results obtained, there is the only known paleopathological case of Chagas disease (American Trypanosomiasis), in addition to atherosclerosis, anthracosis, emphysema and pneumonia. The mummy with Chagas disease was a woman that did not die immediately, so the fact that she has managed to survive despite severe degrees of heart disease, megacolon and megaesophagus, suggests that she was aided through the use of drugs. Only one case of atherosclerosis was found, however important, considering that the person died at an age of about 30 years in a region poor in nutrition. In one mummy was possible to observe the presence of pulmonary exudates and areas with air space expansion and destruction of septa. There are signs of pulmonary exudate in at least two cases. We can speculate that the pneumonia occurred as a complication of the disease that led to the death, or that it was the direct cause of death.

We have found presence of pulmonary anthracosis. The proximity to fires from early childhood produced an early anthracotic tattoo, especially in people who use the bonfire to warm the places.

In the Museum of Anthropology and Ethnology of Florence are conserved several pre-Columbian mummies. These bodies were brought from South America to Italy in the second half of the XIX century.

We performed a histopathological study on mummified tissue specimens from seven pre-Columbian mummies, with hematoxylin-eosin and immunohistochemical stains. The results confirm that the modern techniques of pathological anatomy are successfully applied on mummified tissues.

Among the results obtained, there is the only known paleopathological case of Chagas disease (American Trypanosomiasis), in addition to atherosclerosis, anthracosis, emphysema and pneumonia. The mummy with Chagas disease was a woman that did not die immediately, so the fact that she has managed to survive despite severe degrees of heart disease, megacolon and megaesophagus, suggests that she was aided through the use of drugs. Only one case of atherosclerosis was found, however important, considering that the person died at an age of about 30 years in a region poor in nutrition. In one mummy was possible to observe the presence of pulmonary exudates and areas with air space expansion and destruction of septa. There are signs of pulmonary exudate in at least two cases. We can speculate that the pneumonia occurred as a complication of the disease that led to the death, or that it was the direct cause of death.

We have found presence of pulmonary anthracosis. The proximity to fires from early childhood produced an early anthracotic tattoo, especially in people who use the bonfire to warm the places.

References
The cholera, endemic in Bengal, in the early 19th century spread in the West thanks to the revolution in transportation resulted from the steam engine. Tuscany was struck in 1835 and then, even more violent, in 1854-55. Thanks to Pietro Betti, Superintendent for health of the Grand Duchy of Tuscany, we have a detailed description of the epidemic and a precise estimate of the deaths. 26,327 individuals died in 1855. The disease penetrated in Tuscany by Liguria in July 1854; from the ports of Avenza and Livorno spread towards the Interior of the region, until Florence, and quietened down in December 1854. Then cholera rekindled in devastating form in March-April of 1855, starting from the area immediately west of Florence, a district rich in activities related to the water cycle, and retraced the route in reverse order made last year, always following the way of the Arno and the new railway line opened in 1848: in august-september the cholera was spread to all the Tuscany. The area of Lucca is one of the hardest hit in the region. Between 2007 and 2010, the Division of Paleopathology of the University of Pisa undertook the archaeological exploration of the cholera cemetery of Benabbio, a mountain village near Lucca, where cholera lashed between August and October of 1855 causing 46 deaths in a population of around 900 inhabitants. The excavation made it possible to detect for the first time the material characteristics of a cholera cemetery. The findings provide a new source for anthropologically reading the reaction of a community facing the mortality crisis, between acceptance of regulations imposed by the authorities and local strategies.

References
Tognotti E. Lessons from the History of Quarantine, from Plague to Influenza A. Emerging Infectious Diseases 2013;19.

Archaeoanthropology of the italic-roman necropoleis near L’Aquila (Abruzzo region)

G. MIRANDA

Physical Anthropology, L’Aquila, Italy

The ancient people modulated their livelihood models to the environment in which they lived, often turning it radically; at the same time, the environment exerted a marked influence on human groups, influencing their life conditions and health status. In this context, we present the results of the paleopathological analysis and demographic characteristics derived from a research conducted on 1022 adult individuals found in the necropoleis of Fossa, Bazzano and Capestrano distributed within a radius of 12 Km in the Aterno River plain (Abruzzo region).

