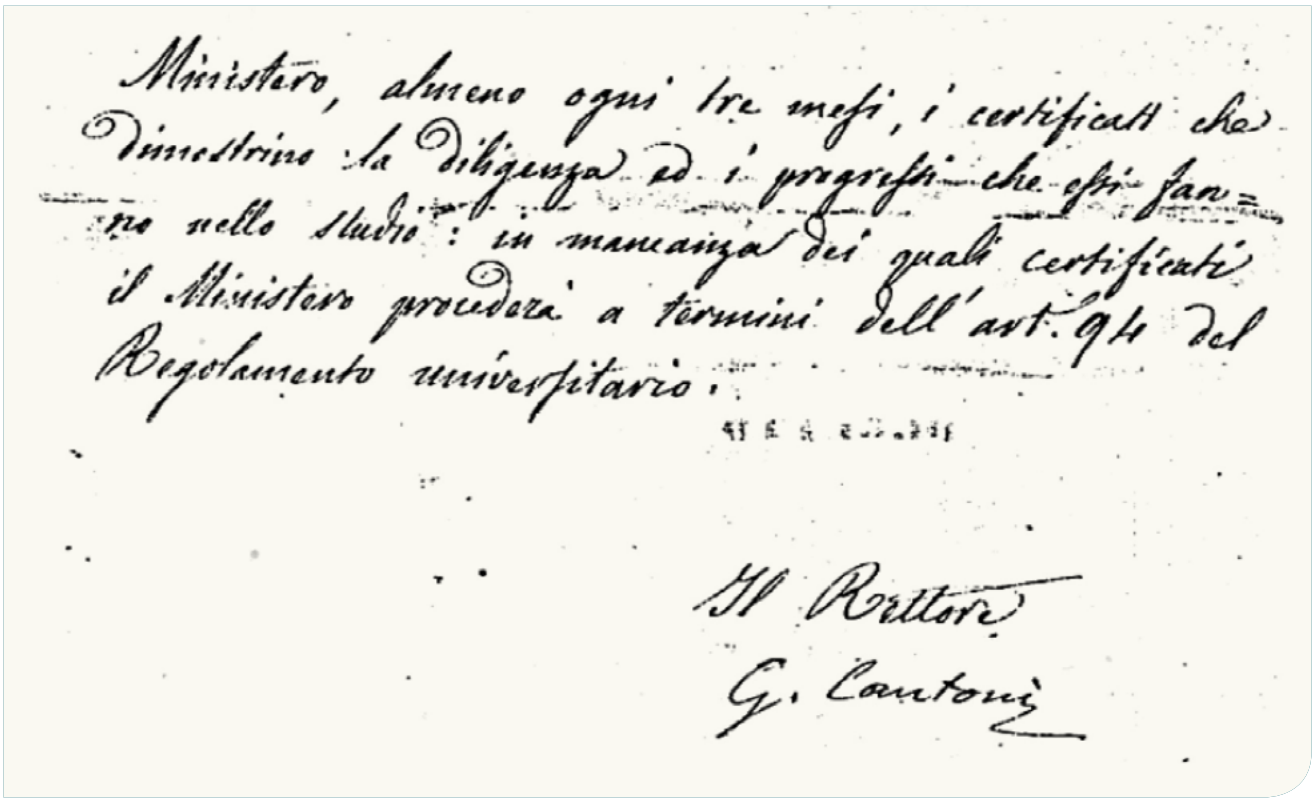
Ospedali Galliera di Genova

Cited in Index Medicus/MEDLINE, BIOSIS Previews, SCOPUS

PATHOLOGICA

JOURNAL OF THE ITALIAN SOCIETY OF ANATOMIC PATHOLOGY AND DIAGNOSTIC CYTOPATHOLOGY,
ITALIAN DIVISION OF THE INTERNATIONAL ACADEMY OF PATHOLOGY



In this issue:

Review

Practical approach to diagnosis of bland-looking spindle cell lesions of the breast

Historical Pathologica

Enrico Sertoli and the supporting cells of the testis. "*Morphology is function*"

Proceedings

V Meeting Nazionale Gruppo Italiano Paleopatologia

Case reports

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

04

VOL. 111

DECEMBER 2019

PACINI EDITORE MEDICINA

Periodico trimestrale - Aut. Trib. di Genova n. 75 del 22/06/1949 - ISSN: 1591-951X (Online)

Ospedali Galliera di Genova

Cited in Index Medicus/MEDLINE, BIOSIS Previews, SCOPUS

PATHOLOGICA

JOURNAL OF THE ITALIAN SOCIETY OF ANATOMIC PATHOLOGY AND DIAGNOSTIC CYTOPATHOLOGY, ITALIAN DIVISION OF THE INTERNATIONAL ACADEMY OF PATHOLOGY

04

Vol. 111

September 2019

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

PACINI EDITORE MEDICINA

Publisher

Pacini Editore S.r.l.
Via Gherardesca, 1
56121 Pisa, Italy
Tel. +39 050 313011
Fax +39 050 3130300
info@pacinieditore.it
www.pacinimedicina.it

UPDATED INFORMATION FOR AUTHORS INCLUDING EDITORIAL STANDARDS FOR THE PREPARATION OF MANUSCRIPTS

Pathologica is intended to provide a medium for the communication of results and ideas in the field of morphological research on human diseases in general and on human pathology in particular.
The journal welcomes contributions concerned with experimental morphology, ultrastructural research, immunocytochemical analysis, and molecular biology. Reports of work in other fields relevant to the understanding of human pathology may be submitted as well all papers on the application of new methods and techniques in pathology. The official language of the journal is Italian. Articles from foreign authors will be published in English.

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

by mail addressed to:

Pathologica – pathologica@pacinieditore.it

The manuscript must have the following enclosures:

1) The manuscript must be submitted by e-mail to the address: pathologica@pacinieditore.it
   The files containing the article, illustrations and tables must be sent in attachment and the statements of the Authors indicated at the previous points 2 and 3 must also be enclosed or sent by air mail.
2) A separate covering letter, signed by every Author, must state that the material submitted has not been previously published, and is not under consideration (in whole or in part) elsewhere, and that it is conform with the regulations currently in force regarding research ethics. The Authors are solely responsible for the statements made in their paper, and must state that they have obtained the informed consent of patients for their participation in the experiments and for the reproduction of photographs. For studies performed on laboratory animals, the authors must state that the relevant national laws or institutional guidelines have been adhered to. Only papers that have been prepared in strict conformity with the editorial norms outlined herein will be considered for publication. Their eventual acceptance is conditional upon a critical assessment by experts in the field, the implementation of any changes requested, and the final decision of the Editor-in-Chief.
3) Conflict of Interests. in the letter accompanying the article, Authors must declare if they got funds, or other forms of personal or institutional financing – or even if they are under contract – from Companies whose products are mentioned in the article. This declaration will be treated by the Editor-in-Chief as confidential, and will not be sent to the referees. Accepted works will be published accompanied by a suitable declaration, stating the source and nature of the financing.

Editorial standards for the preparation of manuscripts:

Pathologica will accept for publication only manuscript in English.
The article, in English, should be written in Microsoft Word™ preferably, saving files in .RTF, .DOC or .DOCX format. Any other programme can be used, including open source programmes: please always save files in .RTF, .DOC or .DOCX format.
Do not use, under any circumstances, graphical layout programmes such as Publisher™, Pacemaker™, Quark X-press™, Adobe Indesign™. Do not format the text in any way (avoid styles, borders, shading …); use only character styles such as italics, bold, underlined. Do not send the text in PDF.
Text and individual tables must be stored in separate files.
The article must include:
(1) a title (in English);
(2) an abstract (in English);
(3) a set of key words (in English);
(4) titles and legends for all of the tables and figures.
The Authors are required to correct and return (within 48 hours of their being sent) the first set of galley proofs of their paper.
On the first page of the manuscript should appear:
A concise *title*; a set of *key words* (no more than 5); the *names* of the authors and the *institution* or *organisation* to which each author is affiliated; the category under which the authors intend the work to be published (although the final decision here rests with the Editor-in- Chief); and the *name*, *mailing address*, and *telephone* and *fax numbers* of the author to whom correspondence and the galley proofs should be sent.
The second page should contain the abstract. At the end of the text should appear the bibliography, the legends to the tables and figures, and specification (where applicable) of the congress at which all or part of the data in the paper may have already been presented.

Tables

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

Figures

Send pictures in separate files from text and tables.
- Software and format: preferably send images in .TIFF or .JPEG format, resolution at least 300 dpi (100 x 150 mm). Will not be accepted for publication manuscript with images of bad quality.
The references must be limited to the most essential and relevant citations, identified in the text by Arabic numbers and listed at the end of the manuscript in the order in which they are cited. The format of the references in the bibliography section should conform with the examples provided in N Engl J Med 1997;336:309-15. The first six Authors must be indicated, followed by et al. Journals should be cited according to the abbreviations reported on Index Medicus.

Examples of the correct format for bibliographic citations:

Journal articles: Jones SJ, Boyede A. *Some morphological observations on osteoclasts*. Cell Tissue Res 1977;185:387-97.
Books: Taussig MJ. *Processes in pathology and microbiology*. Oxford: Blackwell 1984.
Chapters from books: Vaughan MK. *Pineal peptides: an overview*. In Reiter RJ (ed.). *The pineal gland*. New York: Raven 1984:39-81.

Acknowledgements and information on grants or any other forms of financial support must be cited at the end of the references. Notes to the text, indicated by an asterisk or similar symbol, should be shown at the bottom of the page.

Mathematical terms, formulae, abbreviations, units and measures should conform to the standards set out in Science 1954;120:1078.
Drugs should be referred to by their chemical name; the commercial name should be used only when absolutely unavoidable (capitalizing the first letter of the product name and giving the name of the pharmaceutical firm manufacturing the drug, town and country).
The editorial office accepts only papers that have been prepared in strict conformity with the general and specific editorial norms for each survey. The acceptance of the papers is subject to a critical revision by experts in the field, to the implementation of any changes requested, and to the final decision of the Editor in Chief.
The Authors are required to correct and return (within 3 days of their mailing) only the first set of galley proofs of their paper.
Authors may order reprints, at the moment they return the corrected proofs by filling in the reprint order form enclosed with the proofs.

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

Journal registered at “Registro pubblico degli Operatori della Comunicazione” (Pacini Editore srl registration n. 6269 - 29/8/2001).

Articles in open access and Publisher’s note available on-line: www.pathologica.it

Published by PACINI EDITORE, Pisa, Italy - December 2019

C O N T E N T S

REVIEW

Practical approach to diagnosis of bland-looking spindle cell lesions of the breast
G. Magro, L. Salvatorelli, L. Puzzo, E. Piombino, G. Bartoloni, G. Broggi, G.M. Vecchio 344

CASE REPORTS

Pleural chondroid metaplasia in a background of IgG4 plasma cell rich chronic inflammatory infiltrate: report of a rare case with review of literature
M. Spinelli, A. Datta, M.A. Montero Fenandez, P. Viola 361

Malignant peripheral nerve sheath tumor of the bladder. A case report
G. Petracco, C. Patriarca, R. Spasciani, A. Parafioriti 365

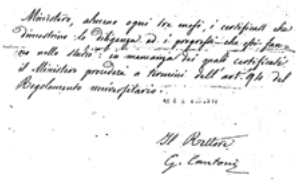
Eosinophilic cytoplasmic inclusions in type 2 papillary renal cell carcinoma
M. Ungari, M. Trombatore, G. Ferrero, M.D. Gusolfino, L. Manotti, G. Tanzi, E. Varotti, R. Bertoni 369

PATHOLOGICA STORICA

Enrico Sertoli and the supporting cells of the testis. "*Morphology is function*"
C. Patriarca, M. Colecchia, C.A. Clerici 375

PROCEEDINGS

V Meeting Nazionale Gruppo Italiano Paleopatologia 382



Front cover: Excerpt from a letter written by the Rector G. Cantoni, dated 18 October 1865. (page 377).

PATHOLOGICA 2019;111:344-360; doi: 10.32074/1591-951X-31-19

Review

Practical approach to diagnosis of bland-looking spindle cell lesions of the breast

G. Magro[1], L. Salvatorelli[1], L. Puzzo[1], E. Piombino[1], G. Bartoloni[2], G. Broggi[1], G.M. Vecchio[1]

[1] Department of Medical and Surgical Sciences and Advanced Technologies, G.F. Ingrassia, Azienda Ospedaliero-Universitaria "Policlinico Vittorio Emanuele", Anatomic Pathology, School of Medicine, University of Catania, Italy; [2] Anatomic Pathology, A.R.N.A.S. Garibaldi-Nesima, Catania, Italy

Summary

The diagnosis of bland-looking spindle cell lesions of the breast is often challenging because there is a close morphological and immunohistochemical overlap among the different entities. The present review will discuss reactive spindle cell nodule/exuberant scar, nodular fasciitis, inflammatory pseudotumor, myofibroblastoma (classic type), lipomatous myofibroblastoma, palisaded myofibroblastoma, benign fibroblastic spindle cell tumor, spindle cell lipoma, fibroma, leiomyoma, solitary fibrous tumor, myxoma, schwannoma/neurofibroma, desmoid-type fibromatosis, dermatofibrosarcoma protuberans, low-grade fibromatosis-like spindle cell carcinoma, inflammatory myofibroblastic tumor and low-grade myofibroblastic sarcoma arising in the breast parenchyma. The pathologist should be aware of each single lesion to achieve a correct diagnosis to ensure patient a correct prognostic information and therapy. Accordingly representative illustrations and morphological/immunohistochemical diagnostic clues will be provided.

Key words

Spindle cell tumors • Breast parenchyma • Differential diagnosis• Diagnostic approach

Introduction

Bland-looking spindle cell lesions of the breast comprise a heterogeneous group of tumor-like and tumor entities, ranging from reactive to low-grade malignant neoplasms with metastatic potential [1]. (Tab. I). Accordingly, differential diagnosis between the benign spindle cell lesions and the potentially aggressive tumors is mandatory to avoid overdiagnosis and overtreatment. However, this distinction is often challenging in daily practice, especially in needle core biopsies, due to the morphological and immunohistochemical overlap exhibited by the different lesions that often share bland-looking spindle cells with the morphological features of fibroblasts/myofibroblasts, arranged haphazardly or in short fascicles or with focal storiform growth pattern, and set in a variable fibro-myxoid stroma. Pathologists should be aware

Tab. I. Bland-looking spindle cell lesions of the breast.

Reactive lesions	
- Reactive Spindle Cell Nodule/Exuberant Scar - Nodular Fasciitis - Inflammatory Pseudotumor	
Benign tumors	
Specific to mammary stroma	**Not specific to mammary stroma**
- Myofibroblastoma, classic-type - Myofibroblastoma, lipomatous variant - Myofibroblastoma, palisaded variant - Benign Fibroblastic Spindle Cell Tumor	- Leiomyoma - Schwannoma/Neurofibroma - Spindle cell lipoma - Solitary fibrous tumor - Myxoma - Fibroma
Low-grade tumors, locally aggressive	
- Desmoid-type fibromatosis - Dermatofibrosarcoma protuberans	
Low-grade tumors with metastatic potential	
- Low-grade fibromatosis-like spindle cell carcinoma - Low-grade myofibroblastic sarcoma - Inflammatory Myofibroblastic Tumor	

How to cite this article: Magro G, Salvatorelli L, Puzzo L, et al. *Practical approach to diagnosis of bland-looking spindle cell lesions of the breast.* Pathologica 2019;111:344-60. https://doi.org/10.32074/1591-951X-31-19.

Correspondence: Gaetano Magro, Department of Medical and Surgical Sciences and Advanced Technologies, G.F. Ingrassia, Section of Anatomic Pathology, University of Catania - santa Sofia 87 street, 95123 Catania, Italy - Tel. +39095 3782022 - Fax +39095 3782023 - E-mail: g.magro@unict.it

of the morphological and immunohistochemical differences between fibroblasts and myofibroblasts when dealing with a spindle cell lesion of the breast. Recognizing a lesion as predominantly fibroblastic or myofibroblastic in nature may help in the diagnostic approach. Fibroblastic lesions are mainly or entirely composed of elongated spindle cells with scant, pale to slightly eosinophilic cytoplasm, elongated nuclei with absent or only inconspicuous nucleoli. Fibroblasts are usually stained with vimentin and CD34, generic mesenchymal markers lacking any specificity of differentiation cell lineage. Focal and weak staining with α-smooth muscle actin can be seen. Conversely myofibroblasts -modified fibroblasts with the capability to contract- are plumper than fibroblasts, showing more abundant slightly to deeply eosinophilic cytoplasm and ovoid nuclei with evident small nucleoli. Unlike fibroblasts, myofibroblasts exhibit a more diffuse and strong staining for α-smooth muscle actin; some lesions, like myofibroblastoma, are typically stained with desmin more than with α-smooth muscle actin. Finally, the pathologist should be aware that the neoplastic cells of the breast carcinoma, as like in other carcinomas, may adopt a spindled morphology raising confusion with benign/low-grade mesenchymal lesions. This phenomenon is related to an epithelial-mesenchymal transition, i.e. a biologic process due to plasticity of the cells, that consists in the progressive loss of epithelial morphological and immunohistochemical features and gain of a mesenchymal cell profile, including the expression of vimentin and α-smooth muscle actin. Low-grade, fibromatosis-like spindle cell carcinoma is a prototypical example of an epithelial-mesenchymal transition [10].

In our opinion, the tumor-like and tumor spindle cell lesions of the breast are often underrecognized, with

Tab. II. Key diagnostic features.

Reactive Spindle Cell Nodule/Exuberant Scar: circumscribed and, at least focally, infiltrative margins; previous biopsy/FNA; α-smooth muscle actin-positive spindle cells; foamy and hemosiderin-laden macrophages, lymphocytes and foreign body giant cells; fat necrosis
Nodular Fasciitis: circumscribed and, at least focally, infiltrative margins; α-smooth muscle actin-positive spindle cells; fibro-myxoid stroma, at least focally, with tissue culture-like appearance
Inflammatory Pseudotumor: circumscribed and, at least focally, infiltrative margins; α-smooth muscle actin-positive spindle cells closely intermingling with lymphocytes and plasma cells; previous history of local trauma/stimuli; ALK-1 is negative
Myofibroblastoma, Classic-type: circumscribed margins; desmin/CD34/α-smooth muscle actin-positive spindle cells; short intersecting fascicles interrupted by keloid-like collagen fibers
Lipomatous Myofibroblastoma: circumscribed margins; desmin/CD34/α-smooth muscle actin-positive spindle cells with finger-like pseudo-infiltration into an intratumoral lipomatous component
Palisaded/Schwannian Myofibroblastoma: circumscribed margins; desmin/CD34/α-smooth muscle actin-positive spindle cells with formation of Verocay-like bodies; S100 protein is negative
Benign Fibroblastic Spindle Cell Tumor: circumscribed margins; CD34-positive fibroblastic-like spindle cells; short intersecting fascicles; thick keloid-like collagen fibers; variable additional lipomatous component
Spindle Cell Lipoma: circumscribed margins; CD34-positive short spindle cells with bipolar cytoplasmic processes; variably admixed mature lipomatous component; at least focally, myxoid stroma with ropey collagen fibers
Fibroma: circumscribed margins; hypocellular, fibrosclerotic nodule with interspersed CD34-positive fibroblast-like spindle cells
Solitary Fibrous Tumor: circumscribed margins; CD34/STAT6-positive fibroblast-like spindle cells, haphazardly arranged (pattern-less growth pattern); branching vessels, often with perivascular hyalinization
Leiomyoma: circumscribed margins; interlacing fascicles of desmin/α-smooth muscle actin/h-caldesmon-positive spindle cells with the features of mature smooth muscle cells_
Myxoma: circumscribed margins; vimentin positive spindle to stellate cells embedded in an abundant myxoid stroma; atypical bizarre cells, along with thick keloid-like collagen fibers, can be seen
Schwannoma/Neurofibroma: circumscribed margins; S100-positive spindle cells with formation of Verocay-bodies and alternating Antoni A and Antoni B areas (schwannoma); cells with wavy nuclei set in myxoid stroma with keloid-like collagen fibers (neurofibroma)
Desmoid-type Fibromatosis: finger-like infiltrative margins**;** α-smooth muscle actin and β-catenin-positive fibroblast/myofibroblast-like spindle cells arranged into long intersecting fascicles; the cells are often aligned parallel and are separated by collagenized stroma
Dermatofibrosarcoma Protuberans: circumscribed and, at least focally, infiltrative margins; CD34-positive fibroblast-like spindle cells; diffuse storiform growth pattern; low mitotic activity; finger-like or honeycomb infiltration of the adjacent fibro-fatty tissue
Low-grade, Fibromatosis-like Spindle Cell Carcinoma: finger-like infiltrative margins; p63/cytokeratin-positive spindle cells with the features of fibroblasts/myofibroblasts; at least focally, small cohesive clusters of cytokeratin/p63-positive epithelioid-polygonal cells
Inflammatory Myofibroblastic Tumor: circumscribed and, at least focally, infiltrative margins; α-smooth muscle actin-positive spindle cells admixed with lymphocytes and plasma cells; no association with previous history of local trauma/stimuli; ALK-1 expression in about 40-50% of cases
Low-grade Myofibroblastic Sarcoma: circumscribed and, at least focally, infiltrative margins; α-smooth muscle actin-positive myofibroblastic-like cells with mild/moderate nuclear pleomorphism and high mitotic activity (7 to 35 mitoses x 10 HPF); fascicular arrangement

Tab. III. Differential diagnoses between benign versus low-grade lesions.

Nodular fasciits *versus* Desmoid-type Fibromatosis
Shared features: at least focally, infiltrative margins with entrapment of mammary ducts/lobules; α-smooth muscle actin-positive spindle cells in a variable fibro-myxoid stroma
Distinguishing features: desmoid-type fibromatosis shows long intersecting fascicles with cells aligned parallel, whereas nodular fasciitis exhibits cells haphazardly arranged or forming short fascicles with focal storiform growth pattern; unlike nodular fasciitis, desmoid-type fibromatosis is usually stained with β-catenin (nuclear staining)
Lipomatous Myofibroblastoma *versus* Desmoid-type Fibromatosis
Shared features: α-smooth muscle actin-positive spindle cells in a fibrous stroma, with finger-like extension into mature adipose tissue
Distinguishing features: desmoid-type fibromatosis exhibits infiltrative margins, whereas lipomatous myofibroblastoma shows pushing borders; adipose tissue is an integral part of the lipomatous myofibroblastoma, whereas adipose tissue in desmoid-type fibromatosis is mammary fat infiltrated by neoplastic cells; lipomatous myofibroblastoma is stained with desmin, CD34 and estrogen/progesterone receptors, whereas desmoid-type fibromatosis is negative to these markers, but positive for β-catenin
Classic-type Myofibroblastoma *versus* Low-grade Myofibroblastic Sarcoma
Shared features: circumscribed borders, α-smooth muscle actin-spindle cells arranged in short fascicles with variable fibro-myxoid stroma
Distinguishing features: low-grade myofibroblastic sarcoma is more cellular and the spindle cells show, at least focally, moderate nuclear pleomorpshism, nuclear overlapping, as well as high mitotic activity (7 to 35 mitoses x 10 HPF); myofibroblastoma is a tumor with absent to low mitotic activity (up to 2 mitoses x 10HPF), that variably co-expresses desmin, CD34 and estrogen/progesterone receptors
Desmoid-type Fibromatosis *versus* Low-grade Fibromatosis-like Spindle Cell Carcinoma
Shared features: infiltrative margins with entrapment of mammary ducts/lobules and fat; α-smooth muscle actin-positive spindle cells set in a fibrous stroma
Distinguishing features: desmoid-type fibromatosis shows long intersecting fascicles with cells often aligned parallel, but lacks pancytokeratins/p63-positive spindle to focally epithelioid-polygonal cells arranged in small cohesive clusters

confusion in the distinction between reactive *versus* neoplastic (benign or low-grade malignant) lesions. Although some difficulties are due the fact that different names are often used to indicate the same entity, it is also true that a single name is applied to biologically different lesions. In addition, we think that potential diagnostic errors are likely to occur because the pathologist: i) is faced with an *unfamiliar lesion (myofibroblastoma; low-grade myofibroblastic sarcoma; low-grade fibromatosis-like spindle cell carcinoma); ii) pathologist is not familiar with soft tissue pathology (nodular fasciitis; desmoid-type fibromatosis; solitary fibrous tumor; low-grade myofibroblastic sarcoma); iii) may encounter diagnostic difficulties when dealing with a typical soft tissue lesion/tumor occurring in an unexpected site, such as in the breast (nodular fasciitis, desmoid-type fibromatosis, solitary fibrous tumor, leiomyoma, schwannoma, spindle cell lipoma).* The present overview focuses on the morphological and immunohistochemical features helpful to recognize each single entity in the wide spectrum of the bland-looking spindle cell lesions of breast parenchyma (Tab. II). Representative illustrations along with the main diagnostic clues are provided (Tab. III).

Reactive lesions

Reactive spindle cell nodule/exuberant scar [11-15]

It should be suspected in presence of a fairly circumscribed nodule, arising after biopsy/FNAC or surgical procedures, composed of α-smooth muscle actin-positive spindle cells with the features of myofibroblasts, set in a variably fibro-myxoid stroma containing both foamy and hemosiderin-laden macrophages, lymphocytes and foreign body giant cells (Fig. 1A). Fat necrosis and entrapment or displacement of normal mammary glands/ducts are frequently encountered (Fig. 1A,B). The reactive spindle cells may be, at least focally, arranged into short fascicles (Fig. 1C), focally exhibiting a storiform growth pattern. Mitotic activity is low, ranging from 1 to 4 mitoses x 10 high power fields.

Nodular fasciitis [16-20]

It should be suspected in presence of a nodule with partially circumscribed margins, composed of a proliferation of α-smooth muscle actin-positive spindle cells with the features of myofibroblasts and brisk mitotic activity; the cells are arranged into short, not well-formed fascicles and focally in whorls or storiform growth pattern (Fig. 2A). The stroma, variably

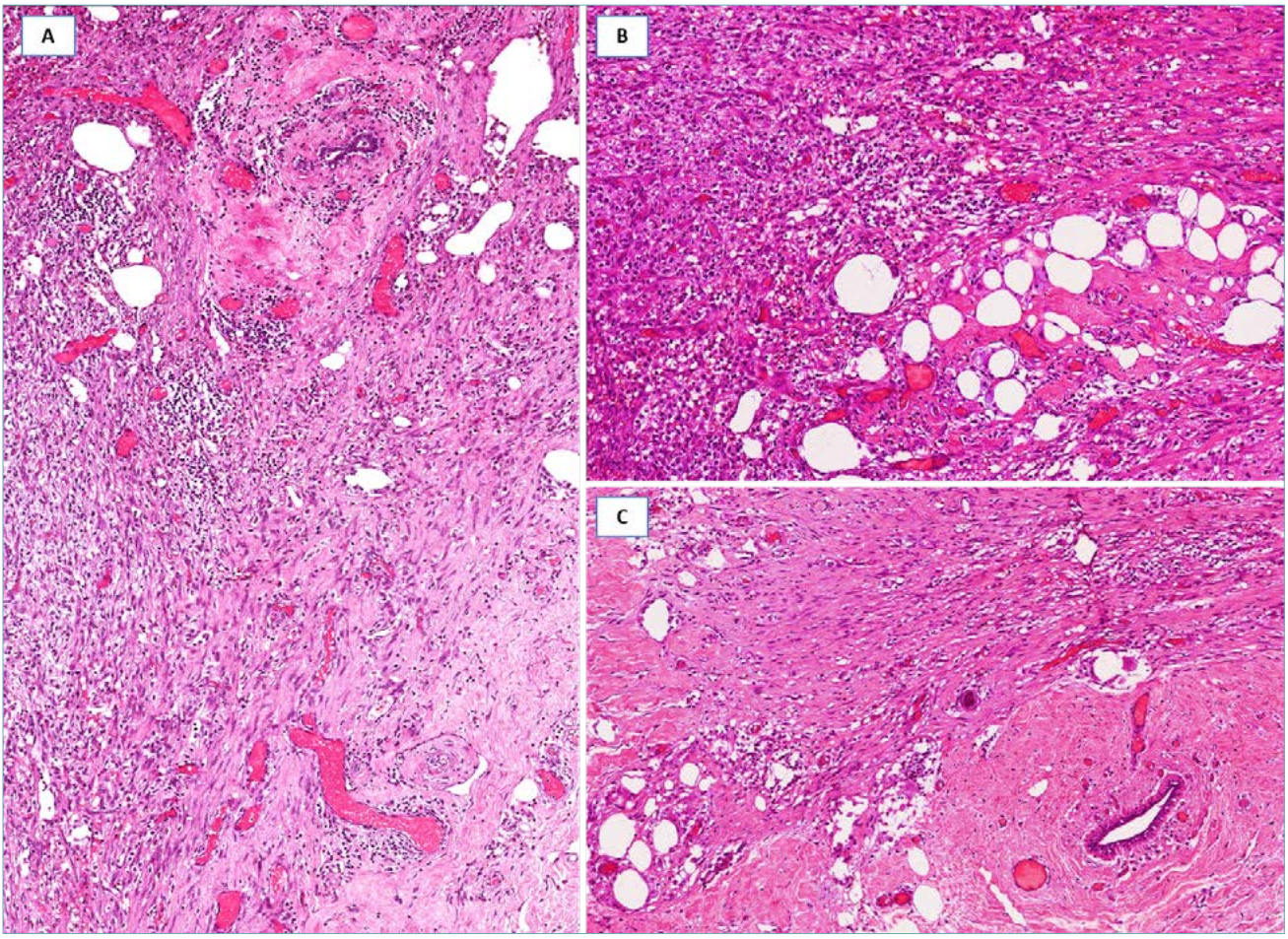


Fig. 1. Reactive spindle cell nodule/exuberant scar. (A) Fibro-inflammatory tissue with spindle cells; (B) fat necrosis is a diagnostic clue; (C) fibro-sclerotic stroma with interspersed spindle cells, entrapping a mammary duct.

myxoid with microcystic degeneration to fibrous in nature (Fig. 2B) and containing red blood cells and lymphocytes, shows, at least focally, a tissue culture-like morphology (Fig. 2C). Mammary ducts/lobules can be entrapped, especially at the periphery of the lesion (Fig. 2A). Surgical excision is curative with rare local recurrence (< 2% of cases, including lesions incompletely excised).

Inflammatory pseudotumor [21-27]

It should be suspected in the presence of a fairly circumscribed nodule arising in association with local trauma/stimuli; it is composed of α-smooth muscle actin-positive spindle cells with the features of myofibroblasts, closely intermingling with lymphocytes and plasma cells (Fig. 3A, B); cells are usually arranged in interlacing short bundles (Fig. 3C) or may exhibit swirling/storiform growth pattern; atypical/bizarre mono- or multi-nucleated cells can be, at least focally, encountered (Fig. 3D); defining the boundaries between a reactive (inflammatory pseudotumor) *versus* a true neoplastic process (inflammatory myofibroblastic tumor) still remains to be established. Surgical excision is curative.

Benign tumors

Myofibroblastoma, classic-type [28 29]

It should be suspected in presence of a well-circumscribed nodule (Fig. 4A) composed of a proliferation of desmin/CD34/α-smooth muscle actin-positive spindle cells with the features of myofibroblasts, arranged into short, haphazardly intersecting fascicles interrupted by thick keloid-like collagen bands (Fig. 4B). Focal storiform or neural-like growth patterns can be seen, including a minor component of neoplastic

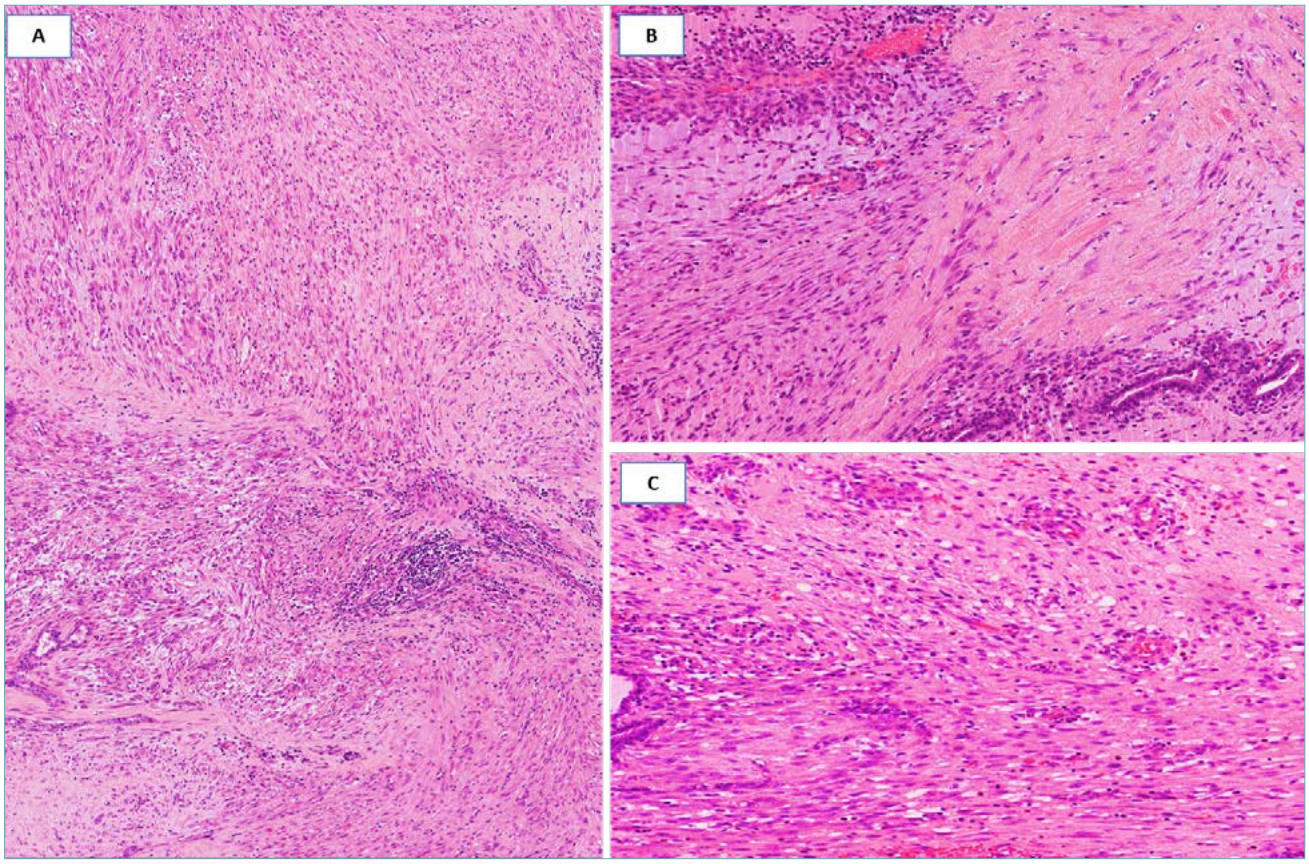


Fig. 2. Nodular fasciitis. (A) Spindle cell proliferation with fibrous stroma and entrapped mammary ducts at the periphery of the lesion; (B) area with myxo-edematous stroma containing inflammatory cells (tissue culture-like appearance); (C) extravasated erythrocytes can be seen.

cells with epithelioid morphology. Mitotic activity is low (0-2 mitoses x 10 high power field). The stroma is usually fibrous to focally myxoid and may contain islands of mature adipose tissue. Mast cells are variably interspersed among neoplastic cells. Usually no entrapment of mammary ducts/lobules is seen. Surgical excision is curative.

Lipomatous myofibroblastoma [30-33]

It should be suspected in presence of a well-circumscribed fibro-fatty nodule (Fig. 5A), composed of a proliferation of desmin/CD34/α-smooth muscle actin-positive spindle cells with the features of myofibroblasts that exhibit a finger-like pseudo-infiltration into an intratumoral lipomatous component (Fig. 5B). Although this pattern is reminiscent of desmoid-type fibromatosis or low-grade (fibromatosis-like) spindle cell carcinoma, tumor margins are pushing and not infiltrative (Fig. 5B); areas with the typical features of myofibroblastoma are identified, at least focally (Fig. 5C). Surgical excision is curative.

Palisaded myofibroblastoma [34-36]

It should be suspected in presence of a well-circumscribed nodule, histologically reminiscent of schwannoma (Fig. 6A). It is composed of desmin/CD34/α-smooth muscle actin-positive spindle cells exhibiting a prominent nuclear palisading, with formation of numerous Verocay-like bodies: two compact rows of well aligned nuclei separated by myxoid matrix (Fig. 6A-C). Mitotic activity is low (0-2 mitoses x 10 high power field). As in other myofibroblastoma variants, keloid-like eosinophilic collagen fibers are dispersed throughout the myxoid stroma and between neoplastic cells; areas with the typical features of myofibroblastoma can be seen, at least focally. Surgical excision is curative.

Benign fibroblastic spindle cell tumor [37-44]

It should be suspected in presence of a well-circumscribed nodule (Fig. 7A) composed of a proliferation of CD34-positive spindle cells with the features of fibro-

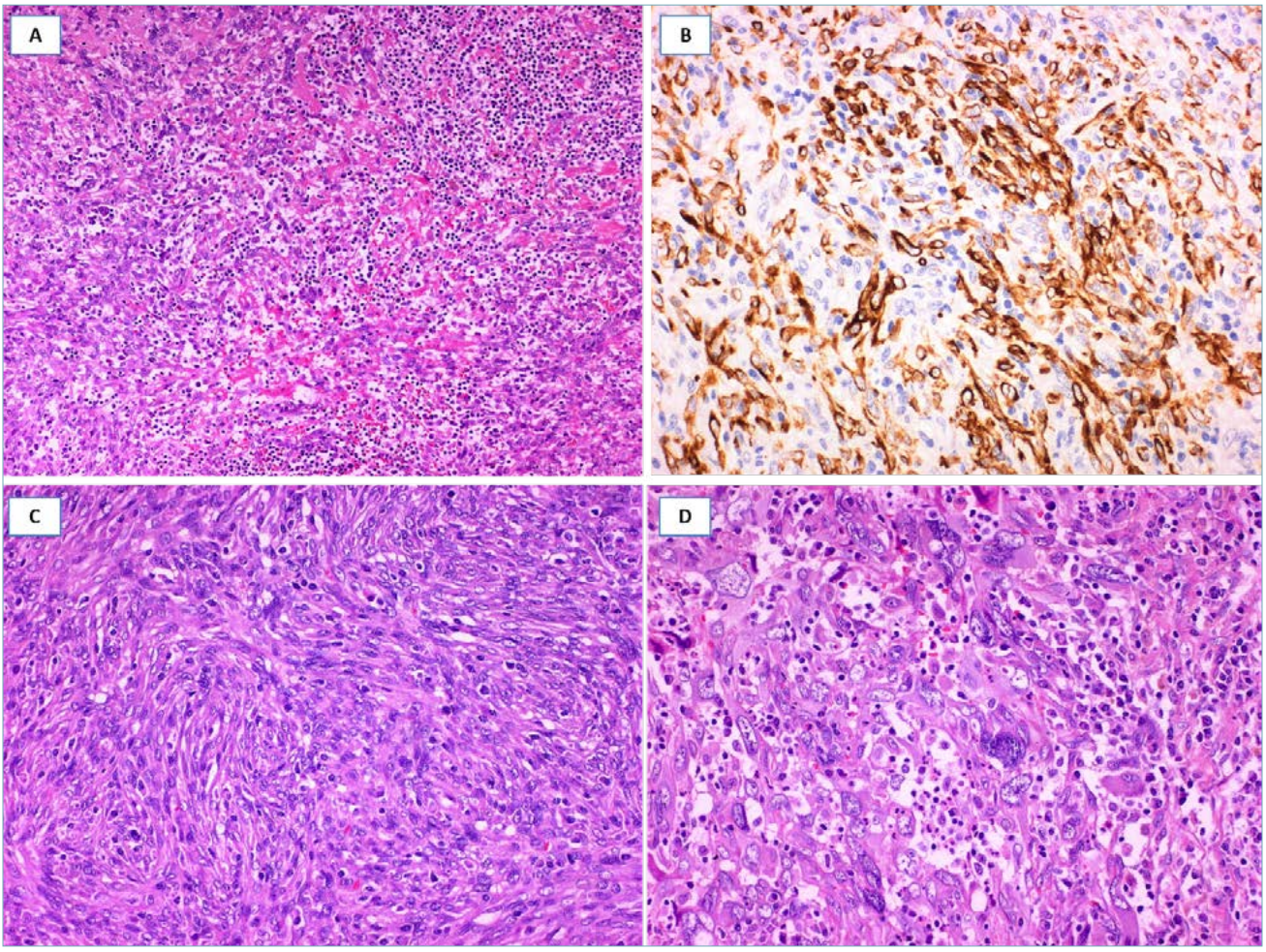


Fig. 3. Inflammatory pseudotumor (male patient with local breast trauma). (A) Spindle cells intermingling with inflammatory cells; (B) spindle cells are stained with α-smooth muscle actin; (C) spindle cells are arranged in a swirling growth pattern; (D) atypical/bizarre cells can be seen.

blasts arranged haphazardly, or in intersecting short fascicles with interspersed keloid-like collagen fibers (Fig. 7B); mitoses are absent to low (0-2 mitoses x 10 high power field). The stroma is collagenized and may contain a prominent lipomatous component (spindle cell lipoma-like morphology). Surgical excision is curative.

Spindle cell lipoma [45-48]

It should be suspected in presence of a circumscribed nodule composed of a proliferation of CD34-positive, short spindle cells, variably admixed with mature adipocytes (Fig. 8A) and set in, at least focally, myxoid stroma containing ropey collagen fibers (Fig. 8B). In the myxoid areas the spindle cells often show long and thin bipolar cytoplasmic processes (Fig. 8B). Mitoses are absent or rare; mast cells are variably scattered throughout the tumor. Surgical excision is curative.

Fibroma [43 49]

It should be suspected in presence of a well-circumscribed, hypocellular nodule (Fig. 9A) composed of CD34-positive spindle cells with the features of fibroblasts, haphazardly dispersed in a heavily collagenized stroma (Fig. 9B) in which mammary ducts/lobules can be entrapped; mitotic activity is absent. Surgical excision is curative.

Leiomyoma [50-53]

It should be suspected in presence of a well-circumscribed nodule (Fig. 10A) composed of interlacing fascicles of desmin/α-smooth muscle actin/h-caldesmon-positive spindle cells with the features of mature

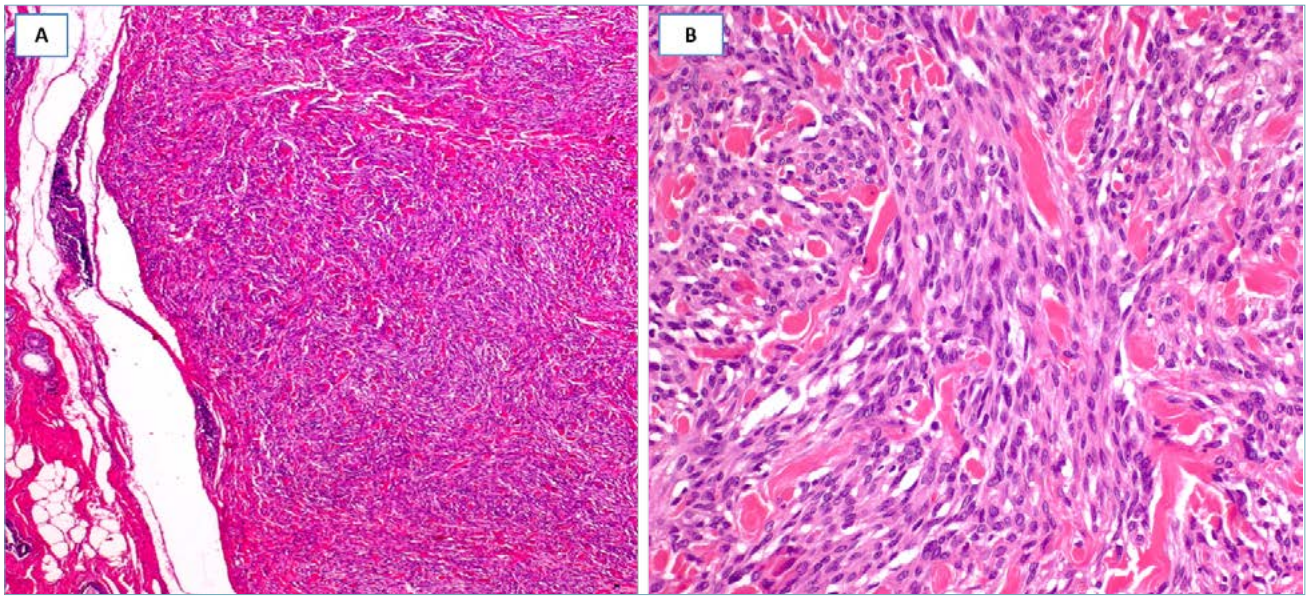


Fig. 4. Myofibroblastoma, classic-type. (A) A spindle cell tumor with pushing margins and numerous keloid-like collagen fibers; (B) cells, with eosinophilic cytoplasm and oval nuclei, are arranged in short fascicles with interspersed keloid-like collagen fibers.

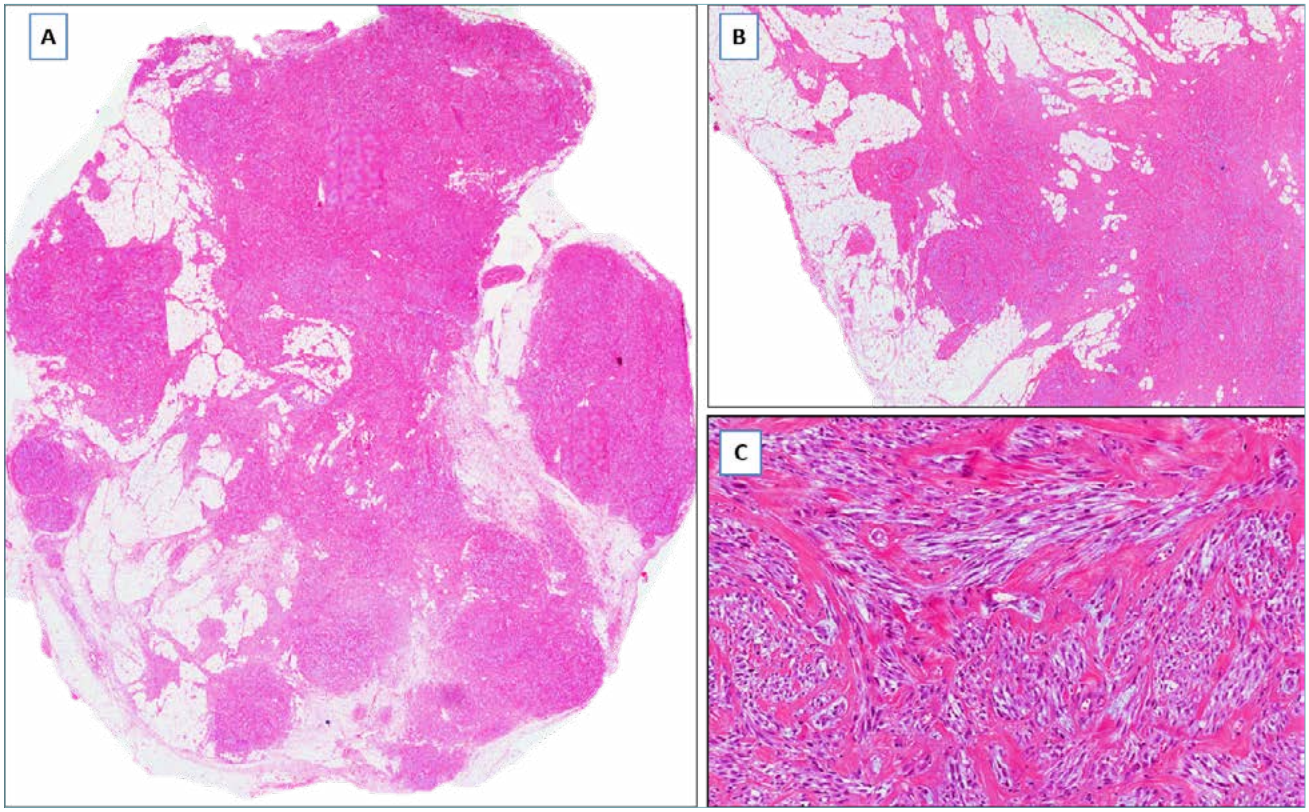


Fig. 5. Lipomatous myofibroblastoma. (A) Fibrolipomatous tumor with pushing borders; (B) the fibrous component exhibits a finger-like infiltration into the lipomatous component, but the margins are circumscribed; (C) tumor area with the characteristics of classic-type myofibroblastoma: fascicles of spindle cells separated by keloid-like collagen bands.

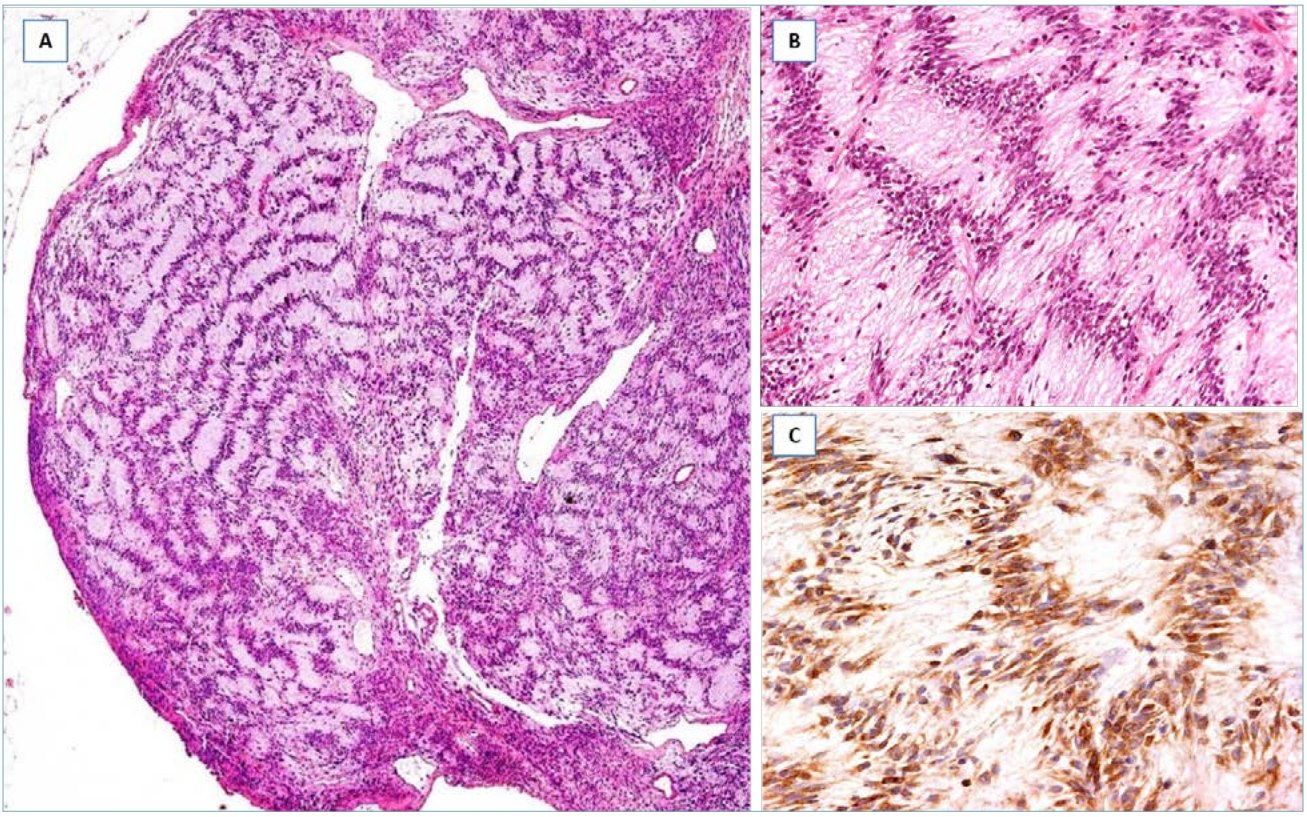


Fig. 6. Palisaded/Schwannoma-like Myofibroblastoma. (A) Tumor with pushing borders, closely reminiscent of Schwannoma; (B) higher magnification showing nuclear palisading with formation of Verocay-like bodies; (C) cells, negative to S100 protein, are stained with α-smooth muscle actin, revealing their myofibroblastic nature.

smooth muscle cells (deeply eosinophilic cytoplasm with elongated nuclei with blunt ends) (Fig. 10B); absent to low mitotic activity. Surgical excision is curative.

Solitary fibrous tumor [43 44 54-61]

It should be suspected in presence of a well-circumscribed nodule (Fig. 11A) composed of CD34/STAT6-positive spindle cells with the features of fibroblasts, low mitotic activity (<4 mitoses x 10 HPF), and haphazardly arranged in a fibrous to focally myxoid stroma containing branching vessels, often with perivascular hyalinization (Fig. 11B, C). Surgical excision is curative. Pathologists should always search for morphological features that can be associated with an aggressive clinical course, including >4 mitoses x 10 HPF, nuclear pleomorphism, hypercellularity, necrosis, sarcomatous dedifferentiation.

Myxoma [62-66]

It should be suspected in presence of a well-circumscribed nodule (Fig. 12A) composed of vimentin-positive spindle- to stellate-shaped cells dispersed in abundant/exclusive myxoid stroma (Fig. 12B); stromal microcystic spaces simulating lipoblasts are seen. Atypical/bizarre cells, as well as keloid-like collagen fibers, can be occasionally observed (Fig. 12C). Mitotic activity is absent. Surgical excision is curative.

Schwannoma/neurofibroma [51 67-76]

It should be suspected in presence of a nodular mass with circumscribed margins, composed of S100-positive spindle cells with wavy nuclei and absent to low mitotic activity; schwannoma shows interlacing fascicles and whorls, as well as palisading nuclei (Verocay bodies) and alternating hypercellular (Antoni A areas) and hypocellular (Antoni B) areas; in neurofibroma the spindle cells are usually haphazardly arranged in a slightly myxoid stroma containing thick collagen fibers. Surgical excision is curative.