The results highlighted a concrete correlation between the sites of Fossa and Capestrano. In both there is a deep respect of earlier burials that are not affected or disrupted by the subsequent ones, but preserved and remembered. From the paleopathological analysis, what emerged in Fossa is a social structure with a working class engaged in agriculture, whereas a leisure class, perhaps more addressed to trade and art, is represented in Capestrano. The social picture from Bazzano appears much more turbulent, in a certainly more aggressive context, with a lower life expectancy, and an economy perhaps not exclusively agricultural. According to the high incidence of facial and cranial traumas, possibly produced with bats and edged weapons, many individuals were to be devoted to military activities.

In conclusion, the differences observed between the three necropoleis in terms of demographics and pathocenosis do not appear linked to geographical and environmental factors. In fact, the environmental homogeneity of each area did not prevent the establishing of so obvious differences between the necropoleis. Probably the differences detected in life conditions of these human groups are related to the existence of different historical and social conditions, regardless of their geographical location.
**Metastatic prostate cancer from the Imperial Age (Rome, I-II Century A.D.)**

S. MINOZZI¹, A. LUNARDINI¹, C. CALDARINI¹, P. CATALANO², G. FORNACIARI¹

¹ Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy;
² Service of Anthropology, Special Superintendence to Archaeological Heritage of Rome, Italy

Archaeological excavations carried out by the Special Superintendence of Archaeological Heritage of Rome between 2007 and 2008 uncovered important ancient structures in Via di Casal Bertone near the Tiburtina Railway Station in Rome. A large manufacturing facility identified as a fullery (fullonica) and a necropolis with 70 burials were found along the ancient tract of the Via Collatina, dating back between 1st and 2nd century AD. The anthropological analysis of skeletal remains showed high frequencies of stress markers and traumas, suggesting that the population had probably been employed as manpower in the near fullonica.

The skeleton of a robust adult man (aged 50-60 years), with marked muscular attachments, was affected by an evident and diffuse neoplastic disease. The most part of the bones showed osteolytic alterations and new bone formations, mainly affecting the axial skeleton: the scapular and pelvic girdle, sternum, ribs, and spine.

The number and size of the multiple lesions and their anatomic distribution, associated with their destructive and proliferative feature, indicated that the lesions were the result of metastases from a primary cancer of the soft tissues. As a matter of fact, metastatic cancer affects most frequently the axial skeleton, rich in hematopoietic marrow, such as vertebrae, sacrum, ribs, sternum, pelvis and skull, as in the case study. The age and sex of the affected individual, as well as the radiographic and histological pictures of the bones, addressed the differential diagnosis of an advanced case of prostate cancer with extensively diffused bone metastases. This malignant tumor frequently spreads to the bones, inducing osteolytic and osteoblastic bone response, especially in the axial skeleton. We do not know whether the presence of this cancer can be linked to the work activity, but the toxic chemicals used in the treatment of the clothes could facilitate the manifestation of the tumor.

**A case of multiple trepanation among the “Martyrs of Otranto”**

V. GIUFFRA, G. FORNACIARI

Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy

The number of multiple trepanations and their anatomic distribution, associated with their destructive and proliferative feature, indicated that the lesions were the result of metastases from a primary cancer of the soft tissues. As a matter of fact, metastatic cancer affects most frequently the axial skeleton, rich in hematopoietic marrow, such as vertebrae, sacrum, ribs, sternum, pelvis and skull, as in the case study. The age and sex of the affected individual, as well as the radiographic and histological pictures of the bones, addressed the differential diagnosis of an advanced case of prostate cancer with extensively diffused bone metastases. This malignant tumor frequently spreads to the bones, inducing osteolytic and osteoblastic bone response, especially in the axial skeleton. We do not know whether the presence of this cancer can be linked to the work activity, but the toxic chemicals used in the treatment of the clothes could facilitate the manifestation of the tumor.