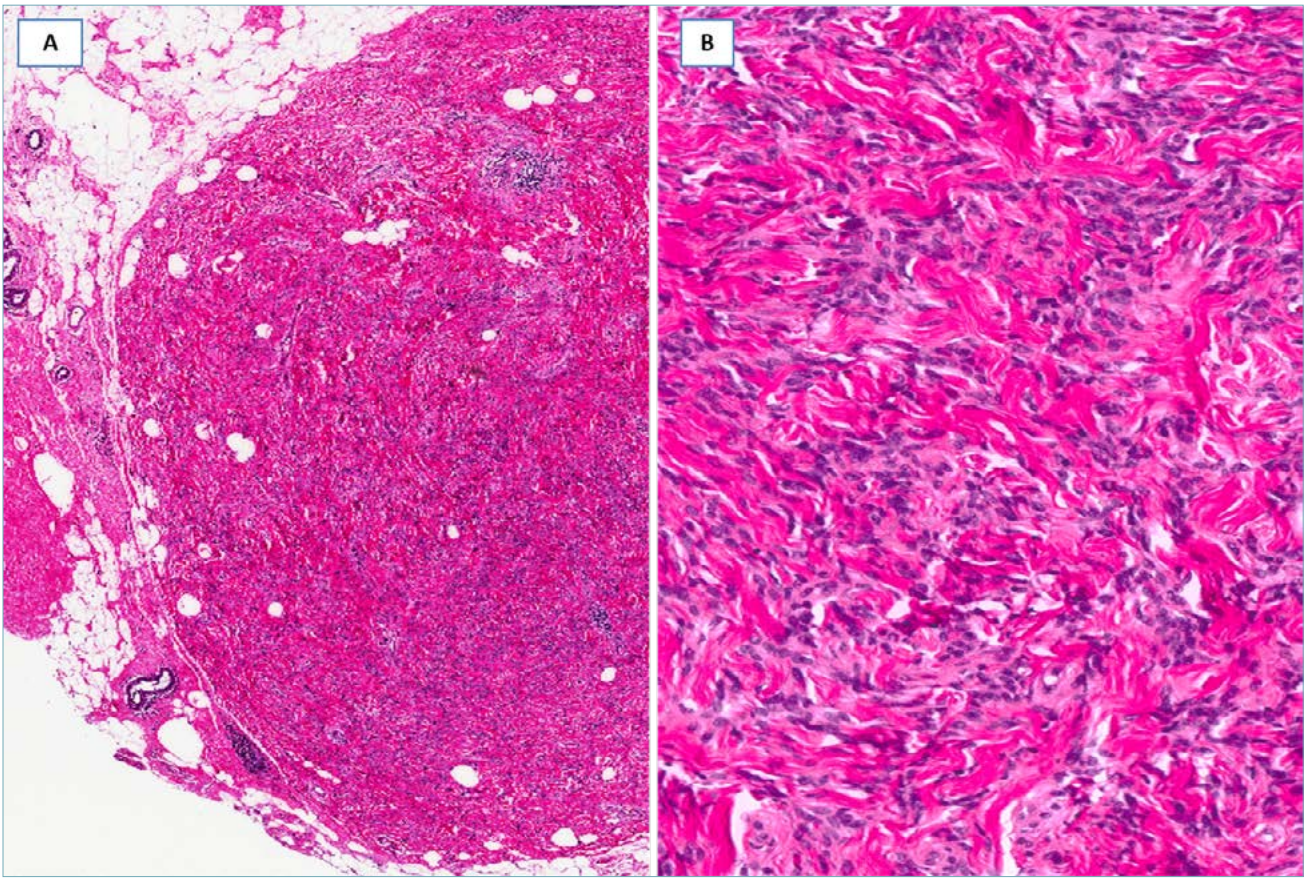


Fig. 7. Benign fibroblastic spindle cell tumor. (A) A fibrous tumor with circumscribed borders; (B) spindle cells look like fibroblasts and are arranged in short fascicles set in a collagenized stroma.

Low-grade tumors, locally aggressive

Desmoid-type fibromatosis[77-82]

It should be suspected in presence of a nodular mass with finger-like infiltrative margins (Fig. 13A), composed of spindle cells with the features of both fibroblasts and myofibroblasts Characteristically the neoplastic cells, often aligned parallel, are arranged in long and sweeping fascicles set in a prominent fibrous to focally myxoid stroma (Fig. 13B,C). Mitoses are rare. These cells are variably stained with α-smooth muscle actin and β-catenin (nuclear staining in about 80% of cases) (Fig. 13C). Desmoid-type fibromatosis is a locally aggressive tumor that can recur locally but with no metastatic potential.

Dermatofibrosarcoma protuberans [83-87]

It should be suspected in presence of a nodular mass with relatively circumscribed margins, composed of CD34-positive spindle cells with the features of fibroblasts, low mitotic activity, and diffusely arranged in a storiform growth pattern with finger-like or honeycomb infiltration of the adjacent fibro-fatty tissue (Fig. 14 A-C). Radical excision is curative. Local recurrence is usually due to incomplete surgical excision.

Low-grade tumors with metastatic potential

Low-grade fibromatosis-like spindle cell carcinoma [88-89]

It should be suspected in presence of a nodular mass with finger-like infiltrative margins (Fig. 15A), composed of p63/cytokeratin-positive spindle cells (Fig. 15B) and low mitotic activity; variable co-expression of α-smooth muscle actin can be seen. The identification, at least focally, of epithelioid-polygonal cells arranged in small cohesive clusters (Fig. 15C), better highlighted by immunohistochemistry (Fig. 15D) is the

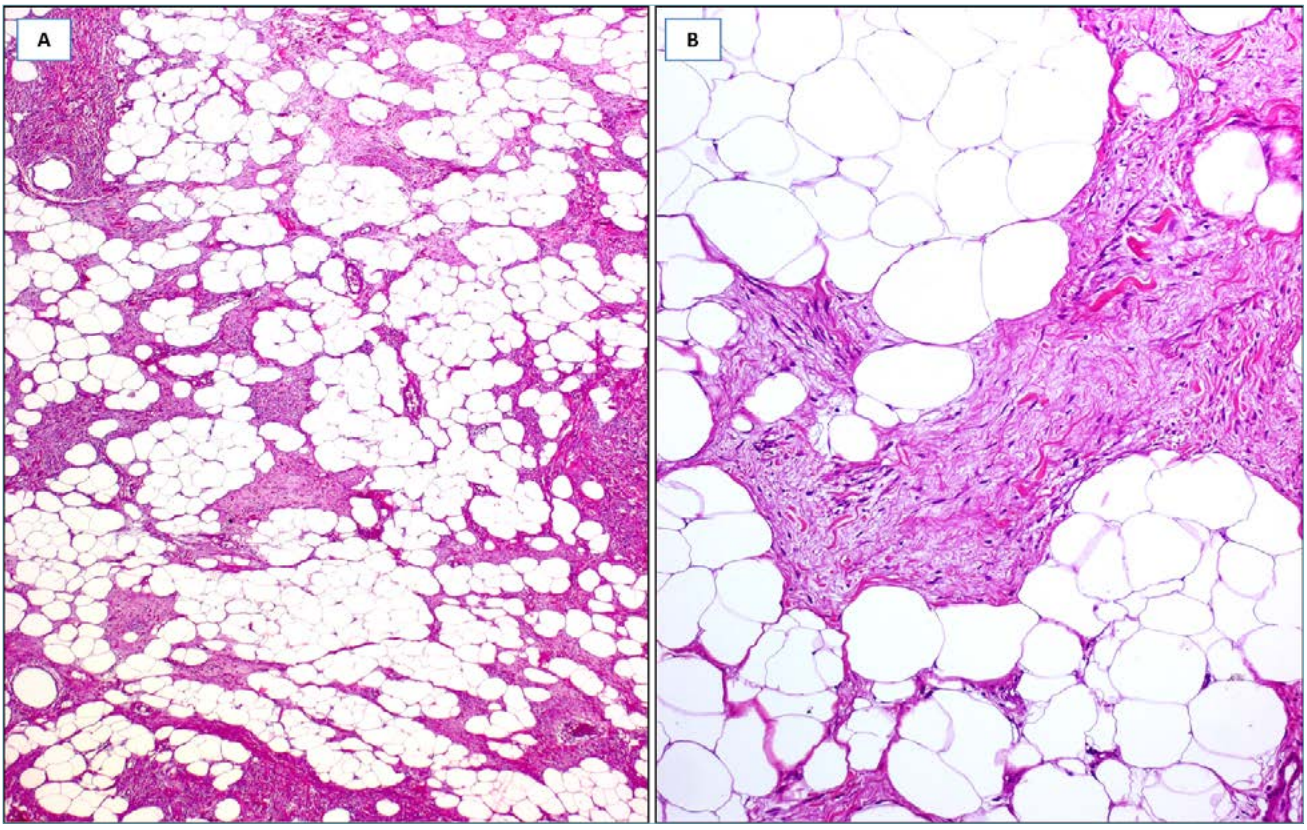


Fig. 8. Spindle cell lipoma. (A) A fatty-tumor with interspersed fibro-myxoid areas; (B) higher magnification: myxoid area showing spindle cells with long cytoplasmic bipolar processes and ropey collagen fibers.

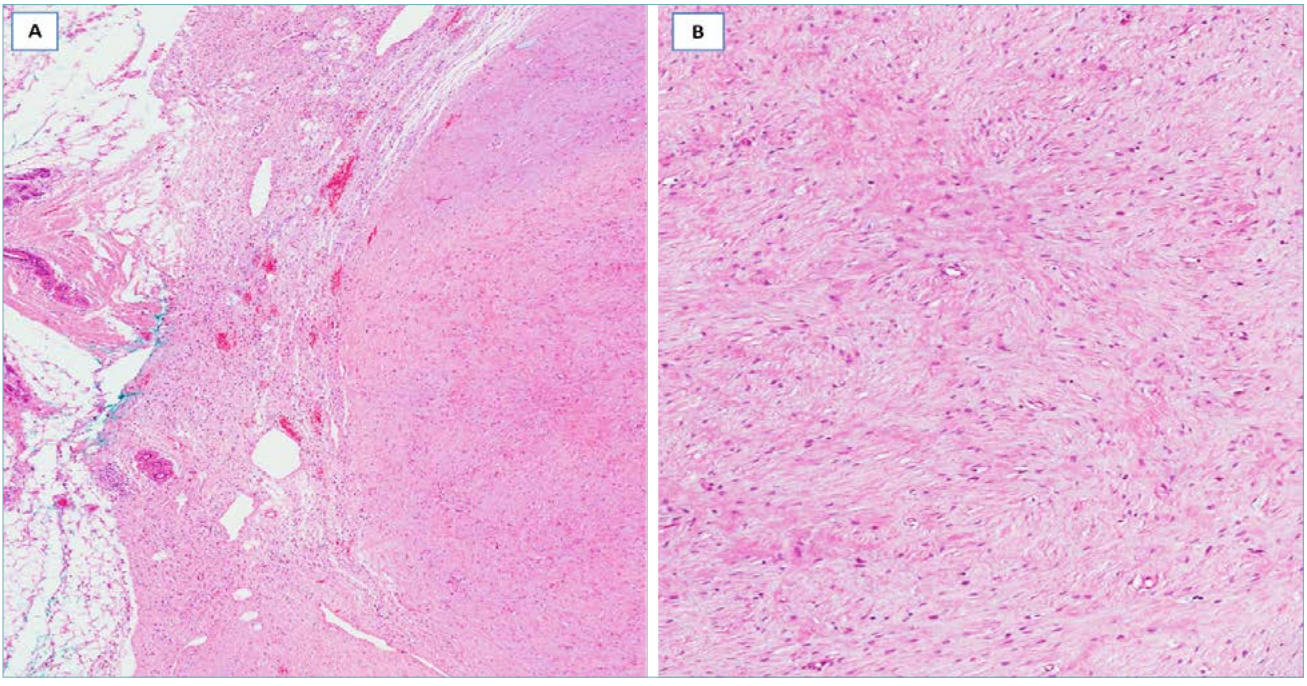


Fig. 9. Fibroma. (A) A fibrous hypocellular tumor with circumscribed margins; (B) higher magnification showing fibroblast-like spindle cells set in a collagenized stroma.

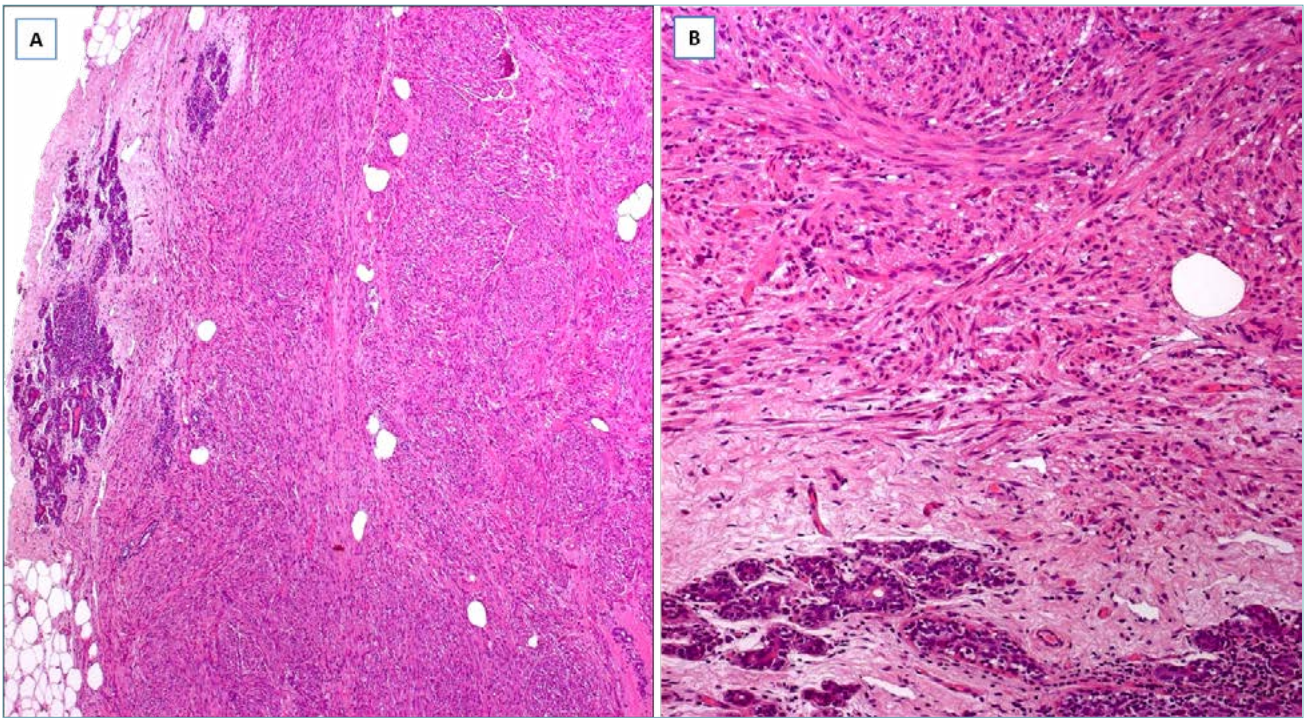


Fig. 10. Leiomyoma. (A) Spindle cell tumor with circumscribed borders and fascicular growth pattern; (B) higher magnification showing smooth muscle cells with deep eosinophilic cytoplasm.

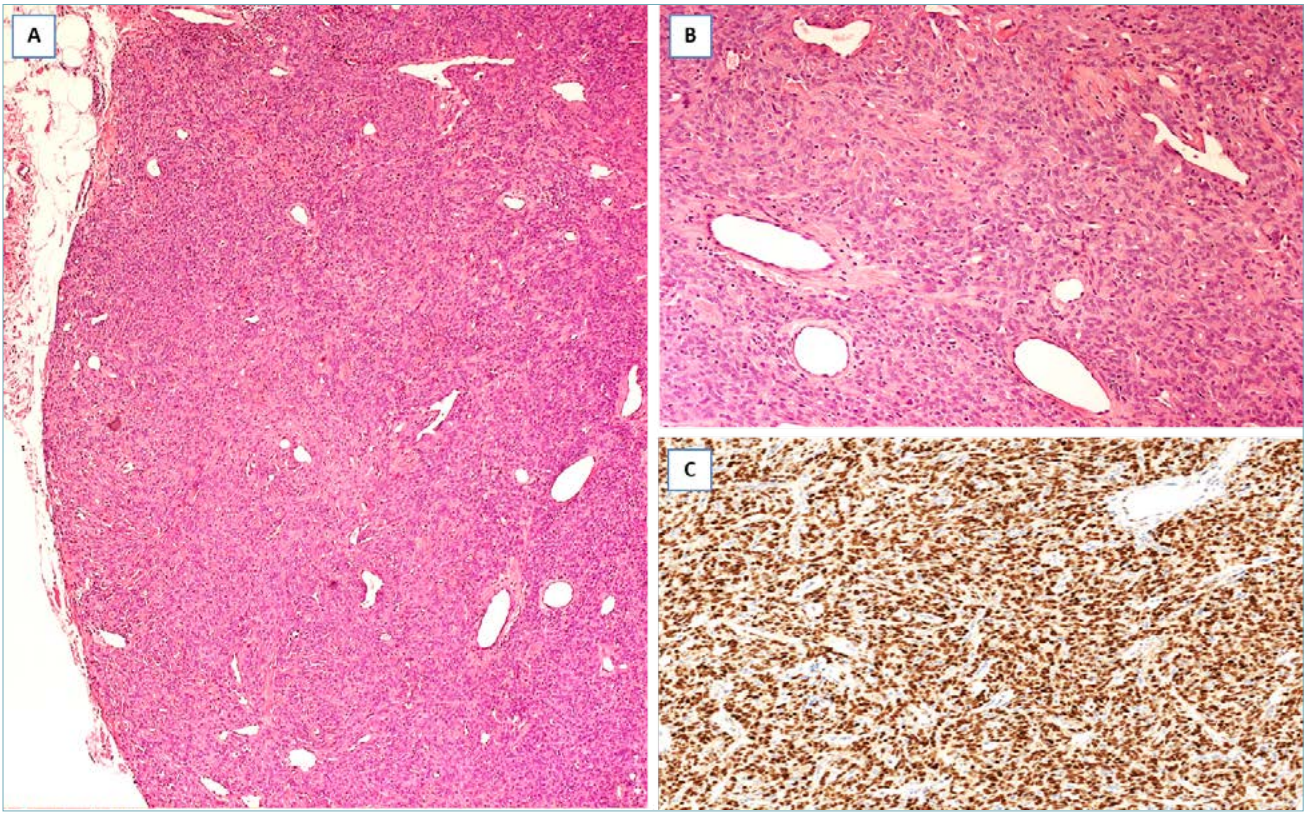


Fig. 11. Solitary fibrous tumor. (A) Spindle cell tumor with pushing margins; (B) neoplastic cells are set in a fibrous stroma containing branching blood vessels with perivascular fibrosis; (C) neoplastic cells show diffuse nuclear staining with STAT-6.

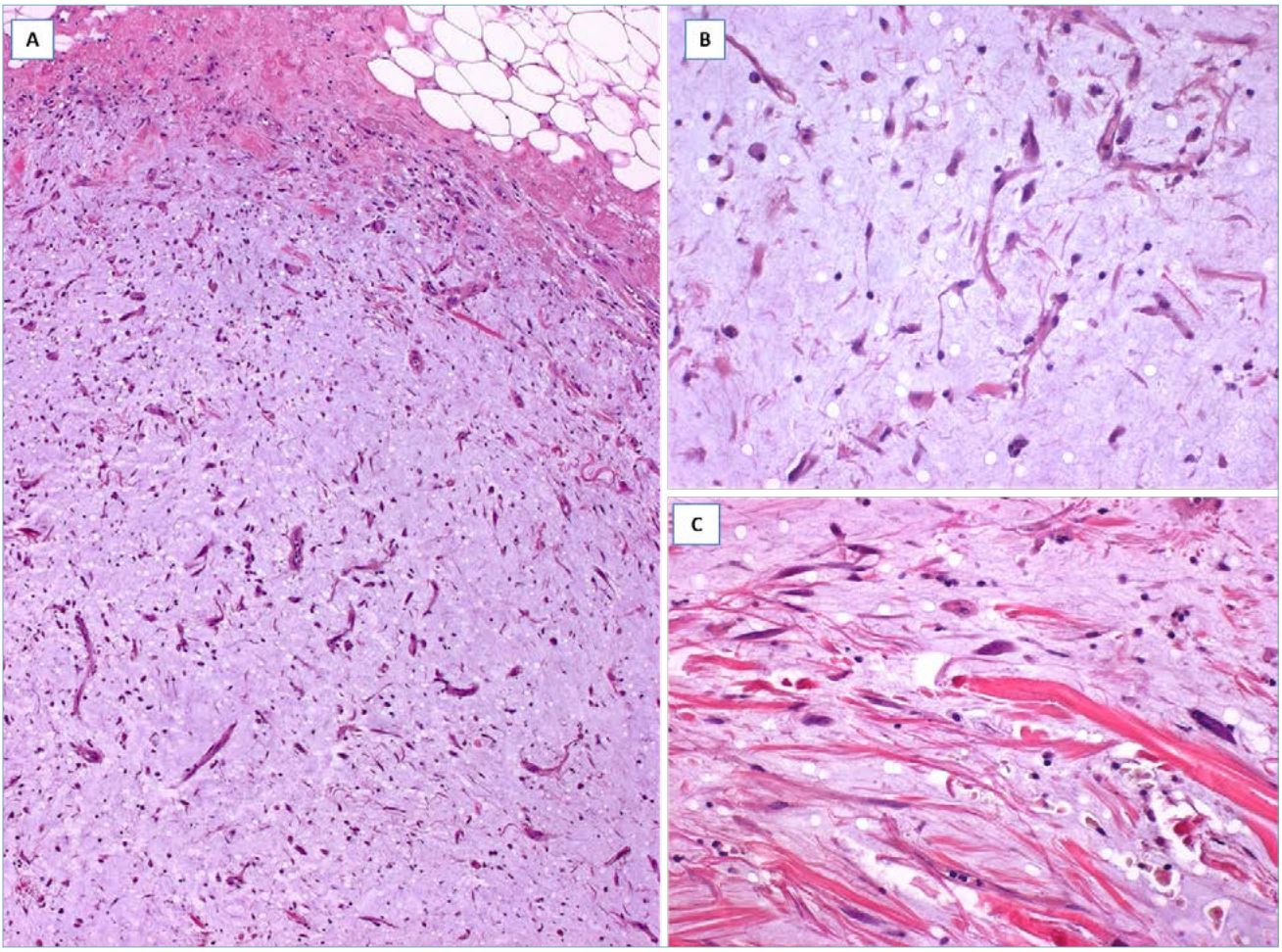


Fig. 12. Myxoma. (A) A myxoid tumor with circumscribed margins; (B) higher magnification showing spindle and stellate cells embedded in abundant myxoid stroma; stromal microcystic spaces look like univacuolated lipoblasts; (C) neoplastic cells may exhibit nuclear atypia (bizarre cells) and the myxoid stroma may contain keloid-like collagen fibers.

main diagnostic clue for this type of carcinoma. A minority of squamous nests and/or small-sized neoplastic glands can be encountered. Estrogen/progesterone receptors and HER2 are negative (triple-negative carcinoma). This carcinoma can recur locally, with low metastatic potential (lymph node or distant metastases). Radical excision is curative in most cases.

Inflammatory Myofibroblastic Tumor [90-94]

It should be suspected in presence of a nodular mass with relatively circumscribed margins, with morphological features similar to inflammatory pseudotumor, arising without apparent correlation with local trauma/stimuli; ALK-1 expression favors this diagnosis. Surgical excision is curative with local recurrence documented in about 15-20% of cases. Rarely distant metastases have been reported.

Low-grade myofibroblastic sarcoma [95-98]

It should be suspected in presence of a nodular mass with relatively circumscribed margins, composed of a proliferation of mitotically active (from 7 to 35 mitoses x 10 HPF) spindle cells with the features of myofibroblasts, showing, at least focally, moderate nuclear pleomorphism, fascicular arrangement and variable staining for α-smooth muscle actin. This tumor, which can recur locally, has metastatic potential.

Conflict of interest statement

None declared.

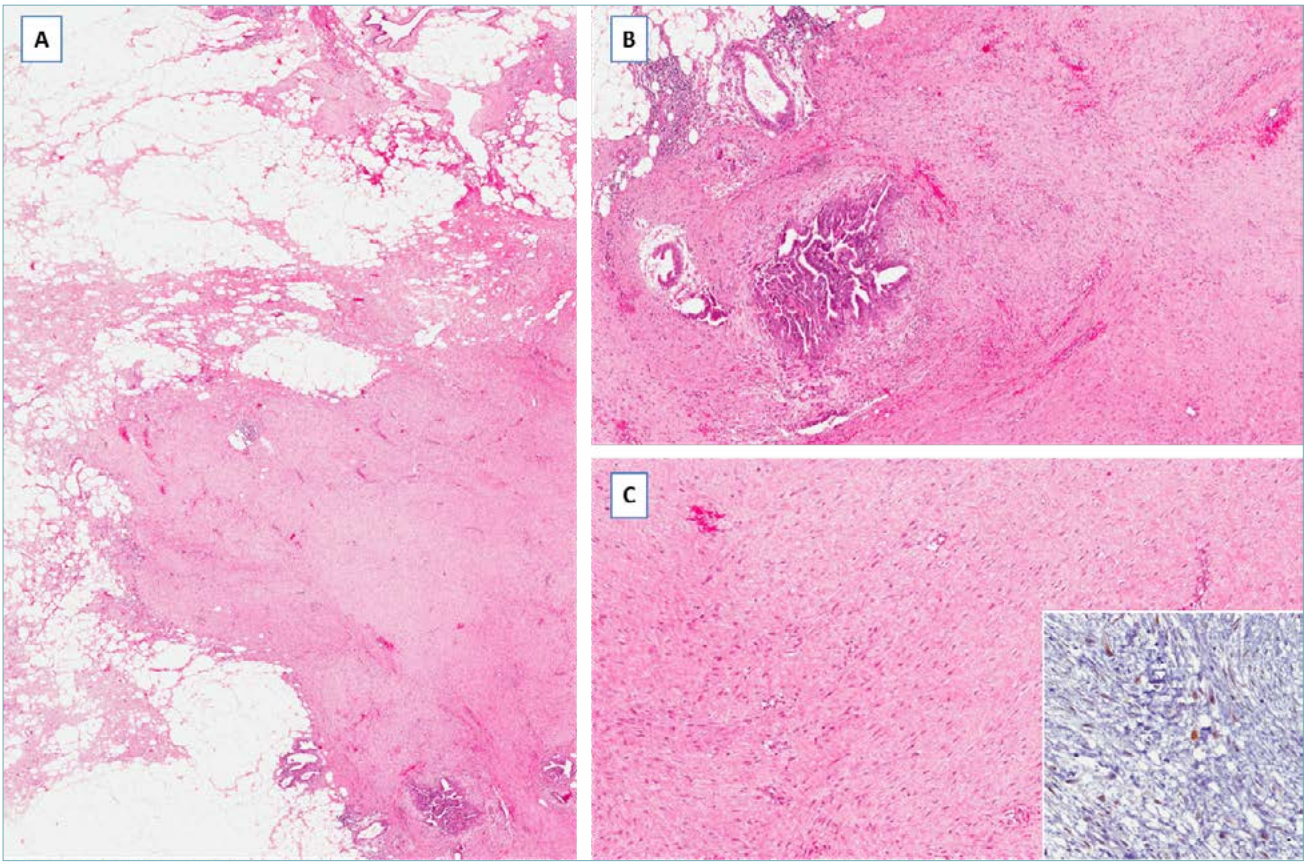


Fig. 13. Desmoid-type Fibromatosis. (A) Fibrous proliferation with infiltrative margins; (B) bland-looking spindle cells entrap pre-existing mammary ducts; (C) higher magnification showing spindle cells aligned parallel and separated by a fibrous stroma. Neoplastic cells show nuclear expression of β-catenin (insert).

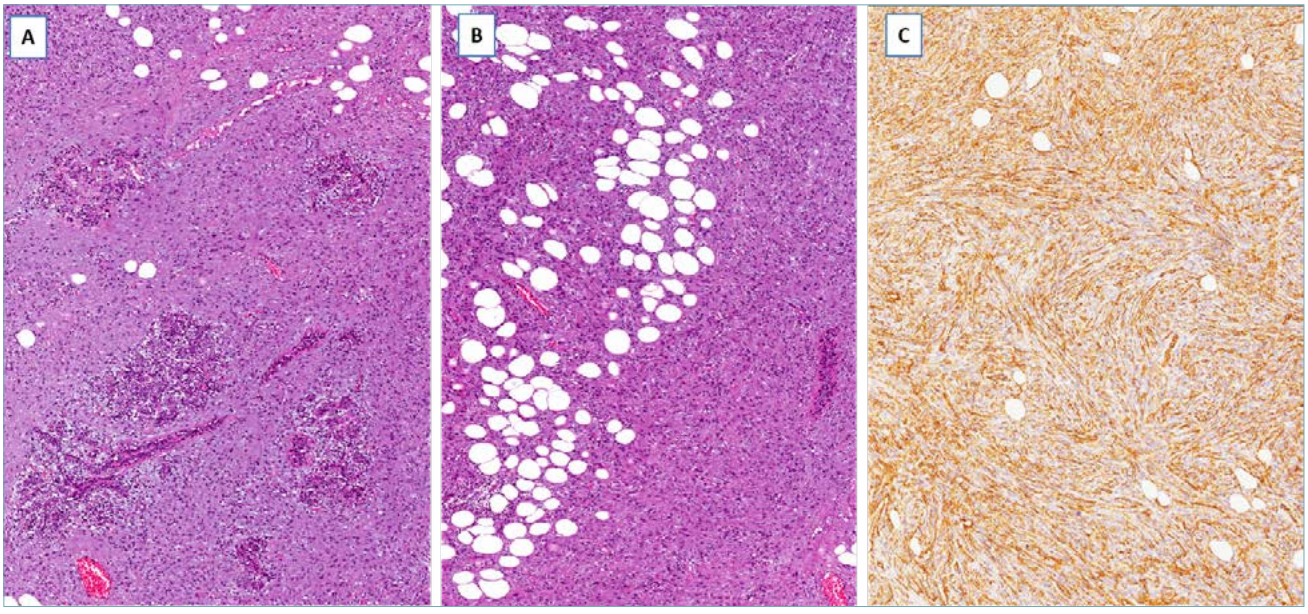


Fig. 14. Dermatofibrosarcoma protuberans. (A) Spindle cell tumor surrounding pre-existing duct/lobular units; (B) the neoplastic cells diffusely infiltrate adipose tissue; (C) neoplastic cells are diffusely stained with CD34.

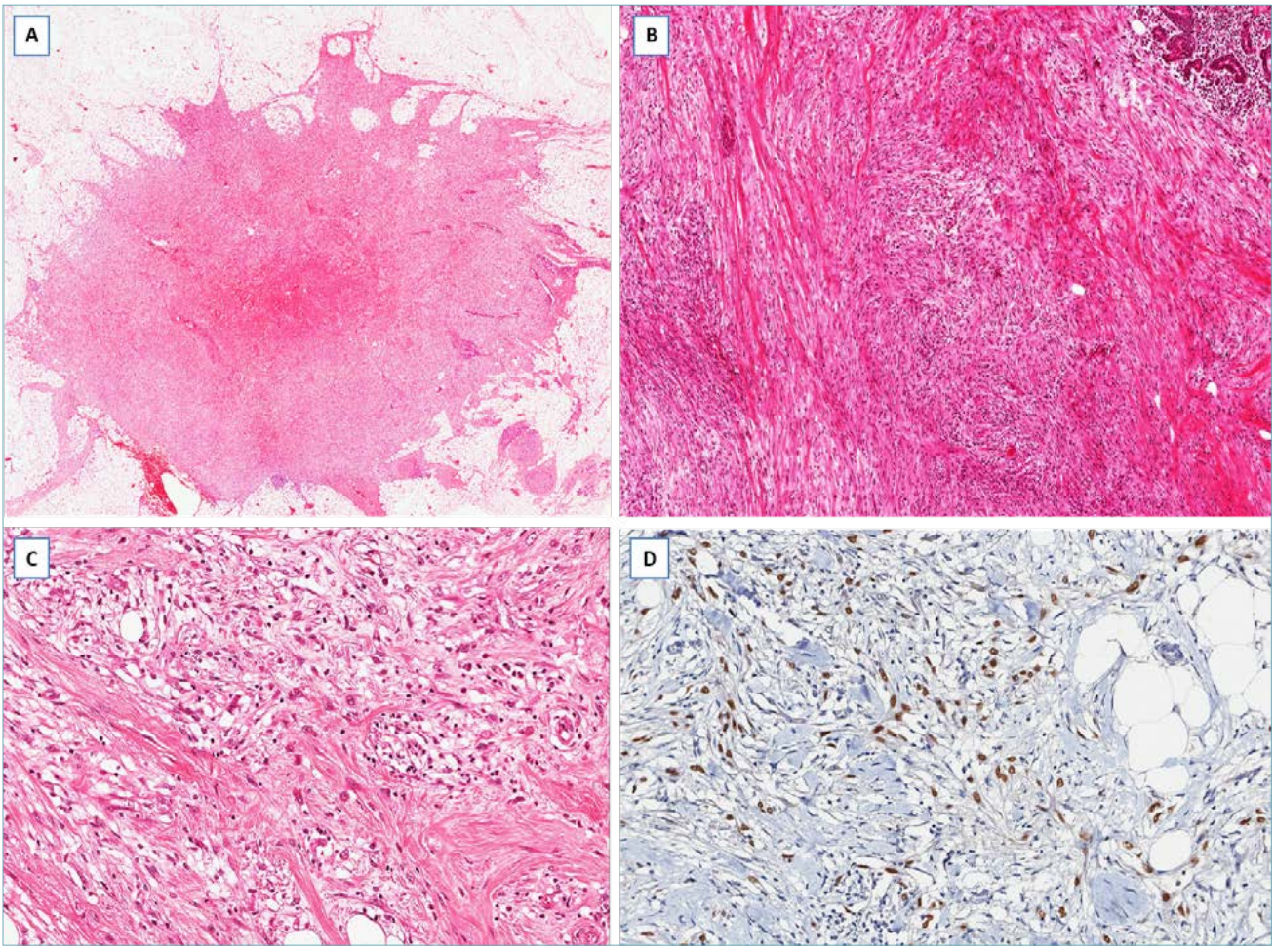


Fig. 15. Low-grade fibromatosis-like spindle cell carcinoma. (A) Low-magnification showing a fibrous tumor with finger-like infiltrative margins; (B) bland-looking spindle cells are set in a fibrous stroma and exhibit a fascicular arrangement; (C) some tumor areas show single or small groups of round to epithelioid cells scattered throughout the fibrous stroma; (D) these neoplastic cells show nuclear expression of p63.

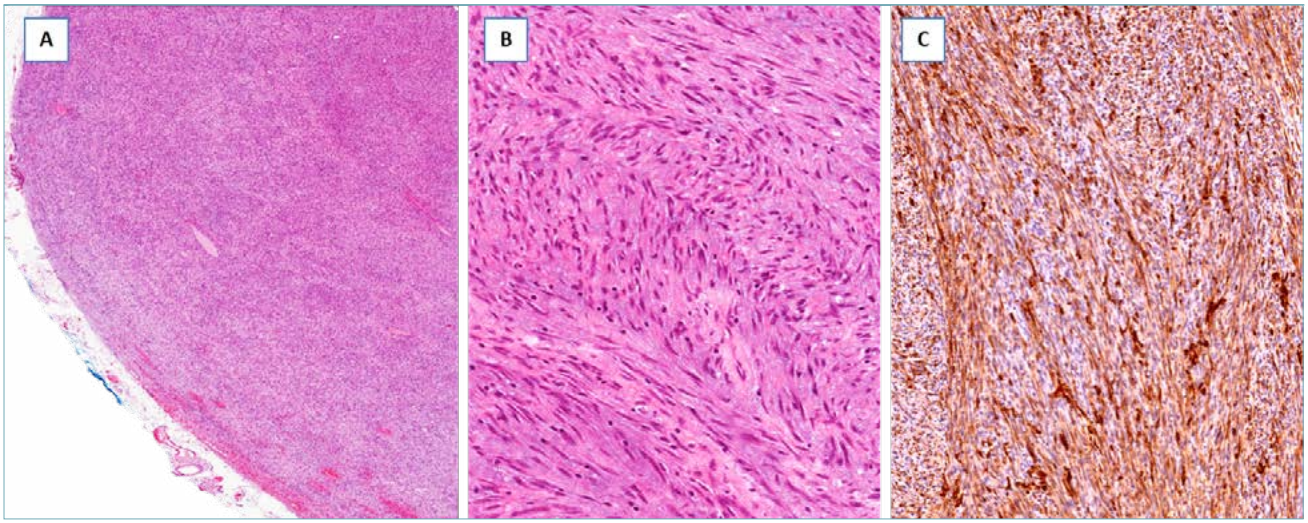


Fig. 16. Low-grade myofibroblastic sarcoma. (A) Low-magnification showing a hypercellular tumor with pushing borders; (B) the neoplastic cells, with the morphological features of myofibroblasts, are arranged in short intersecting fascicles; (C) neoplastic cells are diffusely stained with α-smooth muscle actin.

References

1 Al-Nafussi A. *Spindle cell tumours of the breast: practical approach to diagnosis*. Histopathology 1999;35:1-13. https://doi.org/10.1046/j.1365-2559.1999.00766.x

2 McMenamin ME, DeSchryver K, Fletcher CD. *Fibrous Lesions of the Breast: A Review*. Int J Surg Pathol 2000;8: 99-108. https://doi.org/10.1177/106689690000800204

3 Magro G, Michal M, Bisceglia M. *Benign spindle cell tumors of the mammary stroma: diagnostic criteria, classification, and histogenesis*. Pathol Res Pract 2001;197:453-66. https://doi.org/10.1078/0344-0338-00112

4 Magro G, Bisceglia M, Michal M, et al. *Spindle cell lipoma-like tumor, solitary fibrous tumor and myofibroblastoma of the breast: a clinico-pathological analysis of 13 cases in favor of a unifying histogenetic concept*. Virchows Arch 2002;440:249-60. https://doi.org/10.1007/s00428-001-0572-y

5 Brogi E. *Benign and malignant spindle cell lesions of the breast*. Semin Diagn Pathol 2004;21:57-64. https://doi.org/10.1053/j.semdp.2003.10.007

6 Rakha EA, Aleskandarany MA, Lee AH, et al. *An approach to the diagnosis of spindle cell lesions of the breast*. Histopathology 2016;68:33-44. https://doi.org/10.1111/his.12865

7 Cheah AL, Billings SD, Rowe JJ. *Mesenchymal tumours of the breast and their mimics: a review with approach to diagnosis*. Pathology 2016;48:406-24. https://doi.org/10.1016/j.pathol.2016.05.006

8 Tay TKY, Tan PH. *Spindle cell lesions of the breast - An approach to diagnosis*. Semin Diagn Pathol 2017;34:400-9. https://doi.org/10.1053/j.semdp.2017.05.012

9 Magro G. *Differential diagnosis of benign spindle cell lesions*. Surg Pathol Clin 2018;11:91-121. https://doi.org/10.1016/j.path.2017.09.005

10 Huang J, Li H, Ren G. *Epithelial-mesenchymal transition and drug resistance in breast cancer (Review)*. Int J Oncol. 2015;47:840-8. https://doi.org/10.3892/ijo.2015.3084

11 Tabbara SO, Frierson HF Jr, Fechner RE. *Diagnostic problems in tissues previously sampled by fine-needle aspiration*. Am J Clin Pathol 1991;96:76-80. https://doi.org/10.1093/ajcp/96.1.76

12 Lee KC, Chan JK, Ho LC. *Histologic changes in the breast after fine-needle aspiration*. Am J Surg Pathol 1994;18:1039-47. https://doi.org/10.1097/00000478-199410000-00007

13 Gobbi H, Tse G, Page DL, et al. *Reactive spindle cell nodules of the breast after core biopsy or fine-needle aspiration*. Am J Clin Pathol 2000;113,288-94. https://doi.org/10.1309/RPW4-CXCC-1JHM-0TL7

14 Garijo MF, Val-Bernal JF, Vega A, et al. *Postoperative spindle cell nodule of the breast: pseudosarcomatous myofibroblastic proliferation following endosurgery*. Pathol Int 2008;58,787-91. https://doi.org/10.1111/j.1440-1827.2008.02312.x

15 Sciallis AP, Chen B, Folpe AL. *Cellular Spindled histiocytic pseudotumor complicating mammary fat necrosis*. Am J Surg Pathol 2012;36:1571-8. https://doi.org/10.1097/PAS.0b013e31825faa2b

16 Kang A, Kumar JB, Thomas A, et al. *A spontaneously resolving breast lesion: imaging and cytological findings of nodular fasciitis of the breast with FISH showing USP6 gene rearrangement*. BMJ Case Rep 23: 2015. https://doi.org/10.1136/bcr-2015-213076

17 Paliogiannis P, Cossu A, Palmieri G, et al. *Breast nodular fasciitis: a comprehensive review*. Breast Care (Basel) 2016;11:270-74. https://doi.org/10.1159/000448185

18 Hayashi S, Yasuda S, Takahashi N, et al. *Nodular fasciitis of the breast clinically resembling breast cancer in an elderly woman: a case report*. J Med Case Rep,11,57. https://doi.org/10.1186/s13256-017-1219-1

19 Naso JR, Chiu CG, Goecke ME, et al. *Benign spindle cell lesions of the breast: a diagnostic approach to solitary fibrous tumour, nodular pseudoangiomatous stromal hyperplasia and nodular fasciitis*. J Clin Pathol 2019;72:438-42. https://doi.org/10.1136/jclinpath-2018-205561

20 Erinanc H, Türk E. *The Rare Benign Lesion That Mimics a Malignant Tumor in Breast Parenchyma: Nodular Fasciitis of the Breast*. Case Rep Pathol 2018;30:1612587. https://doi.org/10.1155/2018/1612587

21 Yip CH, Wong KT, Samuel D. *Bilateral plasma cell granuloma (inflammatory pseudotumour) of the breast*. Aust N Z J Surg 1997;67:300-2. https://doi.org/10.1111/j.1445-2197.1997.tb01972.x

22 Ilvan S, Celik V, Paksoy M, et al. *Inflammatory myofibroblastic tumor (inflammatory pseudotumor) of the breast*. APMIS 2005;113:66-9. https://doi.org/10.1111/j.1600-0463.2005.apm1130110.x

23 Kim SJ, Moon WK, Kim JH, et al. *Inflammatory pseudotumor of the breast: a case report with imaging findings*. Korean J Radiol 2009;10:515-8. https://doi.org/10.3348/kjr.2009.10.5.515

24 Park SB, Kim HH, Shin HJ, et al. *Inflammatory pseudotumor (myoblastic tumor) of the breast: a case report and review of the literature*. J Clin Ultrasound 2010;38:52-5. https://doi.org/10.1002/jcu.20637

25 Hill PA. *Inflammatory pseudotumor of the breast: a mimic of breast carcinoma*. Breast J 2010;16: 549-50. https://doi.org/10.1111/j.1524-4741.2010.00967.x

26 Vecchio GM, Amico P, Grasso G, et al. *Post-traumatic inflammatory pseudotumor of the breast with atypical morphological features: a potential diagnostic pitfall*. Report of a case and a critical review of the literature. Pathol Res Pract 2011;207:322-6. https://doi.org/10.1016/j.prp.2011.01.009

27 Greenleaf EK, Williams NC, Leung AM. *Inflammatory Pseudotumor of the Breast*. Am Surg 2016;82:e106-7. PMID: 27215709.

28 Magro G. *Mammary myofibroblastoma: a tumor with a wide morphologic spectrum*. Arch Pathol Lab Med 2008;132:1813-20. https://doi.org/10.1043/1543-2165-132.11.1813

29 Magro G. *Mammary myofibroblastoma: an update with emphasis on the most diagnostically challenging variants*. Histology and Histopathology 2016;31:1-23. 10:14670

30 Magro G, Michal M, Vasquez E, et al. *Lipomatous myofibroblastoma: a potential diagnostic pitfall in the spectrum of the spindle cell lesions of the breast*. Virch Arch. 2000;437:540-4. https://doi.org/10.1007/s004280000297

31 Baxendine-Jones J, Theaker JM, Baldwin LJ. *Predominant fatty variant of myofibrolastoma of breast*. J Clin Pathol 2001;54:568-569. https://doi.org/10.1136/jcp.54.7.568

32 Magro G, Longo FR, Salvatorelli L, et al. *Lipomatous myofibroblastoma of the breast: case report with diagnostic and histogenetic considerations*. Pathologica 2014;106:36-40. PMID: 25291864.

33 Rowe JJ, Cheah AL, Calhoun BC. *Lipomatous tumors of the breast: A contemporary review*. Semin Diagn Pathol. 2017;34:453-61. https://doi.org/10.1053/j.semdp.2017.05.008

34 Magro G, Foschini MP, Eusebi V. *Palisaded myofibroblastoma of the breast: a tumor closely mimicking schwannoma: report of 2 cases*. Hum Pathol 2013;44:1941-6. https://doi.org/10.1016/j.humpath.2013.01.018

[35] Laforga JB, Escandón J. *Schwannoma-Like (Palisaded) Myofibroblastoma: A Challenging Diagnosis on Core Biopsy*. Breast J 2017;23:354-6. https://doi.org/10.1111/tbj.12744

[36] Fabbri VP, Damiani S, Baccarini P, et al. *Cytological Features of Palisaded Mammary-Type Myofibroblastoma*. Int J Surg Pathol 2017;25:173-6. https://doi.org/10.1177/1066896916665699

[37] Toker C, Tang CK, Whitely JF, et al. *Benign spindle cell breast tumor*. Cancer 1981;48:1615-22. https://doi.org/10.1002/1097-0142(19811001)48:7<1615::aid-cncr2820480724>3.0.co;2-i

[38] Böger A. *Benign spindle cell tumor of the male breast*. Pathol Res Pract 1984;178:395-9. https://doi.org/10.1016/S0344-0338(84)80035-7

[39] Chan KW, Ghadially FN, Alagaratnam TT. *Benign spindle cell tumour of breast- a variant of spindled cell lipoma or fibroma of breast?* Pathology 1984;16:331-6. https://doi.org/10.3109/00313028409068546

[40] Magro G, Bisceglia M, Pasquinelli G. *Benign spindle cell tumor of the breast with prominent adipocytic component*. Ann Diagn Pathol 1998;2:306-11. https://doi.org/10.1016/s1092-9134(98)80023-3

[41] Bombonati A, Parra JS, Schwartz GF, et al. *Solitary fibrous tumor of the breast*. Breast J 2003;9:251. https://doi.org/10.1046/j.1524-4741.2003.09315.x

[42] Dragoumis D, Atmatzidis S, Chatzimavroudis G, et al. *Benign spindle cell tumor not otherwise specified (NOS) in a male breast*. Int J Surg Pathol 2010;18:575-9. https://doi.org/10.1177/1066896908328576

[43] Magro G, Angelico G, Righi A, et al. *Utility of STAT6 and 13q14 deletion in the classification of the benign spindle cell stromal tumors of the breast*. Hum Pathol 2018;81:55-64. https://doi.org/10.1016/j.humpath.2018.06.015

[44] Magro G, Spadola S, Motta F, et al. *STAT6 expression in spindle cell lesions of the breast: An immunohistochemical study of 48 cases*. Pathol Res Pract. 2018;214:1544-1549. https://doi.org/10.1016/j.prp.2018.07.011

[45] Lew WY. *Spindle cell lipoma of the breast: a case report and literature review*. Diagn Cytopathol 1993;9:434-7. https://doi.org/10.1002/dc.2840090412

[46] Mulvany NJ, Silvester AC, Collins JP. *Spindle cell lipoma of the breast*. Pathology 1999;31:288-91. https://doi.org/10.1080/003130299105188

[47] Abdel-All HS. *Breast spindle cell tumours: about eight cases*. Diagn Pathol 2006;22:1:13. https://doi.org/10.1186/1746-1596-1-13

[48] Jaffar R, Zaheer S, Vasenwala SM, et al. *Spindle cell lipoma breast*. Indian J Pathol Microbiol 2008;51:234-6. https://doi.org/10.4103/0377-4929.41666

[49] Magro G, Angelico G, Spadola S, et al. *Fibroma of the breast: a rare tumour in the spectrum of the benign spindle cell tumours of the mammary stroma*. Pol J Pathol 2018;69:189-94. https://doi.org/10.5114/pjp.2018.76703

[50] Granic M, Stefanovic-Radovic M, Zdravkovic D, et al. *Intraparenchimal Leiomyoma of the Breast*. Arch Iran Med. 2015;18:608-12. https://doi.org/0151809/AIM.0012

[51] Jones MW, Norris HJ, Wargotz ES. *Smooth muscle and nerve sheath tumors of the breast*. A clinicopathologic study of 45 cases. Int J Surg Pathol 1994;2,85-92. https://doi.org/10.1177/106689699400200202

[52] Vecchio GM, Cavaliere A, Cartaginese F, et al. *Intraparenchymal leiomyoma of the breast: report of a case with emphasis on needle core biopsy-based diagnosis*. Pathologica 2013;105,122-7. PMID: 24466762

[53] Krings G, McIntire P, Shin SJ. *Myofibroblastic, fibroblastic and myoid lesions of the breast*. Semin Diagn Pathol 201;34:427-37. https://doi.org/10.1053/j.semdp.2017.05.010

[54] Khalifa MA, Montgomery EA, Azumi N, et al. *Solitary fibrous tumors: a series of lesions,some in unusual sites*. South Med J 1997;90:793-9. https://doi.org/10.1097/00007611-199708000-00005

[55] Falconieri G, Lamovec J, Mirra M, et al. *Solitary fibrous tumor of the mammary gland: a potential pitfall in breast pathology*. Ann Diagn Pathol 2004;8:121-5. https://doi.org/10.1016/j.anndiagpath.2004.03.002

[56] Meguerditchian AN, Malik DA, Hicks DG, et al. *Solitary fibrous tumor of the breast and mammary myofibroblastoma: the same lesion?* Breast J 2008;14:287-92. https://doi.org/10.1111/j.1524-4741.2008.00588.x

[57] Yang LH, Dai SD, Li QC, et al. *Malignant solitary fibrous tumor of breast: a rare case report*. Int J Clin Exp Pathol 2014;7:4461-6. PMID: 25120834

[58] Salemis NS. *Solitary fibrous tumor of the breast: A case report and the review of the literature*. Breast J 2018;24:78-81. https://doi.org/10.1111/tbj.12841

[59] Tsai SY, Hsu CY, Chou YH, et al. *Solitary fibrous tumor of the breast: A case report and review of the literature*. J Clin Ultrasound 2017;45:350-4. https://doi.org/10.1002/jcu.22415

[60] Park BN, Woo OH, Kim C, et al. *Recurrent solitary fibrous tumor of the breast: Magnetic resonance imaging and pathologic findings*. Breast J 2018;24:1064-5. https://doi.org/10.1111/tbj.13131

[61] Brenes J, Moreno A, Merchán MJ, et al. *Solitary fibrous tumor of the breast: A rare neoplasm*. Breast J 2018;24:417-9. https://doi.org/10.1111/tbj.12925

[62] Tyler GT. *Report of a case of pure myxoma of the breast*. Ann Surg 1915;61:121-7. https://doi.org/10.1097/00000658-191501000-00022

[63] Chan YF, Yeung HY, Ma L. *Myxoma of the breast: report of a case and ultrastrucutual study*. Pathology 1986;18:153-7. https://doi.org/10.3109/00313028609090845

[64] Balci P, Kabakci N, Isil T, et al. *Breast myxoma: radiologic and histopathologic features*. Breast J 2007;13:88-90. https://doi.org/10.1111/j.1524-4741.2006.00370.x

[65] Magro G, Cavanaugh B, Palazzo J. *Clinico-pathological features of breast myxoma: report of a case with histogenetic considerations*. Virch Arch 2010;456:581-6. https://doi.org/10.1007/s00428-010-0902-z

[66] Nel J, O' Donnell M, Bradley R. *Myxoma of the Breast in a Male Patient*. Breast J 2016;22:468-9. https://doi.org/10.1111/tbj.12612

[67] Charu V, Cimino-Mathews A. *Peripheral nerve sheath tumors of the breast*. Semin Diagn Pathol 2017;34:420-6. https://doi.org/10.1053/j.semdp.2017.05.011

[68] Bellezza G, Lombardi T, Panzarola P, et al. *Schwannoma of the breast: a case report and review of the literature*. Tumori 2007;93:308-11. PMID: 17679472

[69] Gultekin SH, Cody HS, Hoda SA. *Schwannoma of the breast*. South Med J 1996;89: 238-9. https://doi.org/10.1097/00007611-199602000-00018

[70] Murat A, Kansiz F, Kabakus N, et al. *Neurofibroma of the breast in a boy with neurofibromatosis type 1*. Clin Imaging 2004;28:415-7. https://doi.org/10.1016/S0899-7071(04)00004-X

[71] Lee EK, Kook SH, Kwag HJ, et al. *Schwannoma of the breast showing massive exophytic growth: a case report*. Breast 2006;15: 562-6. https://doi.org/10.1016/j.breast.2005.11.009

[72] Dialani V, Hines N, Wang Y, et al. *Breast schwannoma*. Case Rep Med 2011;2011:930841. https://doi.org/10.1155/2011/930841

[73] Thejaswini M, Padmaja K, Srinivasamurthy V, et al. *Solitary intramammary schwannoma mimicking phylloides tumor: cytological clues in the diagnosis*. J Cytol 2012;29:258-60. https://doi.org/10.4103/0970-9371.103947

[74] Parikh Y, Sharma KJ, Parikh SJ, et al. *Intramammary schwannoma: a palpable breast mass*. Radiol Case Rep 2016;11:129-33. https://doi.org/10.1016/j.radcr.2016.05.011

[75] Jeyaretna DS, Oriolowo A, Smith MEF, et al. *Solitary neurofibroma in the male breast*. World J Surg Oncol 2007;27;5:23. https://doi.org/10.1186/1477-7819-5-23

[76] Thompson S, Kaplan SS, Poppiti RJ, et al. *Solitary neurofibroma of the breast*. Radiol Case Rep 2012;7:462. https://doi.org/10.2484/rcr.v7i4.462

[77] Wargotz ES, Norris HJ, Austin RM, et al. *Fibromatosis of the breast*. A clinical and pathological study of 28 cases. Am J Surg Pathol 1987;11,38-44. https://doi.org/10.1097/00000478-198701000-00005

[78] Rosen PP, Ernsberger D. *Mammary fibromatosis*. A benign spindle-cell tumor with significant risk for local recurrence. Cancer 1989;63:1363-9. https://doi.org/10.1002/1097-0142(19890401)63:7<1363::aid-cncr2820630722>3.0.co;2-b

[79] Magro G, Mesiti M. *Breast and pectoralis musculo-aponeurotic fibromatosis: two independent lesions occurring in the same patient*. A case report. Pathol Res Pract 1998;194:867-71. https://doi.org/10.1016/S0344-0338(98)80092-7

[80] Devouassoux-Shisheboran M, Schammel MDP, Man YG, et al. *Fibromatosis of the breast*. Arch Pathol Lab Med 2000;124:276-80. https://doi.org/10.1043/0003-9985(2000)124<0276:FOTB>2.0.CO;2

[81] Magro G, Gurrera A, Scavo N, et al. *Fibromatosis of the breast: a clinical, radiological and pathological study of 6 cases*. Pathologica 2002;94:238-46.

[82] Wongmaneerung P, Somwangprasert A, Watcharachan K, et al. *Bilateral desmoid tumor of the breast: case series and literature review*. Int Med Case Rep J 2016;9:247-51. https://doi.org/10.2147/IMCRJ.S106325

[83] Valli R, Rossi G, Natalini G, et al. *Dermatofibrosarcoma protuberans of the breast: a case report*. Pathologica 2002;94:310-3.