The observed lesions are the result of a multiple trepanation carried out by an instrument with a large rounded tip. The different dimensions of the holes allow to suggest the use of tips of different sizes to achieve complete and attempted perforations. Although it is not possible to establish with certainty the reasons for this multiple trepanation, several hypotheses can be proposed. These include experimental surgery and a ritual procedure to obtain relics.

The skull of Otranto is the only evidence of multiple trepanation documented in the skeletal remains belonging to a Saint.

**References**


Advanced morphologic and compositional investigations on samples of paleopathological interest

L. ARRIZZA¹, L. VENTURA²

¹ Centre of Microscopies, University of L’Aquila, Italy ² Department of Pathology, San Salvatore Hospital, L’Aquila, Italy

As a multidisciplinary science, paleopathology is largely based on modern investigation techniques. Among the different approaches employed, advanced morphologic and compositional methods play an important role in paleopathological investigation.

We studied different ancient materials using the following techniques: binocular stereomicroscopy (BSM), phase-contrast microscopy (PCM), scanning electron microscopy (SEM), also with energy dispersive X-ray analysis (EDX), and X-ray diffraction (XRD) analysis. The sample features addressed the choice of the most suitable method.

A fingernail belonged to a XX century Italian mummy was extracted from its bed and submitted to BSM, and SEM/EDX, in order to evaluate its features following a conservative approach. BSM allowed to appreciate differences between dorsal (polished) and ventral (unstained) surfaces, SEM evidenced nail root and free edge contours details, enabling us to select areas for EDX measurements. The latter method displayed calcium sulphate used as a nail polish on the organic structures of the nail, as well as metal remnants from manicure devices.

The renal stones of Pandolfo III Malatesta (1370-1427) and a nobleman from Popoli (XVIII century) were investigated using BSM, SEM/EDX, and XRD. Such methods enabled us to disclose the morphological details of the surface and the inner portions of the stones, along with their chemical compositions (ammonium acid urate and weddelite for Pandolfo; whewellite and hydroxylapatite for Popoli).

The content of four canopic jars belonged to an anonymous Egyptian of the New Kingdom (1550-1069 BC) underwent investigation by BSM, PCM, and SEM/EDX. Such methods enabled us to disclose the morphological details of the surface and the inner portions of the stones, along with their chemical compositions (ammonium acid urate and weddelite for Pandolfo; whewellite and hydroxylapatite for Popoli).

Background. Postmortem fat hydrolysis often results in breasts collapse of spontaneously mummified bodies. They remain recognizable in some circumstances but, to the best of our knowledge, paleopathological studies of the breast are extremely rare with only one case of lactational changes and one of fibroadenoma described in literature. Despite cancer fibrosis should be easily identified in mummified tissues, to date breast cancer has never been reported in ancient human remains.

Methods. A partially (50%) skeletonized mummy dating back to XX century was found in the San Michele Arcangelo church in Sermoneta (central Italy). The female subject was 40-50 years of age at death and her body measured 148 cm, showing good preservation of superficial structures of the inferior and left body region, including her left breast. The latter was entirely submitted to radiology, using a Senographe Essential digital mammograph (GE Medical Systems). Samples from the outer quadrants were rehydrated with Sandison solution, and routinely processed to obtain histological sections stained with hematoxylin-eosin, Masson’s trichrome, von Kossa, and red alizarin stains.

Results. Mammography showed diffuse microcalcifications of the outer breast quadrants, quite similar to those observed in modern patients affected by in situ proliferative lesions. Histology displayed collagen fibers diffusely colonized by fungal spores, and scattered roundish structures with focal calcium deposits.

No firm conclusion can be drawn about the significance of these latter findings, as they may be the result of a pathologic process or related to post-mortem/taphonomic phenomena. Nevertheless, the investigation methods of modern senology may allow an effective approach to ancient breast pathology, disclosing good morphological details in mummified breast specimens.

References