[84] Karcnik TJ, Miller JA, Fromowitz F, et al. *Dermatofibrosarcoma protuberans of the breast: a rare malignant tumor simulating benign disease*. Breast J 1999;5:262-3. https://doi.org/10.1046/j.1524-4741.1999.99003.x

[85] Callery CD, Rosen PP, Kinne DW. *Sarcoma of the breast*. A study of 32 patients with reappraisal of classification and therapy. Ann Surg 1985;201:527-32. https://doi.org/10.1097/00000658-198504000-00020

[86] Norris HJ, Taylor HB. *Sarcomas and related mesenchymal tumors of the breast*. Cancer 1968;22:22-8. https://doi.org/10.1002/1097-0142(196807)22:1<22::aid-cncr2820220105>3.0.co;2-r

[87] Vecchio GM, Broggi G, Mulè A, et al. *Dermatofibrosarcoma protuberans: a tumor in the wide spectrum of the bland-looking spindle cell lesions of the breast*. Pathologica 2019;111:87-91. https://doi.org/10.32074/1591-951X-22-19

[88] Sneige N, Yaziji H, Mandavilli SR, et al. *Low-grade (fibromatosis-like) spindle cell carcinoma of the breast*. Am J Surg Pathol 2001;25,1009-1016. https://doi.org/10.1097/00000478-200108000-00004

[89] Dwyer JB, Clark BZ. *Low-grade fibromatosis-like spindle cell carcinoma of the breast*. Arch Pathol Lab Med 2015;139:552-7. https://doi.org/10.5858/arpa.2013-0555-RS

[90] Zhou Y, Zhu J, Zhang Y, et al. *An inflammatory myofibroblastic tumour of the breast with ALK overexpression*. BMJ Case Rep 2013;4: 2013. https://doi.org/10.1136/bcr-07-2011-4474

[91] Zhao HD, Wu T, Wang JQ, et al. *Primary inflammatory myofibroblastic tumor of the breast with rapid recurrence and metastasis: a case report*. Oncol Lett 2013;5:97-100. https://doi.org/10.3892/ol.2012.948

[92] Bosse K, Ott C, Biegner T, et al. *23-Year-Old Female with an inflammatory myofibroblastic tumour of the breast: a case report and a review of the literature*. GeburtshilfeFrauenheilkd 2014;74:167-70. https://doi.org/10.1055/s-0033-1360185

[93] Markopoulos C, Charalampoudis P, Karagiannis E, et al. *Inflammatory myofibroblastic tumor of the breast*. Case Rep Surg 2015;2015:705127. https://doi.org/10.1155/2015/705127

[94] Choi EJ, Jin GY, Chung MJ, et al. *Primary Inflammatory Myofibroblastic Tumors of the Breast with Metastasis: Radiographic and Histopathologic Predictive Factors*. J Breast Cancer 2015;18:200-5. https://doi.org/10.4048/jbc.2015.18.2.200

[95] Taccagni G, Rovere E, Masullo M, et al. *Myofibrosarcoma of the breast: review of the literature on myofibroblastic tumors and criteria for defining myofibroblastic differentiation*. Am J Surg Pathol 1997;21,489-96. https://doi.org/10.1097/00000478-199704000-00017

[96] Gocht A, Bösmüller HC, Bässler R, et al. *Breast tumors with myofibroblastic differentiation: clinico-pathological observations in myofibroblastoma and myofibrosarcoma*. Pathol Res Pract 1999;195,1-10. https://doi.org/10.1016/S0344-0338(99)80087-9

[97] Lucin K, Mustać E, Jonjić N. *Breast sarcoma showing myofibroblastic differentiation*. Virchows Arch 2003;443:222-4. https://doi.org/10.1007/s00428-003-0853-8

[98] Morgan PB, Chundru S, Hatch SS, et al. *Uncommon malignancies: case 1. Low-grade myofibroblastic sarcoma of the breast*. J Clin Oncol 2005;23,6249-51. https://doi.org/10.1200/JCO.2005.06.213

Received: October 24, 2019 - Accepted: October 28, 2019

PATHOLOGICA 2019;111:361-364; doi: 10.32074/1591-951X-1-19

Case report

Pleural chondroid metaplasia in a background of IgG4 plasma cell rich chronic inflammatory infiltrate: report of a rare case with review of literature

M. Spinelli[1], A. Datta[2], M.A. Montero Fenandez[3], P. Viola[4]

[1] Locum Consultant Cellular Pathologist, Worcester Royal Hospital, Worcester, UK; [2] Consultant respiratory medicine, Northwick Park Hospital/Imperial College healthcare NHS Trust; [3] Consultant Thoracic Pathologist, Manchester University Foundation trust; [4] Consultant Cellular Pathologist, North West London Pathology, based at Imperial College Healthcare NHS Trust

Summary

Mesothelial reactive and degenerative changes secondary to inflammatory conditions, toxin exposure, neoplastic and non-neoplastic disease are often encountered in routine histopathology practice. Herein, we report a very rare case of pleural chronic inflammation with features suggestive for IgG4-related disease associated with the formation of a nest of mature, benign looking cartilage in the pleura of a middle age male patient. The review of the pertinent literature reveals that to date, only few cases of chondroid metaplasia or benign tumor (choristoma) are reported in mesothelium and only occasionally in this change is found in the pleura.

Key words

Chondroid metaplasia • Pleura • Stem cells

Introduction

Mesothelium can react to different stimuli (of inflammatory, chemical, toxic, neoplastic origin) with inflammation, fibrosis necrosis and other degenerative changes. One of the most peculiar, albeit rarely seen changes is the development of mesenchymal derived tissues such as bone or cartilage. The pathogenesis of this condition is still not fully understood and further studies are needed in order to define the mechanisms at the origin of this rare form of metaplasia.

Case report

A 51-years-old male came to clinical attention for a five weeks history of breathlessness. The patient, who worked as a driver and in construction, had a significant smoking habit (30 cigarettes per day), diabetes, hypertension and high cholesterol blood levels under treatment. Imaging showed a moderate left pleural effusion with no evidence of specific features on CT. PET showed moderately avid lymph nodes in the paratracheal and AP window regions but no evidence of activity in the pleura (Fig. 1).

The thoracentesis drained 300 ml of blood stained fluid which showed no evidence of atypical/malignant cells on cytology examination.

During the lung multidisciplinary meeting it was decided to proceed with thoracoscopic pleural biopsies and talc pleurodesis.

The pleural biopsies were represented by multiple fragments of cream and yellow fat tissue with fibrotic consistency, measuring on aggregate up to 30 x 25 x 10 mm. The gross examination did not enlighten obvious pleural plaques or focal abnormalities. The tissue was entirely submitted for histological assessment.

Microscopy examination showed reactive mesothelial

How to cite this article: Spinelli M, Datta A, Montero Fenandez MA , et al. *Pleural chondroid metaplasia in a background of IgG4 plasma cell rich chronic inflammatory infiltrate: report of a rare case with review of literature.* Pathologica 2019;111:361-4. https://doi.org/10.32074/1591-951X-1-19.

Correspondence: Manuela Spinelli, Cellular pathology Department, Worcester Royal hospital, Worcester, UK - Tel. 00447704077613 - E-mail: manuelaspinelli976@gmail.com, Manuela.spinelli@email.it

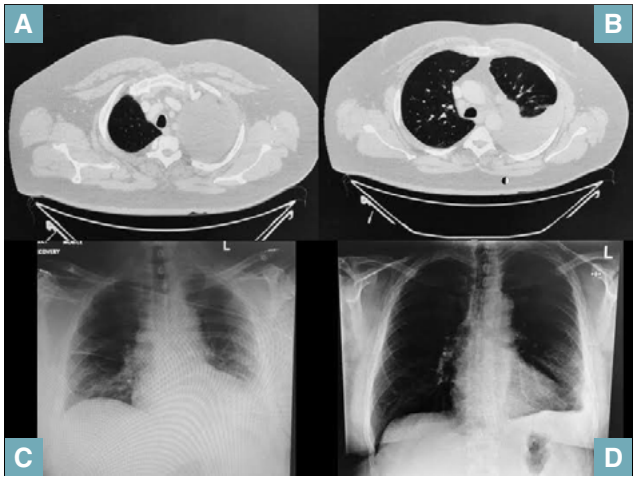


Fig. 1. A and B: CT Thorax with contrast. Large left pleural effusion with underlying compression atelectasis of the basal segment of the left lower lobe and lingual; C and D: Post op Chest X-Ray showing progressive reduction of the effusion.

tissue covering stroma and fat tissue with patchy fibrosis. The fibrotic tissue did not show storiform pattern, collagen necrosis or dysplasia. The fibrosis was associated with a moderate to severe chronic inflammation with a prominent plasma cell component and some pigment-laded histiocytes, but there was no evidence of granuloma formation. The fibrotic stroma showed areas of hyalinization and formation of cartilaginous tissue featuring bland, benign looking chondrocytes. There was no evidence of vasculitis, obliterative phlebitis, necrosis, dysplasia or malignancy.

Immunohistochemistry and special stains confirmed the presence of reactive mesothelial cells (Calretinin and CKAE1/AE3 +), polytypic benign plasma cells (Kappa and Lambda chains stains +) and the absence of amyloid (Congo Red -). The presence of patchy areas of increased, polyclonal plasma cells associated with fibrosis raised the concern of a possible IgG4-related disease. The presence of the chondroid metaplasia itself did not influence this suspect as no data are available in literature to support a relationship between chondroid metaplasia and this entity. The stain for IgG4 showed an increased number of IgG4 + plasma cells focally up to 50 cells/HPF. In these fields the IgG4+/IgG ratio was occasionally more than 40% (Figg. 2-3). The overall appearance, together with the special stains and immunohistochemical features was compatible with moderate to severe chronic inflammation with features suggestive, but not entirely diagnostic for IgG4-related disease. However, the absence of diffuse fibrosis

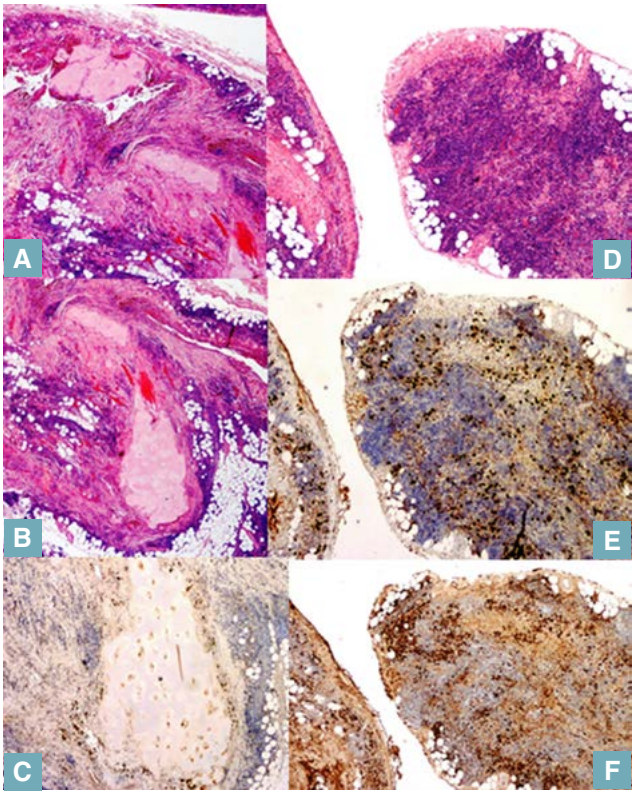


Fig. 2. A and B: Islands of chondroid metaplasia in the pleural tissue (H&E 2X); C: Chondroid tissue highlighted by S100 (IHC 4X); D: Lymphoplasmacellular infiltrate (H&E 2X); E and F: Inflammatory infiltrate IgG and IgG4 staining (2X).

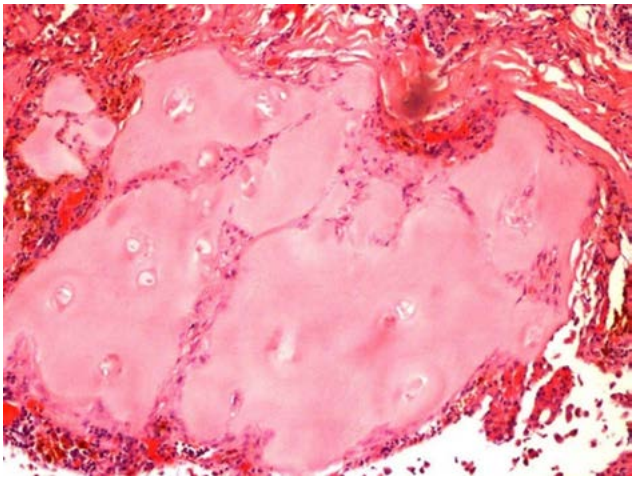


Fig. 3. Magnification of the mature chondroid metaplastic tissue (H&E 10X).

and phlebitis was not entirely supportive for a diagnosis of IgG4 related disease and serology levels were re-

quested for confirmation. Serum IgG4 resulted within normal limits, thus excluding the diagnosis of IgG4-RD.

Discussion and review of literature

The most important features of this biopsy were the plasma cell rich chronic inflammation and the formation of ectopic chondroid tissue. Chondroid metaplasia is a relatively uncommon finding which has been described in different anatomical sites and conditions (both neoplastic and non-neoplastic), but is rarely reported in mesothelium [1] and even more occasionally in pleura. Chondroid metaplasia has been reported in tongue [2], head and neck [3-5], breast [6 7], lung [8] and brain [9]. Formation of ectopic, metaplastic cartilage in the mesothelium is rare and only a few cases are reported in the literature [10-15].

The pathogenesis of benign-looking cartilaginous tissue in mesothelium covered anatomic sites is still not fully understood. Some authors speculate that a population of sub-mesothelial pluripotent stem cells could differentiate towards mesenchymal and chondroid phenotypes [11 13]. Other factors such as previous surgery, trauma, hemorrhage, abortion, ectopic pregnancies, teratomas or other neoplasms (both benign and malignant) have been considered as pathogenetic factors [10-12].

As shown in the Table the presence of metaplastic cartilaginous tissue is rare in pleura: in 1931 Klemperer et al. reported chondroid metaplasia in a series of pleural tumors [13] (although it was not possible to identify the exact number of metaplasia cases in this study), and in 2014 Walsh et al. reported a case of chondroid metaplasia within a rare pleural lipoma [14].

The present case is peculiar for its rarity and the lack of association with neoplasia. It presented itself as a focal area of well defined, mature and S-100 + cartilage on the background patchy, non storiform fibrosis and prominent lymphoplasmacellular inflammatory infiltrate. One of the most relevant features of our case is the association with several, polytypic active plasma cells which, in areas, were expressing the IgG4 phenotype. The presence of this specific plasma cellular phenotype raised the possibility of a systemic IgG4-related disease, thus clinical and serological correlation was strongly recommended in the histopathology report. The serology results came back as negative for pathologic IgG4 levels in blood, and thus a diagnosis of IgG4-Related disease was excluded.

Our case showed only one of three histopathological criteria, but clinical and serological results were negative. The review of the pertinent literature [15-17] did not enlighten the development of chondroid metaplasia in the specific settings of IgG4-RD. However, it is possible that inflammatory-related molecules produced by activated plasma cells, or the specific micro-environment favored by the chronic inflammation may lead to a local increased expression of matrix and osteo-cartilaginous molecules (such as ostepontin, osteonectin, CD68, different cytokines and interleukins) which could promote the differentiation of fibroblasts and mesenchymal stem cells into the chondroid phenotype [10 11 15 18 19]. A similar pathway is known in the development of heterotopic ossification in several organs (thyroid, soft tissue, gastro-intestinal and genito-urinary tracts) as a reactive/dystrophic reaction to many different neoplastic and non-neoplastic disorders including ischemia, necrosis, hemorrhage and fibrosis [19].

Conclusions

We report here a rare case of pleural cartilaginous metaplasia on the background of a chronic inflammation rich in IgG4+ plasma cells. Our case can be considered of interest for the rarity of the chondroid metaplasia itself and for its possible association with a chronic systemic inflammatory disease such as IgG4-

Tab. I. Chondroid metaplasia in mesothelium: review of literature.

Author	Year	Site	Surgery/trauma	No. of cases
Jacubowitz et al.	1930	Peritoneum	Not known	Not known
Klemperer et al.	1931	Pleura	Not known	Not known
Roth et al.	1966	Uterine serosa	Not reported	2
Fadare et al.	2002	Peritoneum	Not reported	2
Houang et al.	2010	Pelvic peritoneum	Yes	1
Walsh et al.	2014	Pleura (within pleural lipoma)	No	1
Kaur et al.	2017	Pelvic peritoneum, broad ligament	Yes	1
Nwanze et al.	2018	Peritoneum surface at porta hepatis	No	1
Franceschi et al.	2018	Peritoneum	Not reported	2
Present case	2018	Pleura	No	1

RD. Only occasional cases of pleural chondroid metaplasia have been reported in the literature to the best of our knowledge and none was related with such a peculiar background.

Conflict of interest statement

None declared.

References

1 Houang M, Merkur M, Russell P. *Chondroid metaplasia of the peritoneum*. Pathology 2010;42:585-7. https://doi.org/10.3109/00313025.2010.508737

2 Vasudevan JA, Somanathan T, Patil SA, et al. *Primary systemic amyloidosis of tongue with chondroid metaplasia*. J Oral Maxillofac Pathol 2013;17:266-8. https://doi.org/10.4103/0973-029X.119753

3 Tenorio F, Campos P, Paramo JI, et al. *Chondroid metaplasia in a fibroepithelial polyp of gingiva*. J Clin Diagn Res 2016;10:ZD9-ZD10. https://doi.org/10.7860/JCDR/2016/20007.8481

4 Bharti JN, Ghosh N, Arora P, et al. *Chondroid choristoma of palatine tonsil. A rare enity*. J Clin Diagn Res 2013;7:1700-1. https://doi.org/10.7860/JCDR/2013/5540.3258.

5 Ide F, Saito I, Horie N, et al. *Othokeratinized odontogenic cyst of the mandible with heterotopic cartilage*. Head Neck Pathol 2009;3:150-2. https://doi.org/10.1007/s12105-009-0116-3

6 Kim YJ, Shim HS, Lee H, et al. *Metaplastic carcinoma with extensive chondroid differentiation in the breast (choristoma)*. Yonsei Med J 2006;47:259-63. https://doi.org/10.3349/ymj.2006.47.2.259

7 Fernández Pérez MA, Rodriguez IV, Tello Royloa A, et al. *Metaplastic breast carcinoma with unusual presentation: review of three cases*. Breast care (Basel) 2015;10:404-7. https://doi.org/10.1159/000441288

8 Flanagan P. *Squamous carcinoma of the lung with osteocartilagineous stroma*. J Clin Pathol 1955;18:403-7. https://doi.org/10.1136/jcp.18.4.403

9 Kepes JJ, Rubinstein LJ, Chiang H. *The role of astrocytes in the formation of cartilage in gliomas. An immunohistochemical study of four cases*. Am J Pathol 1984;117:471-83.

10 Nwanze J, Trivedi D, Crawford B, et al. *Cartilagineous differentiation/chondroid metaplasia in the peritoneum not associated with prior surgery: a case report and review of literature*. Am J Clin Pathol 2018;150:543.

11 Kaur K, Kaur A, Chopra N, et al. *A rare case of cartilagineous heterotopia in broad ligament of uterus*. J South Asian Feder Obst Gynae 2017;9:192-4.

12 Franceschi T, Allias F, Mauduit C, et al. *Chondroid nodule in the female peritoneum arises from normal tissue and not from teratoma or conception product*. Virchows Archiv 2018;473:115-9. https://doi.org/10.1007/s00428-018-2295-3

13 Klemperer P, Coleman BR. *Primary neoplasms of pleura. A report of five cases*. Arch Pathol 1931;11:385-412. https://doi.org/10.1002/ajim.4700220103

14 Walsh J, Prendeville S, Bejoy P, et al. *Massive pleural lipoma with chondroid metaplasia in an asymptomatic male*. Am J Resp Crit Care Med 2014;189: A2912.

15 Yadlapati S, Verheyen E, Efthimiou P. *4.IgG4-related disease: a complex under diagnosed clinical entity*. Reumathol Int 2018;38:169-77. https://doi.org/10.1007/s00296-017-3765-7

16 Umehara H, Nakajima A, Nakamura T, et al. *IgG4-related disease and its pathogenesis. Cross-talk between innate and acquired immunity*. Int Immunol. 2014;26:585-95. https://doi.org/10.1093/intimm/dxu074

17 Opriţă R,Opriţă B, Berceanu D, et al. *Overview of IgG4-related disease*. Journal of Medicine and Life 2017;10:203-7.

18 Liu K, Tripp S, Layfield LJ. *Heterotopic ossification: review of histologic findings and tissue distribution in a 10 years experience*. Path Res Pract 2007;203:633-40. https://doi.org/10.1016/j.prp.2007.05.014

19 Imai N, Iwai A, Hatakeyama S, et al. *Expression of bone morphogenetic proteins in colon carcinoma with heterotopic ossification*. Pathol Int 2001;51:643-8. https://doi.org/10.1046/j.1440-1827.2001.01243.x

Received: November 21, 2018 - Accepted: October 20, 2019

PATHOLOGICA 2019;111:365-368; doi: 10.32074/1591-951X-33-19

Case Report

Malignant peripheral nerve sheath tumor of the bladder
A case report

G. Petracco[1], C. Patriarca[1], R. Spasciani[2], A. Parafioriti[3]

[1] Department of Pathology and [2] Urology, Ospedale S. Anna, ASST Lariana, Como; [3] Department of Pathology, ASST PINI-CTO, Milano, Italy

Summary

Malignant peripheral nerve sheath tumor (MPNST) is an uncommon malignant tumor often associated with Neurofibromatosis type 1 (NF1). Although different soft tissue mesenchymal tumors may arise in the bladder, MPNST is a very rare occurrence. Here, we present a case of MPNST of the bladder in a 50 year old patient with NF1 with involvement of the entire wall of the organ leading to a functional exclusion. The principal differential diagnoses and a short review of the literature are presented.

Key words

Malignant peripheral nerve sheath tumor • Bladder

Introduction

Urothelial cell carcinoma is the most frequent malignant tumor of the bladder and may show various divergent differentions, most frequently squamous cell carcinoma or adenocarcinoma differentiations. Sarcomatoid features are also a possible finding, particularly in muscle infiltrating urothelial cell carcinoma. Hence, when dealing with a malignant mesenchymal-like tumor of the bladder a sarcomatoid carcinoma must be ruled out [1], and this is a frequent diagnostic experience in the daily practice of microscopical examination of the chips of transurethral resection of the bladder (TURB).

Nevertheless, besides rhabdomiosarcoma, which is the most common malignant bladder tumor in infancy [2], primary bona fide sarcomas of the bladder in adult are a rare but real occurrence and have been described in the literature, although only in small series or isolated case reports [3]. They are mainly represented by leyomiosarcoma [4] and angiosarcoma [5]. MPNST is the among the rarest sarcomas arising in the urinary bladder and only a few cases have been documented, predominantly in middle aged patients, with complaints of hematuria [6 7]. Some of those cases arose in the setting of neurofibromatosis type 1 (NF1), probably originating from neurofibromas of autonomic nerve plexuses of the bladder wall [8 9].

In this paper, we describe a case of MPNST of the bladder in a patient affected by NF1, with the involvement of the entire organ. The differential diagnoses of the case are presented.

Case report

A 50-year-old man without history of cigarette smoking and affected by NF1, with a previous diagnosis of skin neurofibroma, was admitted to the Urology Division of Sant'Anna Hospital of Como for numerous episodes of urinary retention and sepsis. After radiological investigations, a prostate trans-uretral resection (TURP) was performed with a histologic diagnosis of fibroleyomiomatous hyperplasia. Nevertheless, urinary function did not improve; therefore, laboratory tests and further radiological investigations were performed. CT scan showed a full thickness neoplastic growth of the bladder with a diffuse involvement and irregular polipoid

How to cite this article: Petracco G, Patriarca C,Spasciani R, et al. *Malignant peripheral nerve sheath tumor of the bladder. A case report.* Pathologica 2019;111:365-8. https://doi.org/10.32074/1591-951X-33-19.

Correspondence: Carlo Patriarca, Servizio di Anatomia Patologica, Ospedale Sant'Anna di Como, via Ravona, 22020 San Fermo della Battaglia (CO), Italy - E-mail: carlo.patriarca@asst-lariana.it

thickening of the wall and involvement of the entire organ, without a distinctive mass (Fig. 1). Since bladder function was compromised by the tumor, the patient underwent a cistoprostatectomy.

On gross examination, the bladder wall was diffusely thickened, and showed hard consistency with grey to white tissue on cut surface, with subtotal vanishing of the normal detrusor muscle, although without the finding of appreciable nodules in muscolaris propria and/or mucosal lesions; hence a broad sampling was performed.

At histological examination we observed normal and intact urothelium that was lining a diffuse spindle cell proliferation with bland aspect in the more superficial layers of the wall, with comma-shaped nuclei and collagenous extracellular matrix (Fig. 2); more deeply in the wall a denser cellular proliferation, particularly around vessels, with prominent cytological atypia, mitoses, and infiltrative pattern of growth were present (Fig. 3). The tumor was in close contact with peripheral nerves.

Considering the possible differential diagnoses, the absence of a residual urothelial carcinoma in situ together with the absence of cytokeratin immunoreactivity of the tumor, excluded a mixoid variant of sarcomatoid carcinoma. Moreover, the diffuse involvement of the organ without polipoid masses, the absence of inflammation and the presence of prominent atypia were not compatible with a myofibroblastic inflammatory tumor. Likewise, atypia and infiltrative pattern of growth were not compatible with a large neurofibroma or with other benign entities. Moreover, absence of fascicles and cytological features were not suggestive of a leyomiosarcoma; finally, the lack of lipoblasts and a proper vascular network ruled out a liposarcoma.

We performed a wide panel of immunohistochemical stainings that showed tumor immunoreactivity for S100, p16, neurofilaments, CD56 and negative

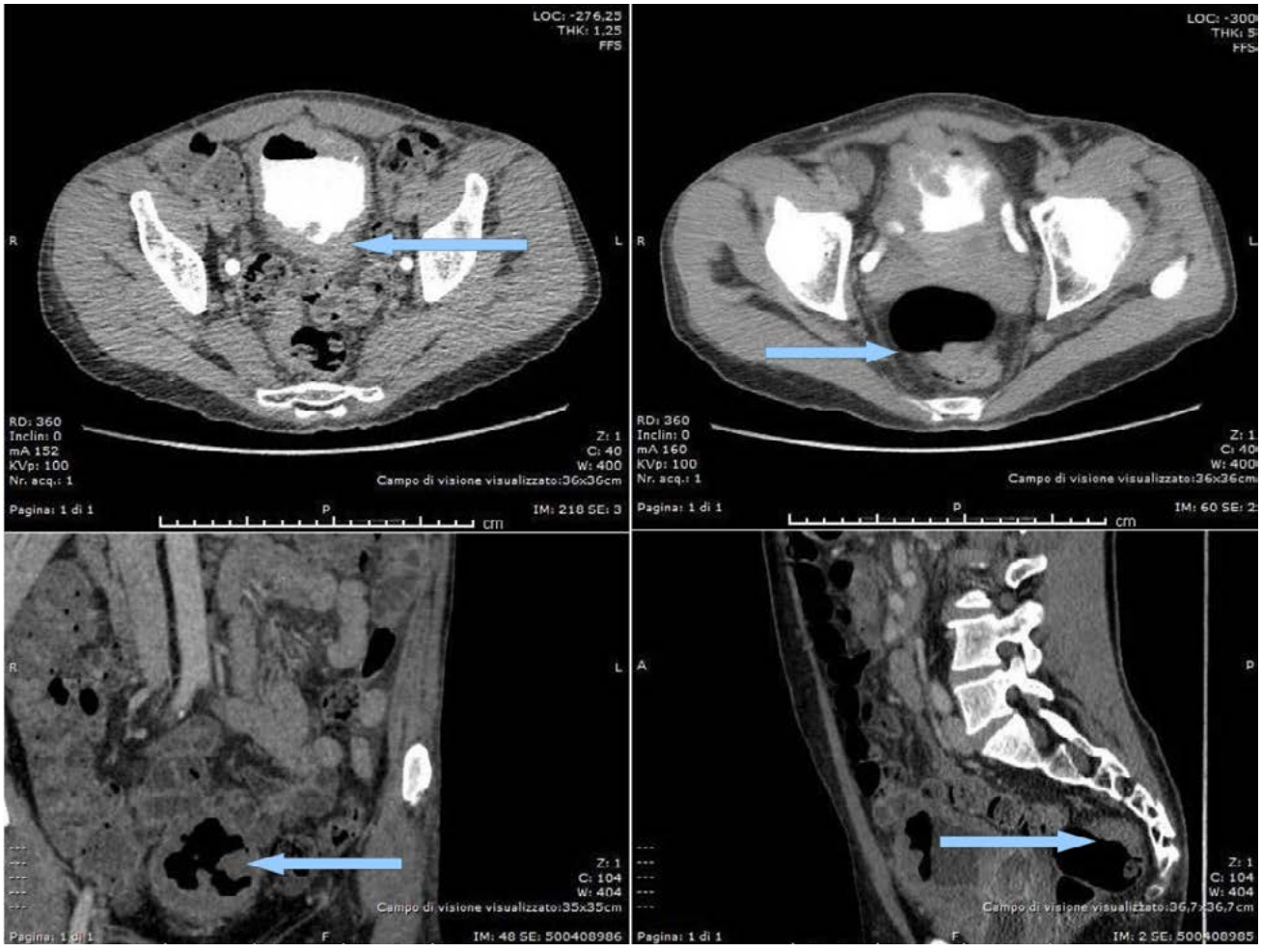


Fig. 1. CT scan diffuse involvement of the bladder with uneven thickening of the entire wall.

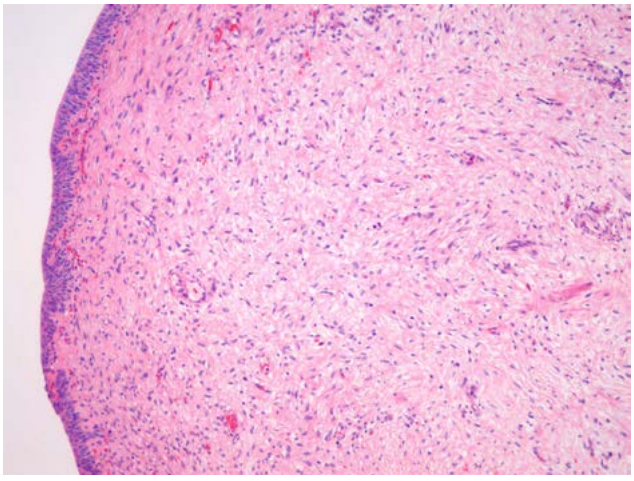


Fig. 2. Mesenchymal hypocellular proliferation of the subepithelial more superficial fascicles of the tumor (H&E 10x PF).

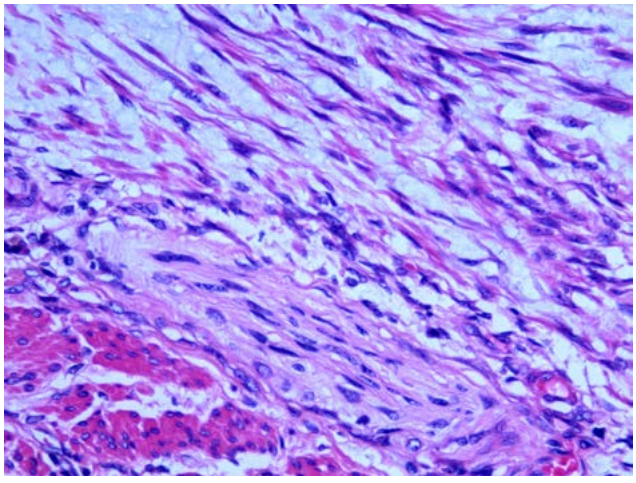


Fig. 3. Hypercellular proliferation of MPNST (H&E 40x PF) with involvement of muscolaris propria bundles and nuclear atypia.

staining for cytokeratin pool, EMA, MDM2, p63, ALK, CD117, DOG1, myogenin, desmin and actin smooth muscle.

On the basis of the morphological and immunohistochemical features of the tumor, as well as on the basis of the presence of an association with NF1, we reached a diagnosis of malignant peripheral nerve sheath tumour.

Interestingly, during a surgical bowel recanalization performed after a few months, numerous small nodules were found, histologically identified as multiple gastrointestinal stromal tumor (GIST).

Discussion

Malignant peripheral nerve sheath tumours mainly affect adults with a roughly equal sex distribution and tend to occur at a younger age in patients with NF1 [10 11], with a peak incidence in the fourth decade. NF1, called also von Recklinghausen disease from the first clinical description, is an autosomal dominant disorder characterized by cafè au lait spots, axillary and inguinal freckels, Lisch nodule of the iris, two or more neurofibromas including plexifom neurofibroma, distinctive bone lesions such as sphenoid dysplasia and coexistence with a variety of neoplasms such as optic gliomas, higher grade astrocytic neoplasms and pheochromocytomas.

NF1 is a common genetic disease with a high rate of penetrance and affects approximately 1 in 3500 newborns. At the basis of this disease are germ line mutations (i.e. deletions, insertions, stop mutations and splicing mutations) causing inactivation of the NF1 tumor suppressor gene located on 17q11.2. Interestingly, the large size of the NF1 gene, the different mutations and the complexity of the disease are at the base of the extremely variable expressivity of the disease [10 11].

A very important problem in patients with NF1 disease is posed by malignant neoplastic occurrences. The exact incidence is not determinated but the lifetime risk of developing MPNST in NF1 patients is 5 to 10-13% [10 11 13], while it is 0.001% in the general population; moreover, individuals with NF1 microdelection have 16-26% risk [12] of malignant transformation. MPNST in NF1 mainly arise in the extremities, followed by the trunk and the head/neck area. Patients with MPNST associated with NF1 have a poorer outcome than sporadic cases and this neoplasm is the main cause of death in patients with NF1 [13].

The occurrence of MPNST arising in the bladder is extremely rare with only a few cases reported in the literature [6-9], and never with such an entire organ involvement. Two of them arose from a malignant transformation of previous neurofibroma in the setting of NF1 [8 9], and two cases occurred sporadically [6 7], in one case [7] presenting at the microscope in an epithelioid variant. In these former cases, MPNST have been observed arising from the trigone and from the lateral or posterior walls of the bladder, with multiple nodules with surface ulceration and areas of necrosis. The tumor sometimes infiltrated focally the entire thickness of the bladder wall, with involvement of perivesical soft tissues or pelvic peritoneum, or may exhibit metastases [7].

In our patient there were urological symptoms due to a mass effect and we had no information about previous lesions or deep-seated plexiform neurofibroma. How-

ever, in our case the presence of von Recklinghausen disease helped us to frame the lesion in the correct clinical context and the findings of mitosis and atypia as well as the phenotype supported us in achieving the diagnosis of MPNST. Moreover, the multiple gastrointestinal stromal tumors (GIST), identified during a surgical bowel recanalization performed after a few months, represent described gastrointestinal manifestations of NF1 [14].

The differential diagnosis between neurofibroma and low grade MPNST is difficult [15] because these lesions represent a histological continuum without clear histological demarcation. Indeed, in the literature no clear cut-off for mitotic index or atypia indicating malignancy is reported, although a poorer prognosis is associated with large tumors (with size varying from 5 to 7 cm in different studies), association with NF1, a mitotic index of greater than 6/10x high-power fields and incomplete resection [10].

Effective targeted molecular treatments are still lacking, hence surgical resection remain the main treatment, also in young patients [10 16]. The recurrence rate is up to 40%, frequently with subsequent hematogenous metastasis. Five-year survival has varied in series from 26 to 60%, and 10-year survival is approximately 45% [10]. At 18 months after surgery our patient had no signs of recurrence.

CONFLICT OF INTEREST STATEMENT

None declared.

References

1 Cheng L, Zhang S, Alexander R, et al. *Sarcomatoid carcinoma of the urinary bladder: the final common pathway of urothelial carcinoma dedifferentiation*. Am J Surg Pathol 2011;35:e34-e46. https://doi.org/10.1097/PAS.0b013e3182159dec

2 Leuschner I, Harms D, Mattke A, Koscielniak E, Treuner J. *Rhabdomyosarcoma of the urinary bladder and vagina: a clinicopathologic study with emphasis on recurrent disease: a report from the Kiel Pediatric Tumor Registry and the German CWS Study*. Am J Surg Pathol 2001;25:856-64. https://doi.org/10.1097/00000478-200107000-00003

3 Cheng L, Lopez-Beltran A, Bostwick DG. *Bladder Pathology*. Wiley-Blackwell. New Jersey 2012.

4 Martin SA, Sears DL, Sebo TJ, Lohse CM, et al. *Smooth muscle neoplasms of the urinary bladder: a clinicopathologic comparison of leiomyoma and leiomyosarcoma*. Am J Surg Pathol 2002;26:292-300. https://doi.org/10.1097/00000478-200203000-00002

5 Engel JD, Kuzel TM, Moceanu MC, Oefelein MG, Schaeffer AJ. *Angiosarcoma of the bladder: a review*. Urology 1998;52:778-84. https://doi.org/10.1016/s0090-4295(98)00286-6

6 Sari A, Bal K, Tunakan M, Ozturk C. *A case of a sporadic malignant peripheral nerve sheath tumor of the urinary bladder with concomitant in situ urothelial carcinoma treated by transurethral resection*. Indian J Pathol Microbiol 2011;54:147-9. https://doi.org/10.4103/0377-4929.77376

7 Eltoum IA, Moore RJ 3rd, Cook W, et al. *Epithelioid variant of malignant peripheral nerve sheath tumor (malignant schwannoma) of the urinary bladder*. Ann Diagn Pathol 1999;3:304-8. https://doi.org/10.1016/s1092-9134(99)80027-6

8 Kalafatis P, Kavantzas N, Pavlopoulos PM, et al. *Malignant peripheral nerve sheath tumor of the urinary bladder in von Recklinghausen disease*. Urol Int 2002;69:156-9. https://doi.org/10.1159/000065568

9 Rober PE, Smith JB, Sakr W, et al. *Malignant peripheral nerve sheath tumor (malignant schwannoma) of urinary bladder in von Recklinghausen neurofibromatosis*. Urology 1991;38:473-6. https://doi.org/10.1016/0090-4295(91)80242-y

10 Thway K, Fisher C. *Malignant peripheral nerve sheath tumor: pathology and genetics*. Ann Diagn Pathol 2014;18:109-116. https://doi.org/10.1016/j.anndiagpath.2013.10.007

11 Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. *Malignant peripheral nerve sheath tumours in neurofibromatosis 1*. J Med Genet 2002;39:311-4. https://doi.org/10.1136/jmg.39.5.311

12 De Raedt T, Brems H, Wolkenstein P, et al. *Elevated risk for MPNST in NF1 microdeletion patients*. Am J Hum Genet 2003;72:1288-1292. https://doi.org/10.1086/374821

13 Ducatman BS, Scheithauer BW, Piepgras DG, et al. *Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases.* Cancer 1986;57:2006-21. https://doi.org/10.1002/1097-0142(19860515)57:10<2006::aid-cncr2820571022>3.0.co;2-6

14 Agaimy A, Vassos N, Croner RS. *Gastrointestinal manifestations of neurofibromatosis type 1 (Recklinghausen's disease): clinicopathological spectrum with pathogenetic considerations*. Int J Clin Exp Pathol 2012;5:852-62.

15 Miettinen MM, Antonescu CR, Fletcher CDM, et al. *Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1-a consensus overview*. Hum Pathol 2017;67:1-10. https://doi.org 10.1016/j.humpath.2017.05.010

16 Bergamaschi L, Bisogno G, Manzitti C, et al. *Salvage rates and prognostic factors after relapse in children and adolescents with malignant peripheral nerve sheath tumors*. Pediatr Blood Cancer 2018;65:10. https://doi.org/10.1002/pbc.26816

Received: October 29, 2019 - Accepted: October 30, 2019

PATHOLOGICA 2019;111:369-374; doi: 10.32074/1591-951X-28-19

Case report

Eosinophilic cytoplasmic inclusions in type 2 papillary renal cell carcinoma

M. Ungari, M. Trombatore, G. Ferrero, M.D. Gusolfino, L. Manotti, G. Tanzi, E. Varotti, R. Bertoni

Department of Pathology, ASST Cremona

Summary

A case of a patient with type 2 papillary renal cell carcinoma with eosinophilic cytoplasmatic inclusions is presented. About 50% of tumor cells were characterized by a well-circumscribed intra-cytoplasmatic round-to-oval or irregular inclusion/globule. Inclusions were 7-30 micron in diameter. They were glassy and pale to slightly eosinophilic in color in hematoxylin and eosin, were stained red by trichrome and were negative for periodic acid-Schiff reaction. Immunohistochemically, globules were negative for PAX8, epithelial membrane antigen, Carbonic Anhydrase IX, pan-cytokeratin (AE1/AE3), CD10, S100 protein, α-smooth-muscle actin, cytokeratin 7 and cytokeratin 34βE12. Glassy hyaline globules were not detected in any adjacent normal kidney cells. The presence of eosinophilic cytoplasmic inclusions in renal cell carcinoma, especially in papillary renal cell carcinoma, has been rarely emphasized in the literature. In this article, we review similar cases in the literature and discuss the nature of eosinophilic globules.

Key words

Ppppillary renal cell carcinoma • Eosinophilic cytoplasmatic inclusions • Sarcomatoid differentiation • Aggressive behavior

Introduction

Papillary renal cell carcinoma (PRCC) is the second most commonly encountered morphotype of renal cell carcinoma (RCC). Among adults the mean age distribution is 59-63 years [1]. Tumors are usually confined to the cortex within the renal capsule at the time of nephrectomy [2]. The cut surface of PRCC vary in color from gray to yellow to red-brown. The tumor is well circumscribed with a fibrous pseudo-capsule. It may contain intra-tumoral hemorrhage and necrosis and/or cystic degeneration. It is derived from renal tubular epithelium and has a papillary or tubulo-papillary architecture. Some tumors show a predominantly tubular architecture or a solid appearance caused by papillae tightly packed [3]. Papillae have delicate or hyalinized fibrovascular cores, that often contain psammoma bodies and foamy macrophages, covered by single layer or by pseudostratified layers of epithelial cells. PRCC, although not reported by I.S.U.P/W.H.O. 2016, has been separated in two subtypes, based on morphologic features [4 5]: a) Type 1 carcinoma have papillae covered by single layer of small epithelial cells with scanty, pale, usually basophilic cytoplasm; b) Type 2 carcinoma have papillae covered by pseudo-stratified large cells with voluminous eosinophilic cytoplasm and higher nucleolar grade. Approximately 5% of PRCCs show sarcomatoid changes [4]. Renal tumors showing papillary architecture but also features of recognized morphotypes of RCC (i.e. collecting duct carcinoma, mucinous tubular and spindle cell carcinoma, hereditary leiomyomatosis and RCC-associated RCC, and MiT family translocation RCC) should not be diagnosed as PRCC [1]. Immunohistochemical studies of PRCC show positive reactions for cytokeratin AE1/AE3, CAM5.2, high molecular weight cytokeratins, epithelial membrane antigen, AMACR, RCC antigen, vimentin, CD10, PAX8, PAX2 [3-8]. Eosinophilic inclusions are the light microscopic manifestations of aggregates of cytoplasmic products or organelles, visualized with

How to cite this article: Ungari M, Trombatore M, Ferrero G, et al. *Eosinophilic cytoplasmic inclusions in type 2 papillary renal cell carcinoma.* Pathologica 2019;111:369-74. https://doi.org/10.32074/1591-951X-28-19.

Correspondence: Marco Ungari, Department of Pathology, ASST Cremona, viale Concordia 1, CR 26100, Italy - Tel. +39 0372 405513 - Fax +39 0372 405738 - E-mail: m.ungari@asst-cremona.it

hematoxylin and eosin (H&E). The presence of glassy hyaline globules (GHG) in renal carcinomas has been rarely emphasized in the literature, in particular in PRCC [9-15]. Here, we report a case of eosinophilic cytoplasmic inclusions in PRCC, with review of the literature.

Materials and methods

Fixation of tissue is carried out in 10% neutral buffered formalin for 24 hours. Once tissue is embedded in paraffin, a 3-4 micron tissue section is cut onto charged glass slides. Positive and negative controls were present into the tissue or added on the glass.
The detection system for immunostaining is BOND Polymer Refine Detection on staining platform LEICA BOND III. 30 min at 100°C in Bond Epitope Retrieval Solution 1 used to antigen retrieval for antibodies: α-smooth-muscle actin (clone AB1-1A4; Dako; 1:1000), Epithelial Membrane Antigen (clone E29; Dako; 1:500), Immunoglobulin light chains λ (polyclonal; Dako; 1:100.000), CD10 (clone 56C6; Leica; 1:50); 20 min for antibody PAX8 (clone Mrq50; Menarini; 1:800) and Immunoglobulin light chains κ (polyclonal; Dako; 1:50.000); 15 min for antibody Carbonic Anhydrase IX (clone TH22; Leica; 1.50); 40 min for antibody Cytokeratin 34βE12 (clone 34βE12; Menarini; 1:100); 30 min at 100°C in Bond Epitope Retrieval Solution 2 for antibody alpha-methylacyl-CoA racemase (clone 13H4; Dako; 1:1000); 10 min at 37°C with Bond Epitope Retrieval Enzyme for antibodies Cytokeratin 7 (clone OV-TL 12/30; Menarini; 1:500) and pan-cytokeratin AE1/AE3 (clone AE1/AE3; Cell Marque; 1:1000); nothing retrieval for S100 protein (polyclonal; Leica; 1:1000).

Case report

In July 2019 a 71-year-old man with a past history of prostatic hyperplasia came to our hospital for hematuria and flank pain. Computer tomography (CT) detected a renal mass. Extensive clinical examination revealed no signs of metastasis. The patient underwent right radical nephrectomy. The patient's post-operative course was without complications. The specimen obtained by nephrectomy weighed 1200 g, with a 12 x 10 x 8 cm well circumscribed mass in the kidney. The cut surface varied from light gray to red-brown, with intratumoral hemorrhage and necrosis (80% of tumor). The tumor showed extension to peri-renal tissues, without extension to fascia of Gerota (distance 1 mm), renal vein, vena cava and lymph nodes (pT3a, A.J.C.C. 8th Ed.) [16]. Histologically the tumor was circumscribed by a fibrous pseudo-capsule (Fig. 1A). The pseudo-capsule was incomplete, with tumor tissue focally intermixed with renal parenchyma. The tumor showed a predominantly tubule-papillary architecture with occasional solid areas (Fig. 1B). Papillae showed delicate or hyalinized fibrovascular cores, that sometimes contained foamy macrophages. No psammoma bodies were detected. Papillae were covered by a single layer or pseudo-stratified layers of large epithelial cells with voluminous eosinophilic cytoplasm, with large and spherical or light irregular nuclei, with prominent nucleoli (grade 3; I.S.U.P./W.H.O. 2016) [1] (Fig. 1C). Approximately 5% of tumor showed sarcomatoid change (grade 4; I.S.U.P./W.H.O. 2016) [1] (Fig. 1D). About 50% of tumor cells were characterized by a well-circumscribed intracytoplasmatic round-to-oval or irregular inclusion/globule with a halo. Inclusions were 7-30 micron in diameter. They were glassy and pale to slightly eosinophilic in color in H&E ("glassy hyaline globules"). Glassy hyaline globules were stained red by trichrome staining, but stained negatively with periodic acid-Schiff (PAS) with or without diastase treatment (Fig. 3), silver and Hale's colloidal iron. Glassy hyaline globules were not detected in any adjacent normal kidney cells. A moderate number of small lymphocytes and plasma cells were interspersed throughout the tumor. Immunohistochemical studies showed diffuse expression of PAX8, alpha-methylacyl-CoA racemase and epithelial membrane antigen, only focal reaction for Carbonic Anhydrase IX and pan-cytokeratin (AE1/ AE3), and negative staining for immunoglobulin light chains κ and λ, CD10, S100 protein, α-smooth-muscle actin, cytokeratin 7 and cytokeratin 34βE12 (Fig. 2). Immunohistochemistry indicated that the eosinophilic inclusions were negative for all antibodies studied. We did not perform electron microscopy evaluation of eosinophilic inclusions. At the time of nephrectomy total body nuclear magnetic resonance was negative. Four months later nuclear magnetic resonance showed bilateral pleural effusions, subcapsular hepatic nodule, suspected for metastasis, and multiple bone metastasis. A bone biopsy of iliac crest showed epithelial atypical cells organized in cellular cords with "renal phenotype" by immunohistochemistry: expression of cytokeratin (AE1/AE3), CD10, PAX8, Carbonic Anhydrase IX, and Vimentin (Fig. 3).

Discussion

In the literature, the presence of glassy hyaline globules in renal carcinomas has been rarely discussed, in particular in PRCC [9-15]. Eosinophilic inclusions can be positive or negative in PAS with or without diastase treatment [9 13 15] and are usually stained red with tri-

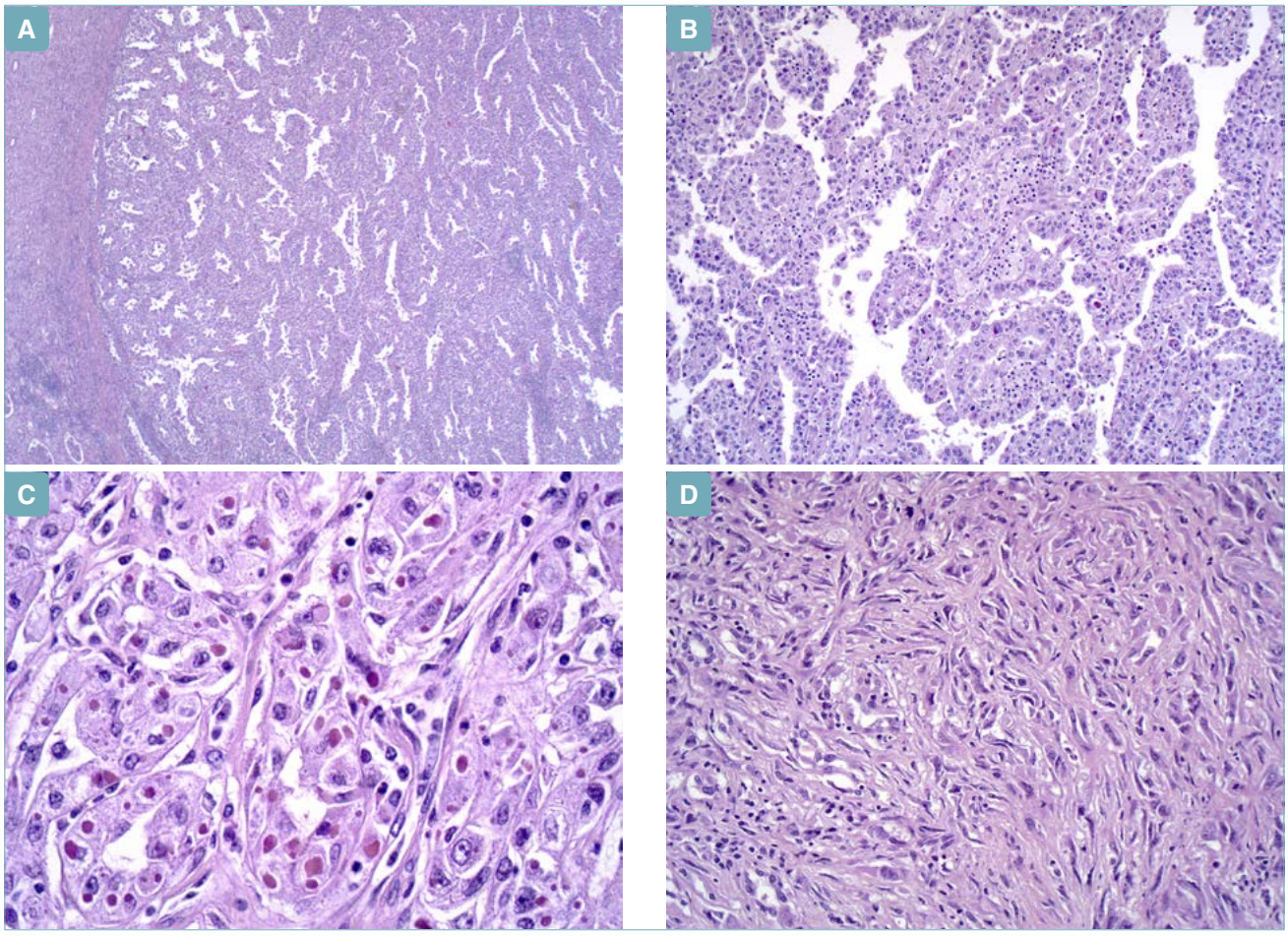


Fig. 1. Hematoxylin and eosin. (A) The tumor was well circumscribed with a fibrous pseudo-capsule. (B) Tubule-papillary architecture; papillae showed delicate or hyalinized fibrovascular cores, with inflammatory cells and occasional foamy macrophages. (C) Papillae were covered by atypical epithelial cells characterized by a well-circumscribed intra-cytoplasmatic round-to-oval or irregular inclusion with a halo. (D) Approximately 5% of tumor showed sarcomatoid changes.

chrome [9 13 15]. The eosinophilic cytoplasmic inclusions in the present case were PAS negative but stained red with trichrome. It has been suggested GHG may be aggregations of intermediate filaments (i.e. Mallory bodies), but no antigenic expression has been detected in immunohistochemistry. Ultrastructurally, intracytoplasmic eosinophilic inclusions consist of a central dense granular layer surrounded by membrane-bound oval organelles containing dense substances, and an outermost layer of clear space between the inclusion and cytoplasmic organelles [11 17]. They may consist of accumulation of an amorphous secretion of stellate shape inside of the cisternae of the rough endoplasmic reticulum [9 10]. Eosinophilic cytoplasmic inclusions were detected in 49 of 64 clear cell RCCs and in 5 of 33 PRCCs, but no GHG were found in 22 cases of chromophobe cell carcinomas and 26 renal oncocytomas [9]. In clear cell RCC, Paneth cell-like granules, which are closely packed, variably sized eosinophilic cytoplasmic inclusions, have been detected. They are confined to the apical portion of the cytoplasm, positive with PAS with and without diastase digestion and negative with trichrome stain. Ultrastructurally, these inclusions consist of an electron-dense single membrane-bound structure, which is consistent with lysosomes [16]. Also, in RCC and oncocytoma PAS-positive spherical globules, which are accumulations of basement membrane material, have been detected in extracellular location [19]. Several types of inclusions have been identified in renal neoplasms. GHG are a characteristic feature of clear cell RCC and in a small minority of papillary renal cell carcinomas, but not in chromophobe RCC and oncocytoma. Thus, the presence of GHG in a renal cell tumor may be useful for

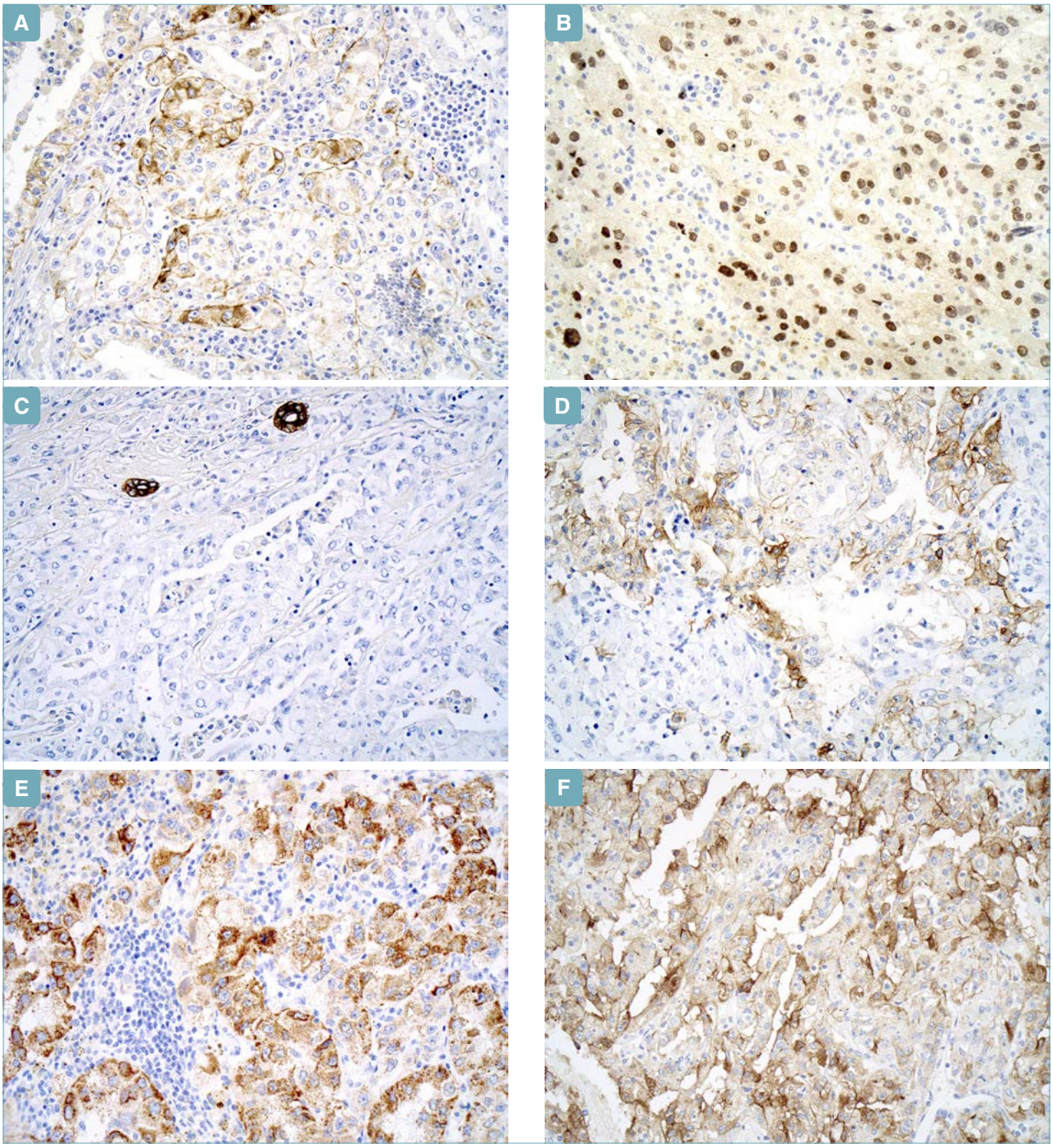


Fig. 2. Immunohistochemistry reactions. (A) Focal expression of pan-cytokeratin AE1/AE3. (B) Diffuse expression of PAX 8. (C) Negative staining for cytokeratin 7 (two normal tubules positive). (D) Focal expression of carbonic anhydrase IX. (E) Diffuse expression of alpha-methylacyl-CoA racemase, with negative eosinophilic cytoplasmic inclusions. (F) Diffuse expression of epithelial membrane antigen, with negative eosinophilic cytoplasmic inclusions.

excluding a diagnosis of chromophobe cell carcinoma or oncocytoma.

In conclusion, we present a rare case of papillary type 2 carcinoma with intra-cytoplasmic glassy hyaline globules, focal sarcomatoid differentiation, and aggressive behavior.

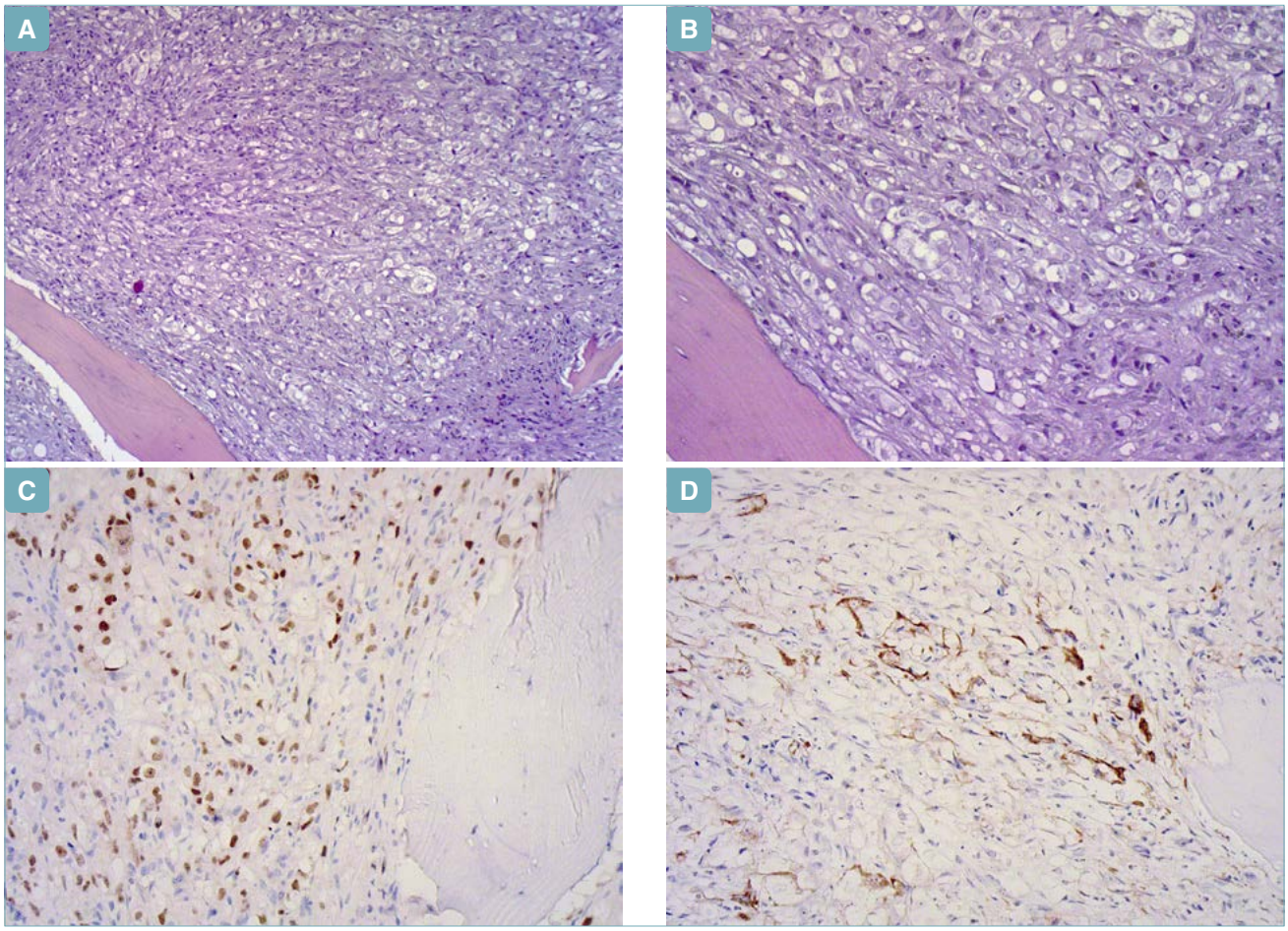


Fig. 3. (A) Bone localization of epithelial atypical cells organized in cellular cords (hematoxylin and eosin). (B) Large atypical cells with irregular nucleus and clear, abundant cytoplasm (hematoxylin and eosin). (C) Diffuse expression of PAX 8. (D) Focal expression of Carbonic Anhydrase IX.

Conflict of interest statement

None declared.

References

1. Delahunt B, Algaba F, Eble J, et al. *Papillary renal cell carcinoma*. In Moch H, Humphrey PA, Ulbright TM, et al, eds. *WHO Classification of Tumours Of the Urinary System nd Male Genital Organs*. 4th ed. Lyon, France; IARC Press 2016.
2. Murphy WM, Grignon DJ, Perlman EJ. *Tumors of the kidney, bladder, and related urinary structures*. AFIP - Atlas of Tumor Pathology. 4th Series; Fascicle 1. Washington DC 2004.
3. Renshaw AA, Zhang H, Corless CL, Fletcher JA, Pinis MR. *Solid variants of papillary (chromophil) renal cell carcinoma: clinicopathologic and genetic features*. Am J Surg Pathol 1997;21:1203-9. https://doi.org/10.1097/00000478-199710000-00011
4. Delahunt B, Eble JN. *Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors.* Mod Pathol 1997;10:537-44.
5. Delahunt B, Eble JN, McCredie MR, et al. *Morphologic typing of papillary renal cell carcinoma: comparison of growth kinetics and patients survival in 66 cases*. Hum Pathol 2001;32:590-5. https://doi.org/10.1053/hupa.2001.24984
6. Avery AK, Beckstead J, Renswa AA, Corless CL. *Use of antibodies to RCC and CD10 in the differential diagnosis of renal neoplasms.* Am J Surg Pathol 2000; 24:203-10. https://doi.org/10.1097/00000478-200002000-00006
7. Tretiakova MS, Sahoo S, Takahashi M, et al. *Expression of alpha-methylacyl-CoA racemase in papillary renal cell carcinoma*. Am J Surg Pathol 2004;28:69-76. https://doi.org/10.1097/00000478-200401000-00007
8. Kuroda N, Tanaka A, Ohe C, Nagashima Y. *Recent advances of immunohistochemistry for diagnosis of renal tumors.* Pathol Int Aug 2013;63:381-90. https://doi.org/10.1111/pin.12080
9. Hes O, Michal M, Sulc M, et al. *Glassy hyaline globules in granular cell carcinoma, chromophobe cell carcinoma, and oncocytoma of the kidney.* Ann Diagn Pathol 1998;2:12-8. https://doi.org/10.1016/s1092-9134(98)80030-0
10. Hes O, Benakova K, Vanecek T, et al. *Clear cell type of renal cell carcinoma with numerous hyaline globules: a diagnostic pit-*

fall. Pathol Int Mar 2005;55:150-4. https://doi.org/10.1111/j.1440-1827.2005.01805.x

11. Dambara N, Shikata N, Kawamura H, et al. *Eosinophilic cytoplasmic inclusions in papillary renal cell carcinoma*. Med Mol Morphology 2005;38:262-6. https://doi.org/10.1007/s00795-005-0286-3

12. Kuroda N, Satake H, Miyazaki E, et al. *Collecting duct carcinoma exhibiting diastase resistant PAS-positive globular cytoplasmic inclusions and rhabdoid features arising in adult polycystic kidney disease: a case report*. Int J Surg Pathol 2004;12:171-7. 10.1177/106689690401200215

13. Jacirdar J, Irie T, French SW, et al. *Globular Mallory-like bodies in renal cell carcinoma: report of a case and review of cytoplasmic eosinophilic globules.* Hum Pathol 1985;16:949-52. https://doi.org/10.1016/s0046-8177(85)80135-0

14. Unger P, Perino G, Dchiff H, et al. *Eosinophilic globules resembling Mallory bodies in a renal cell carcinoma.* NY State J Med 1992;92:18-20.

15. Gatalica Z, Miettinen M, Kovatich A, McCue PA. *Hyaline globules in renal cell carcinoma and oncocytomas.* Hum Pathol 1997;28:400-3. https://doi.org/10.1016/s0046-8177(97)90026-5

16. A.J.C.C. *Cancer Staging Manual*. 8th ed. American College of Surgeons 2018.

17. Krishnan B, Truong LD. *Renal epithelial neoplasms: the diagnostic implications of electron microscopic study in 55 cases.* Hum Pathol 2000;33:68-79. https://doi.org/10.1053/hupa.2002.30210

Received: September 26, 2019 - Accepted: November 11, 2019

PATHOLOGICA 2019;111:375-381; doi: 10.32074/1591-951X-32-19

Historical Pathologica

Enrico Sertoli and the supporting cells of the testis

"Morphology is function"

C. Patriarca[1], M. Colecchia[2], C.A. Clerici[3]

[1] Pathology Unit, St Anna Hospital (ASST Lariana), Como, Italy; [2] Pathology Department, SSD Uropathology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; [3] Department of Oncology and Hemato-oncology, University of Milan, and SSD Clinical Psychology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Summary

In 1865, Enrico Sertoli, at the age of 23, published an article in his own name entitled: "About the existence of special branched cells in the seminiferous tubules of the human testis". These were Sertoli's ideal cells; in this paper he arrived at a perspicacious description of the morphology and function of these cells and in the subsequent articles he investigated the topic of spermatogenesis. Despite the importance of Sertoli's discovery, the attention of the scientific literature remained very limited after Sertoli's death for half a century and the partial eclipse finished only in the 1970s of the twentieth century.

"*Il testicolo dell'uomo è costituito, come ognun sa, nella sua parte più essenziale,*
da un numero stragrande di piccoli canaletti, tortuosi e tra loro aggomitolati,
che terminando nella loro estremità a fondo cieco oppure ad anse,
a due e tre superiormente si uniscono in unici canaletti non tortuosi"
Enrico Sertoli, 1865

Morphology is function

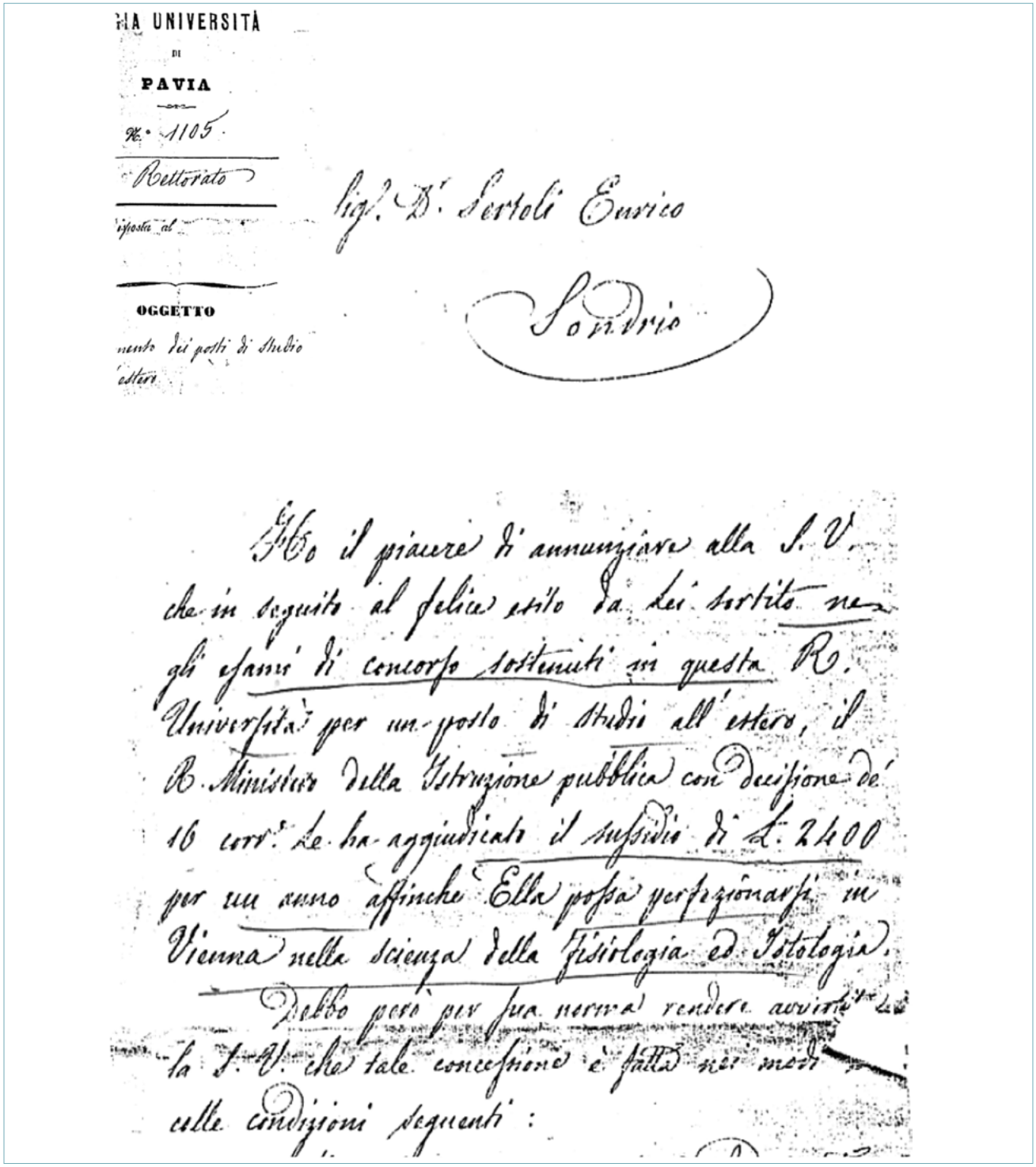
Before an audience of students at the *Scuola Superiore di Medicina Veterinaria* during the opening ceremony for the academic year 1872-73, Enrico Sertoli concluded his talk by exhorting his listeners to study histology. In somewhat bombastic tones, typical of the rhetoric of his time, he said: "Take courage, young scholars! Don't be deterred by the difficulties you will encounter in studying such an important part of medical knowledge". After giving the students a good scolding ("*Le forme microscopiche, che voi forse non sapete ancora abbastanza apprezzare, almeno se arguir debbo dalla riluttanza che molti di voi mostrarono nel sottoporsi a prove concernenti questa parte tanto essenziale dello scibile medico*"), he dedicated a part of his talk to emphasizing the close relationship between form and function [1]. He judged that the most effective models to use to persuade his audience could be drawn "*by taking for example the nerve cell, with its multiple branches*". Although the central nervous system was not his field of study, while working and studying in the laboratories at the Botanical Gardens in Pavia, he had happened to come into close contact with a student a year younger than him, Camillo Golgi. This was the man who, in 1873, was adding the final touches to his "black reaction" technique for staining neurons that – many years later – earned him a Nobel Prize that he shared with Ramon J Cajal. "*Form acquires its real importance when it is acknowledged as a function",* Sertoli said, and again: *"the functional manifestations are closely related to the morphological composition".* ("*La vita inchiude il concetto della forma (…) e la conoscenza della forma (…) ci rappresenta graficamente le leggi secondo le quali i processi della vita si compiono (…). Dice il prof. Oehl che la forma (…) determina la direzione, come un filo telegrafico la direzione del movimento*").

How to cite this article:Patriarca C, Colecchia M, Clerici CA. *Enrico Sertoli and the supporting cells of the testis. "Morphology is function"*. Pathologica 2019;111:375-81. https://doi.org/10.32074/1591-951X-32-19.

Correspondence: Carlo Patriarca, Servizio di Anatomia Patologica, Ospedale Sant'Anna di Como, via Ravona, 22020 San Fermo della Battaglia (CO), Italy - E-mail: carlo.patriarca@asst-lariana.it

It was under the guidance of the histologist and physiologist Eugenio Oehl that Enrico Sertoli graduated in 1865 [2]. His virtual peers Camillo Golgi and Giulio Bizzozzero were also attending Oehl's laboratory during the same period. Still in 1865, Sertoli published an article in his own name in the journal *il Morgagni* entitled: "*Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano*" [3]. These were Sertoli's ideal cells, one of the most beautiful examples of the relationship between form and function to be found in nature.

Enrico Sertoli (Sondrio 1842-1910) was the sec-



RIA UNIVERSITÀ
DI
PAVIA

N.° 1105.

Rettorato

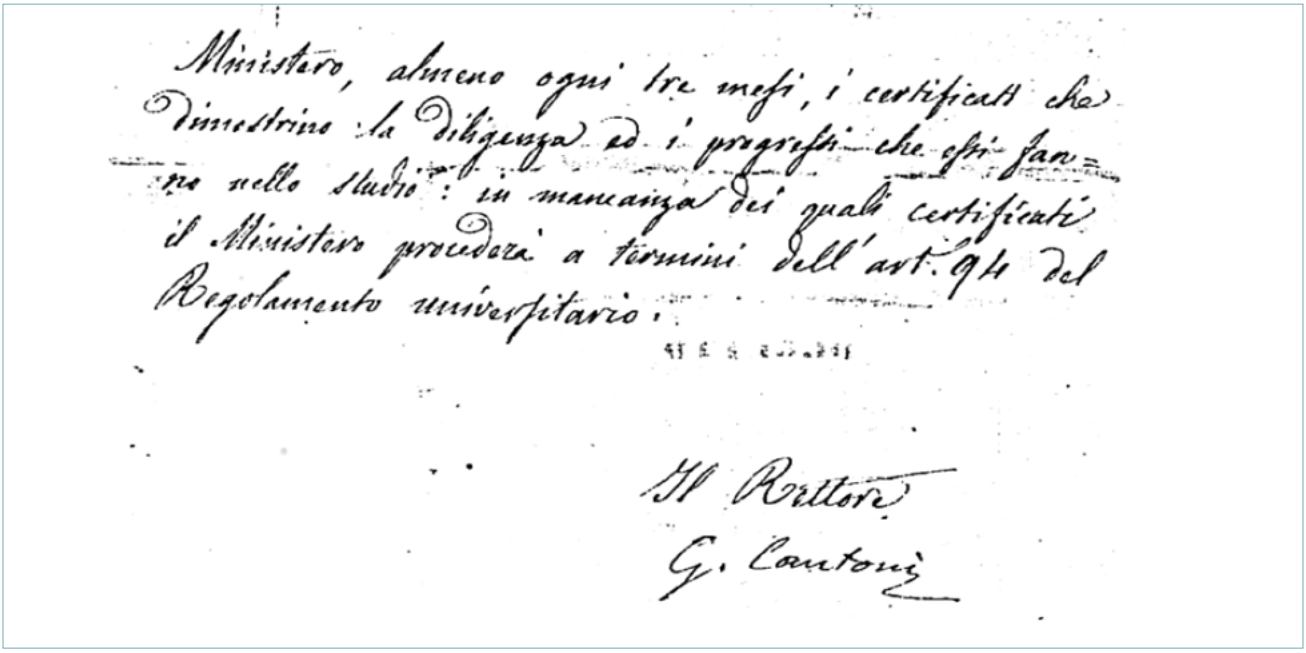
Risposta al

OGGETTO

mento dei posti di studio
estero

Sig.r D.r Sertoli Enrico
Sondrio

Ho il piacere di annunziare alla S. V.
che in seguito al felice esito da Lei sortito ne
gli esami di concorso sostenuti in questa R.
Università per un posto di studii all'estero, il
R. Ministero della Istruzione pubblica con decisione de'
16 corr.e le ha aggiudicato il sussidio di L. 2400
per un anno affinchè Ella possa perfezionarsi in
Vienna nella scienza della Fisiologia ed Istologia.
Debbo però per sua norma rendere avvisata
la S. V. che tale concessione è fatta nei modi
colle condizioni seguenti :


Ministero, almeno ogni tre mesi, i certificati che
dimostrino la diligenza ed i progressi che essi fan-
no nello studio: in mancanza dei quali certificati
il Ministero procederà a termini dell'art. 94 del
Regolamento universitario.

Il Rettore
G. Cantoni

Fig. 1. Excerpt from a letter written by the Rector G. Cantoni, dated 18 October 1865: *in seguito al felice esito da lei sortito negli esami (…) per un posto di studio all'estero, il Regio Ministero dell'Istruzione pubblica (…) le ha aggiudicato un sussidio di lire 2400 per un anno, affinchè ella possa perfezionarsi in Vienna nella scienza della Fisiologia e Istologia. (…) I sussidi saranno pagati in dodici rate mensili eguali posticipate (…)* (by kind concession of the Sertoli family).

ond-born son of a noble family. He had already graduated in medicine in 1865, at the age of 23, when he departed for Vienna to further his studies at the laboratories of Ernst Brucke, one of the most famous physiologists at the time.

Enrico probably received only a part of the 2400 lire bursary he had been awarded (Fig. 1), because he hastily returned to Italy the next year, when he had the chance to join the Lombard patriots and fight beneath the Stelvio pass against the Austrians [4]. These were the early days of June 1866, at the time of the first military operations in the Third Italian War of Independence. With the 68th Infantry Regiment, Enrico was then sent to Palermo, where the army was engaged in quelling a revolt. When an outbreak of cholera spread from Naples to Sicily, he was finally able to return to being a doctor, albeit in uniform.

After his time in the army, Sertoli returned to his studies, heading this time to Tübingen and the physiology laboratory directed by Hoppe-Seyler, where he obtained a post as assistant and focused his interest on blood proteins [2].

In 1870, at just 28 years old, he was called to teach anatomy and physiology at the *Scuola Superiore di Medicina Veterinaria* in Milan and, once there, he did not forget *his* cells.

The institute had been established by Eugenio Beauharnais in 1805, growing from what was originally the *Scuola Minore di Anatomia Veterinaria e Mascalcia* created by the previous Austrian government. Sertoli was appointed director of physiology and kept occupied full time in running the laboratory [5], but this did not prevent him from returning to debating the function of the supporting cells in the seminiferous tubules. He reiterated their trophic and supporting role, but rejected the idea that these cells might "*take part directly in the formation of the sperm cells)*" [6]. There was a widespread conviction among histologists of the 19th century that, in addition to the known germ cells, there were epitheliomorphic elements that the famous Swiss histologist Kolliker erroneously defined as polygonal [2 7]. It was believed that these elements originated from germ cells, or else that part of them gave rise to germ cells. But Sertoli had never seen spermatozoa inside such cells, which also had a shape too dissimilar from that of the seminiferous cells. He argued thus in his article of 1865, concluding that he was not convinced that the branched cells produced spermatids.

One wonders what means Sertoli had used to arrive at such a perspicacious description of the morphology and function of these cells. As was common practice

in his time, at 20 years old, Sertoli had bought himself a Belthle microscope (a German instrument that was then top of the range, with 10x and 20x lenses) - and he must have been very fond of it because it was recently still in an excellent state of repair and in the hands of his family [2].

Sertoli was in the habit of fixing tissue samples in a solution of mercury salts (mercuric chloride, which went by the name of 'corrosive sublimate') and ammonia. After three or five days, he would then proceed as follows: "*From it I remove a piece of tubule that I tear with the aid of fine needles over a glass dish, adding a drop of distilled water; I cover the preparation with a glass lid and then submit it to observation*" [3]. That was it - no formalin (which was still not commercially available), and no modern microtome sectioning; and, as for hematoxylin, Waldeyer was still fine-adjusting it around the time, and Sertoli was not using it yet.

This is how Sertoli described the 'branched cells' in his article of 1865: "*These cells are irregularly cylindrical or conical with delicate borders and with nuclei that invariably contain a nucleolus. The cytoplasm is transparent, homogeneous, and always contains fine fat droplets. These cells are almost always furnished with very transparent fine processes in which fat droplets are also found (….). In some of these cells one sees a bifurcation or other secondary processes. Other cells of this type send out more processes that branch and sometimes envelop other cells"* (…).

The description goes on in great detail ("*Ho inoltre osservato che questi prolungamenti discostandosi dalla cellula formano delle curve, le quali corrispondendo ad altre formate o ad altri prolungamenti o da incurvamento all'interno di un lato della cellula, costituiscono come delle nicchie semicircolari nelle quali vengono ricettate le cellule seminifere* (…). *Le cellule seminifere sono fortemente aderenti alle pareti dei rispettivi ricettacoli, giacchè per il rotolamento io non riuscii mai a distaccarle*"), and Sertoli comments on how acetic acid and iodine tincture affect the visibility of the cell. Figure 2 shows Sertoli's original drawings, which perfectly illustrate the branched form of these cells and spermatogenesis-supporting function that he envisaged for them, based on a detailed description (here reported only in part) of how these cells cluster to form concave niches. Not only the fat content in the cytoplasm, but also the adhesion of spermatogons and spermatids (that we can now attribute to the role of the adhesion molecules) had already been clearly recognized.

Another significant passage, but an erroneous observation in this case, is documented in Figure 3, accompanied by Sertoli's comment that: "*The processes have also a further function: they guarantee the communication among the branched cells (….) so that the content of the cells is fused"* (…).

Although Sertoli never mentioned the word 'syncitial', and some deny that he would have defined these branched cells as such [7], it seems to us that his own hand drawing leave little space for doubt on the matter. After all, a precise definition of the boundaries of these branched cells must have sometimes been very hard to achieve using the microscopes of Sertoli's time. Even in 1902, von Ebner (a convinced supporter of their nature as precursors of spermatids) [8] agreed that Sertoli's cells were syncytial [2 7]. Whether they were

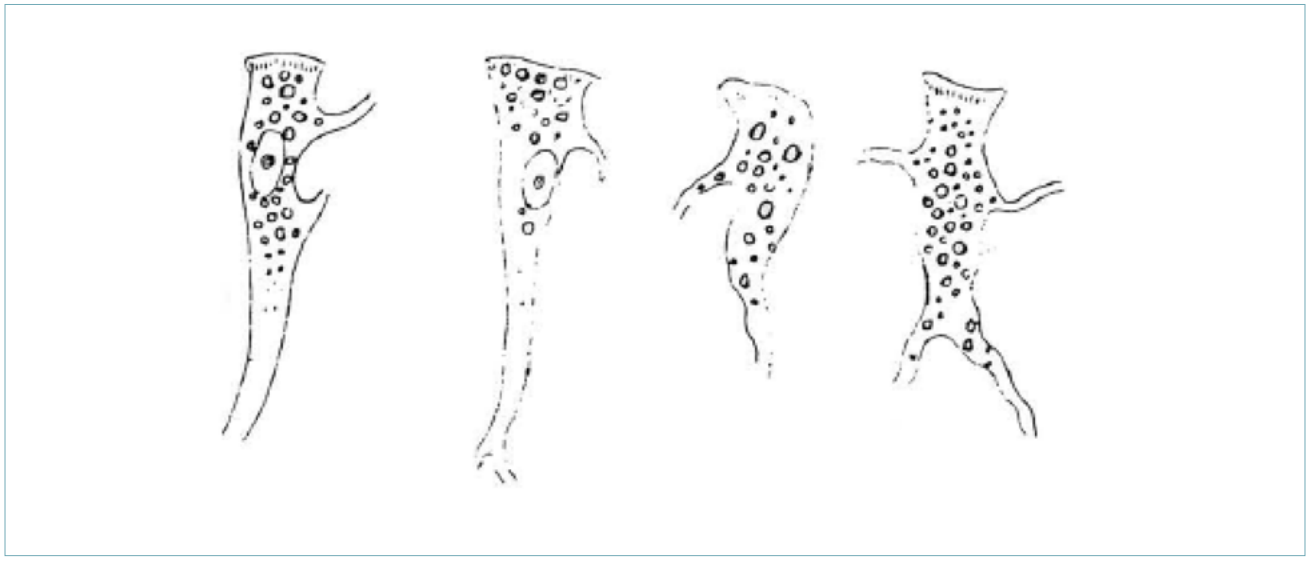


Fig. 2. (*From Sertoli E. Il Morgagni 1865*).

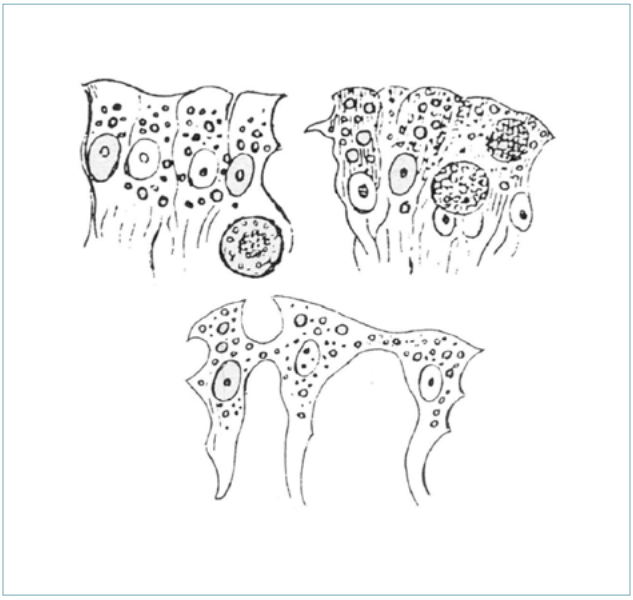


Fig. 3. (*From Sertoli E. Il Morgagni 1865*).

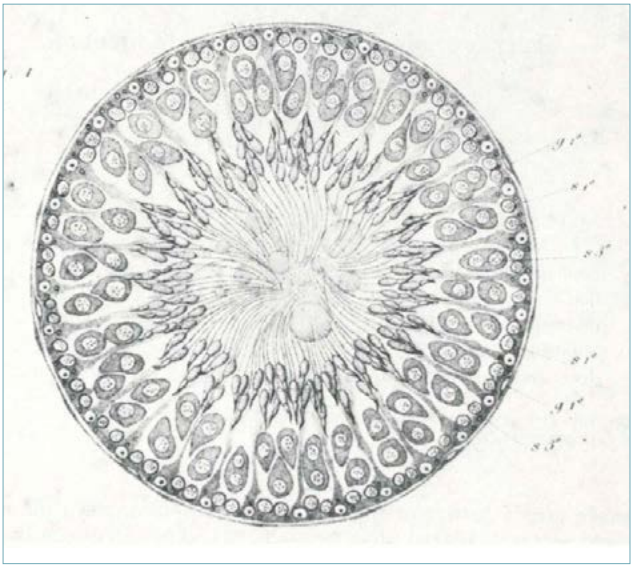


Fig. 4. Sertoli's idealized drawing of 1878 representing a cross section of a seminiferous tubule.

syncitial or not, according to Sertoli, the branched cells "*are joined together in parallel*": this finding was also a massive leap forward, and was only confirmed after 1960, in the modern anatomo-functional concept of the hemato-testicular barrier [7 8].

Sertoli returned to the topic of branched cells in the 1870s [6 9 10], when he clarified their post-mitotic nature, the so-called *fixed cells* (*Dans les cellules fixes (…) décrites sur le nom de cellules ramifiées, on n'observe pas le plus petit indice de caryokinèse, quel que soit le point du canalicule où on le examine* [10]. This was when he started adding Muller fixative and silver nitrate, improving the quality of his lab technique. His work was so clear in its approach and scientific precocious, even on the topic of spermatogenesis (Sertoli provided an accurate description of its various stages) that what he wrote in 1878 [11] was recently translated into English [8]. These are writings in which, among other things, Sertoli quibbled with von Ebner, who called his cells 'spermatoblasts'. They also provide further topographical details of his studies, when he wrote that, adjacent to the *rete testis*, "*I found that the cells in question little by little stopped presenting extensions*. It is worth adding that, even today, pathologists daily engaging in diagnostics are still astounded by Sertoli's ability to describe the jagged contours of *his* cells using the means available to him in the 19th century.

For teaching purposes, Sertoli also tried to summarize his observations in the shape of a pattern, which became the forerunner of all subsequent schematic representations in the histology manuals [12] (Fig. 4).

In later years Sertoli saw his name firmly attached to the branched cells (in 1888 von Ebner spoke of "Sertoli's cells"), and turned his keen gaze elsewhere. In

Fig. 5. Enrico Sertoli (photo from around 1900).

Number of publications with "Sertoli cell" in the title or abstract (NLM search).

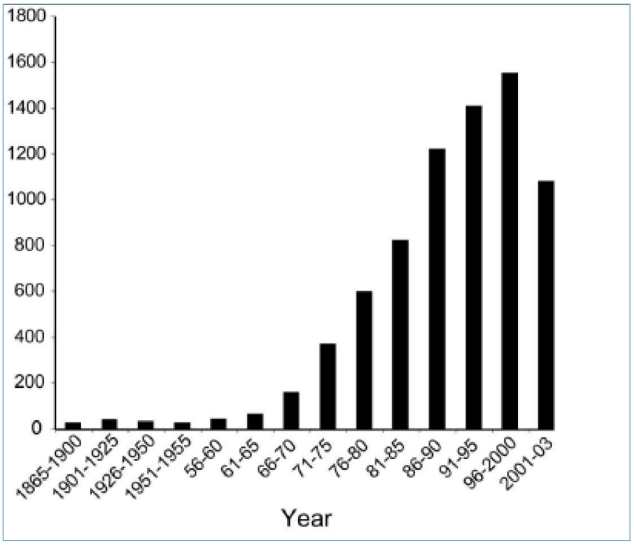


Fig. 6. (*Modified from Rex A Hess 2005* Ref 2).

addition to running the institute of which he became director, his efforts became focused on new research fields (the lymphatic system, lung, muscle, kidney, etc.) that kept him busy for several years (Fig. 5) [2 13 14], until he decided to leave his university teaching post in 1907.

Despite the importance of Sertoli's discovery, the attention demonstrated by the scientific literature remained very limited for roughly half a century after Sertoli's death (in Sondrio in 1910). On average, Sertoli cells were only mentioned in about one publication a year (Fig. 6) up until the early 1950s, when Gunnar Teilum published his descriptions of Sertoli cell tumors [15 16].

Also in *Pathologica*, Sertoli's name almost eclipsed, surviving as a substantivized (*sertolization*) name in the descriptions of testicular atrophy [17].

Some of the reasons for Sertoli's partial eclipse during the first half of the 20th century include: the introduction of electron microscopy only after the 1930s; the great interest in biology of germ cell maturation rather then of Sertoli cells; the lack of understanding in the field of endocrinology; the limited development of andrology and scarce interest in the topic of male infertility; and the rarity of tumors of the sexual cords.

Today we know that Sertoli cell tumor (SCT) is a well characterized entity composed of cells resembling embryonal, prepubertal, and adult Sertoli cells. SCT accounts for < 1% of all testicular tumors, yet it is the second most common sex cord–stromal tumor. SCT occurs in a wide range of age, most occurring in adults. Its rarity and some overlapping features with seminoma and other sex cord stromal tumors (Leydig, unclassified tumors) is a matter of difficult interpretation in the routinary practice. Orchiectomy remains the only therapeutic option, because radiotherapy and chemotherapy have been unsuccessful to date. About 12% of SCTs are malignant [18]. Gynecomastia seems to be more frequent in malignant cases. Features predictive of aggressive behavior include extratesticular spread, size > 5 cm, high-grade cytological atypia, > 5 mitoses per 10/HPFs, and necrosis and lymphovascular invasion. Recently the occurrence of *CTNNB1* gene mutations and nuclear displacement of β-catenin has been considered a driver mutation in the oncogenesis of this tumor in the majority of Sertoli cell tumors [19].

In summary, what we label with Sertoli's name is a well known tumor. However, it has to be said that what happened to Sertoli is a far from an unusual case of posthumous acknowledgement of the value of a scientific discovery. Thinking of how a remarkable intuition can fade into oblivion brings to mind sadder cases, such as Ignac Semmelweis [20]: the forerunner of antisepsis, he was rejected and forgotten by the scientific community of his time, only to gain his well-deserved place among the great names of medicine after his death.

Conflict of interest statement

None declared.

References

1 *Discorso inaugurale dell'anno scolastico 1872-1873 della Reale Scuola Superiore di Veterinaria di Milano*. Milano: Tipografia L. Vietti, via Fiori Chiari 8.

2 Hess RA, Franca LSR. *History of the Sertoli cell discovery*. In: *Sertoli cell biology*. Chapt. 1. Griswold M, Skinner M, eds. Elsevier Inc. 2005, pp. 3-13. https://doi.org/10.1016/B978-012647751-1/50002-7

3 Sertoli E. *Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano*. Il Morgagni 1865;7:31

4 Patriarca PL. *Storia della Medicina e della Sanità in Valtellina*. Sondrio: L'Officina del Libro 1998.

5 Usuelli F. Enrico Sertoli. Estratto dall'"Annuario Veterinario Italiano" 1934-35. Roma.

6 Sertoli E. *Osservazioni sulla struttura dei canalicoli seminiferi del testicolo*. Gazzetta Medica Italiana. Lombardia 1871;52:413-5.

7 França LR, Hess RA, Dufour JM, et al. *The Sertoli cell: one hundred fifty years of beauty and plasticity*. Andrology 2016;4:189-212. https://doi.org/10.1111/andr.12165

8 Geyer CB. *A historical perspective on some "new" discoveries on spermatogenesis from the laboratory of Enrico Sertoli in 1878.* Biol Reprod 2018;99:671. https://doi.org/10.1093/biolre/ioy146

9 Sertoli E. *Sulla struttura dei canalicoli seminiferi del testicolo studiata in rapporto allo sviluppo dei nemaspermi*. Gazzetta Medica Italiana. Lombardia 1875;2:401-3.

[10] Sertoli E. *Sur la caryokinèse dans la spermatogénèse*. Archives Italiennes de Biologie. 1886;7:369-75.

[11] Sertoli E. *The structure of seminiferous tubules and the development of stermatids in rats*. Archivio per le scienze mediche. Turin: Vincenzo Bona 1878;1-76.

[12] Ober WB, Sciagura C. Leydig. *Sertoli and Reinke: three anatomists who were on the ball*. Pathology annual 1981;16:1-13.

[13] Patriarca PL. *Enrico Sertoli: istologo e fisiologo*. Valtellina Med 1995;XI,2:47-50.

[14] Baratelli G, Lanzani A, Sacco RN. *Biography of Enrico Sertoli*. Urology 2002;60:196-8.

[15] Teilum G. *Estrogen-producing Sertoli cell tumors (Androblastoma tubular lipoides) of the human testis and ovary. Homologous ovarian and testicular tumors*. J Clin Endocrinol Metab 1949;9:301-18. https://doi.org/10.1210/jcem-9-4-301

[16] Teilum G. *Classification of testicular and ovarian androblastoma and Sertoli cell tumors*. Cancer 1958;11:769-82. https://doi.org/10.1002/1097-0142(195807/08)11:4<769::aid-cncr2820110415>3.0.co;2-y

[17] Badini A, Gambaro GC. *Considerazioni anatomo patologiche e cliniche sul cosiddetto pseudoseminoma del testicolo*. Pathologica 1968;LX:431-42.

[18] Young RH, Koelliker DD, Scully RE. *Sertoli cell tumors of the testis, not otherwise specified: A clinicopathologic analysis of 60 cases*. Am J Surg Pathol 1998;22:709-21. https://doi.org/10.1097/00000478-199806000-00008

[19] Perrone F, Bertolotti A, Montemurro G, et al. *Frequent mutation and nuclear localization of β-catenin in sertoli cell tumors of the testis*. Am J Surg Pathol 2014;38:66-71. https://doi.org/10.1097/PAS.0b013e31829cdbc6.

[20] Céline LF. *Il dottor Semmelweis*. Milano: Adelphi 1975.

Received and accepted: October 25, 2019

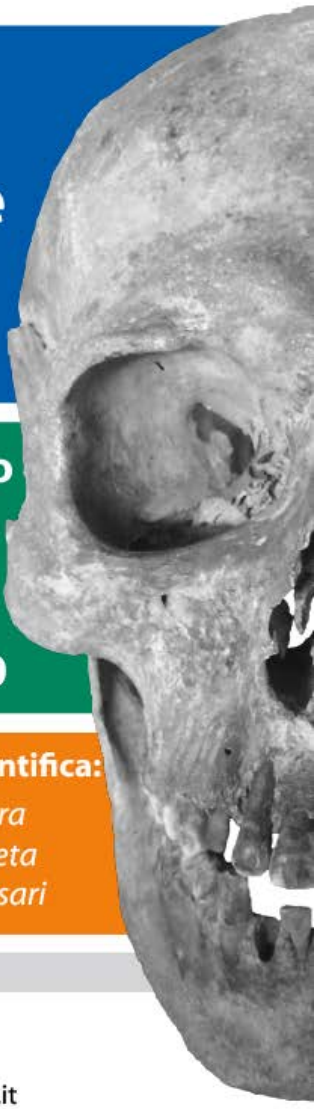
GIPaleo

SIAPEC - IAP

GIPaleo

V Meeting Nazionale

Gruppo Italiano di Paleopatologia



in foto: cranio di San Mercuriale

Centro Residenziale Universitario di Bertinoro

SALA DEL TEATRO

SABATO 18 MAGGIO 2019 ore 8:30

Segreteria Organizzativa:

Luca Saragoni
Mirko Traversari
Maria Giovanna Belcastro

Segreteria Scientifica:

Luca Ventura
Raffaele Gaeta
Mirko Traversari

Segreteria locale:

Centro Residenziale Universitario

Centro Universitario di Bertinoro
via Frangipane, 6 - Bertinoro (FC)
Monica Michelacci: 0543 446556 · mmichelacci@ceub.it

Ingresso libero. Info: www.ceub.it

Con il contributo di:

Daniele Versari di Estados cafè

ESTADOS cafè solidale

IL PANIFICIO DI CAMILLO

AT.ED.2 ATTREZZATURIFICIO

Con il patrocinio di:

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI BENI CULTURALI

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale della Romagna

Provincia di Forlì-Cesena

Comune di Forlì

Comune di Bertinoro

Proceedings of the V National Meeting of the Italian Group of Paleopathology (GIPaleo)

Editor: Luca Ventura

Division of Pathology, San Salvatore Hospital, L'Aquila, Italy

Lecture

The wound and the leg amputation of Giovanni dalle Bande Nere (1498-1526): life and death of a mercenary captain of the italian renaissance

G. FORNACIARI

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

The tomb of Giovanni and his wife Maria Salviati at Cappelle Medicee in Florence was explored to investigate the skeletal remains. Anthropological and paleopathological examination defined: age at death, physical constitution and activity, skeletal diseases. The stump of the right leg was studied macroscopically, under stereoscopic microscope, at X-ray and CT scans, to detect type of injury and level of amputation. 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 anconeus insertions were all present, as well as gluteal insertions to the femur, confirming he was a highly skilled horseman. 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 enthesopathies were found at the clavicular insertions of deltoid and pectoralis major, as well as at the small trochanter (psoas 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 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 best as he could in conformity with surgical knowledge of that period.

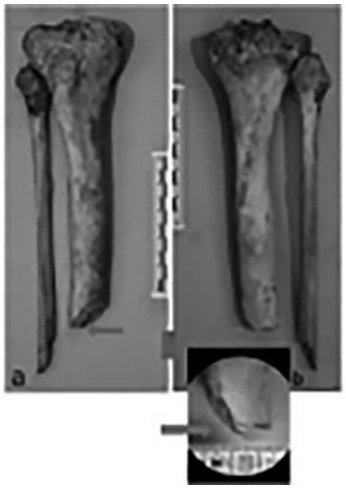


Figure. Anterior (a) and posterior (b) view of right tibia and fibula reveal the characteristics of amputation. The lesion caused by a cannonball from falconet is shown at the same level of the horizontal surgical cut (arrow).

Session 1

Chairmen: L. Saragoni (Forlì) e G. Ercolani (Forlì)

Biomechanical and kinesiological analyses of a femur fracture in paleopathology: reconstruction of injury mechanisms, care and functional outcomes

M. LICATA[1], A. RUSPI[1], S. IORIO[2], O. LARENTIS[1], C. TESI[1], P. BENAGLIA[1], A TOSI[1], G. ARMOCIDA[1], M. RONGA[1], C. ROSSETTI[1]

[1] Center of Research in Osteoarchaeology and Paleopathology, Department of Biotechnology and Life Sciences, University of Insubria; [2] Unit of History of Medicine, Department of Molecular Medicine, Sapienza University, Rome

Biomechanical and kinesiological reasoning allow us to investigate traumas in Paleopathology. The focus of our analysis is to reconstruct pathomechanics, treatment and gait of a subject from the Late Medieval femur, which presents an important bone callus (1).
The femur was discovered in a funerary crypt of the Sanctuary of Sacro Monte (Varese, Northern Italy), an important archaeological context inserted into the UNESCO heritage since 2003.
The femur was studied with computer tomography and the reconstruction of the static and dynamic fictional outcomes of the lesion was performed by the Observation Gait Analysis (OGA).
The OGA is the computerized analysis of the gait. This technique permits to observe movements of each articulation in the space, the posture and the gait underlining a movement strategy.
The femur presents an important callus at the middle third proximal of the shaft. The fracture is oblique and caused by a direct trauma probably associated with occupational activities. The alignment of the segments in the frontal plane leads us to assume that the fracture was treated and the femur was immobilized with splints.
The use of OGA allows us to understand the subject's kind of gait after healing.
Our analysis demonstrated that there was no reduction in bone mass. The deposition of new cortical bone near the fracture determines that the individual has gradually resumed loading the leg and was walking although with significant effects on posture and movement.

References

Licata M, Iorio S, Benaglia P, et al. *Biomechanical analysis of a femur fracture in osteoarchaeology: reconstruction of pathomechanics, treatment and gait*. J Forensic Leg Med 2019;61:115-21. https://doi.org/10.1016/j.jflm.2018.11.009

Demographic analysis of the plague cemetery of Alghero (1582-1583)

V. GIUFFRA, E. BARTOLOTTA[1], M. MILANESE[2]

[1] Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa; [2] Department of History, Human Sciences and Education, University of Sassari, Italy

In 2008, archaeological excavations carried out in the inner courtyard of the former Jesuit College of Alghero brought to light the San Michele cemetery. Characteristic of the site are some burial trenches, narrow and long pits containing the remains of 10 to 30 individuals, and some multiple tombs, which can be related to the plague epidemic that ravaged the city in 1582-83. The present study is focused on the demographic analysis of the 16 trenches containing 185 individuals and of one multiple tomb (T.141) with 14 individuals.
In the case of the trenches it was possible to determine the sex of 178 individuals: 37 are males (20.7%), 53 females (29.8%) and 88 of undetermined sex (49.5%). As for the first two groups, 35.6% of individuals has an age comprised between 20 and 29 years; the two age ranges 30-39 and 40-49 years present the same percentage (25.6%), 8.9% are aged between 17 and 19, and finally 4.4% are over the age of 50. The undetermined sex category is represented by 81 subadults and 7 adults. As for the subadults, the most representative age group is that between 7 and 12 years old with 39.8%, followed by the 23.9% between 13 and 19 years, 18.2% between 2 and 6 years, and finally, 10.2% between 0 and 1 years.
The multiple tomb 141 includes an adult individual (20-29 years) of undetermined sex, a woman of about 17 years and with a 35-week fetus in her womb, and finally 12 subadults in an age range between 0-1 years (21.4%), 2-6 years (7.1%) and 7-12 years (50.0%).
The cemetery of San Michele presents some similarities with the French cemetery of Martigues struck by the plague in 1720. Also in this cemetery this type of trench burials was found, 5 in this case, with 199 individuals. The comparison between the paleodemographic curves for both cemeteries evidences a similarity of the mortality trend. The difference between normal and catastrophic cemeteries, related to a severe epidemic event, consists in the fact that while in the former there is a greater presence of infants and elderly, in the latter there is a certain homogeneity of mortality, proof of the fact that the plague kills in a random way and therefore all individuals present the same risk of death.

References

Milanese M. *Lo scavo del cimitero di San Michele ad Alghero (fine XIII-inizi XVII secolo). Campagna di scavo giugno 2008-settembre 2009*. Pisa: Felici 2010.
Tzorzis S, Signoli M. *Les tranchées des Capucins de Ferri-*

ères (Martigues, Bouches-du-Rhône, France). Un charnier de l'épidémie de peste de 1720 à 1722 en Provence. Comptes Rendus Palevol 2009;8:749-60.
Signoli M, Séguy I, Biraben JN, et al. *Paleodemography and Historical Demography in the Context of an Epidemic: Plague in Provence in the Eighteenth Century*. Population 2002;57:829-54.

Ankylosing Spondylitis (AS) and Diffuse Idiopathic Skeletal Hyperostosis (DISH): a challenging issue. Differential diagnosis considerations based on two observed cases

R. BOANO[1], S. PALLECCHI[2], E SANTORO[3], S. BERTOLINO[2], V.G. VELLONE[4,5], F BUFFELLI[6], E. FULCHERI[4,6]

[1] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino; [2] Dipartimento di Antichità, Filosofia e Storia, Università di Genova; [3] Libero Professionista; [4] Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate, Università di Genova; [5] UO Anatomia Patologica Universitaria, IRCCS Ospedale Policlinico San Martino, Genova; [6] UOSD Patologia Feto-Placentare, IRCCS Istituto Giannina Gaslini, Genova

Ossification of spinal ligaments can persist on skeletal remains requiring a differential diagnosis between diseases that can lead to spinal column ankylosis. The most relevant diseases that can lead to this condition are represented by ankylosing spondylitis (AS) and diffuse idiopathic skeletal hyperostosis (DISH). The differential diagnosis between these two conditions is discussed in two cases of our observation.

Case n. 1: Policastro Bussentino, US 112 - The burial (US 112) was found in the convent of San Francesco in Policastro Bussentino, during an emergency excavation carried out in 2015. The skeleton on a stratigraphic basis is dated between 1846 and 1892. The skeleton is affected by the fusion of 12 vertebrae of the vertebral column, from the fusion of some costovertebral joints and of the sacroiliac joints.

Case n. 2: Turin, Collection Marro, skeleton n.16809 - The skeleton belongs to the Egyptian Collection of the Museum of Anthropology and Ethonography of the University of Turin. It was collected during the archaeological campaign at Gebelein (Upper Egypt) in 1920 and dates back to the First Intermediate Period (2150-1990 BC). In this case, the pathology involves only the vertebral column in which the fusion of numerous vertebrae is found.

AS is a progressive inflammatory disease of unknown etiology primarily affecting the diarthrodial joints of the spine, the costovertebral joints and the sacroiliac joints and usually begins in the second or third decade of life. First it affects the lumbar spine and the sacroiliac joints and progressively ascends until the entire spine and all costovertebral joints are affected. The result of this ankylosing process is the rigid, so-called bamboo spine with loss of its physiological curvatures. DISH is an ossifying diathesis producing ankylosis of the spine due to ligament ossification without intervertebral disk disease. It is not a true arthropathy because neither cartilage nor synovium are involved. It is rarely detected before the age of 40 years and the cause is unknown. The two pictures macroscopically can be confused because both involve the spine with ossification and ankylosis of it. The differential diagnosis on skeletal remains can be based on extimated age and sex and on the elements involved.

AS occurs in the second or third decade of life and mainly affects men. DISH, instead, appears not before the fourth decade of age. DISH only affects the spine, while ankylosing spondylitis also affects the cost-vertebral joints and the sacro-iliac joint. The ligaments involved in the fusion of the spine are different in the two cases. DISH does not affect the intervertebral discs unlike AS which instead involves them and thus has a total fusion of the vertebral bodies.

Although DISH and AS manifest in a similar manner, they are separate diseases. Both pathologies are quite common in mild and initial forms, but are rather rare in the full-blown, severe forms, which involve the whole spine. This topic appear poorly covered both in paleopathological and clinical literature, mainly consisting in small series and case reports. As a consequence, a wide inter-individual variability is present and only rarely an accurate report of the different involved ligaments is provided. In most of the cases the description is limited to a generic attribution to spinal ligaments. Differential diagnosis may be challenging if limited to anthropological examination of the skeletal remains and further radiologic and genetic tests are necessary to confirm our findings.

References

Aufderheide AC, Rodriguez-Martin C. *The Cambridge encyclopedia of human paleopathology*. Cambridge: University Press 1998.
Brothwell D, Sandison AT. *Diseases in Antiquity*. Springfield: Charles C Thomas, 1967.
Fornaciari G, Giuffra V. *Lezioni di Paleopatologia.* Genova: ECIG, 2009.
Ortner DJ, Putschar WGJ. *Identification of pathological conditions in human skeletal remains*. Washington: Smithsonian Institution Press, 1981.
Rogers J, Waldron T. *A field guide to joint disease in archeology.* John Wiley & sons, 1995.

The green mummy of Bologna: FTIR spectroscopy offers new insight into the mummification process

A. ALABISO[1], M.G. BRIDELLI[1], M.C. MAZZOTTI[2], F. FERSINI[2], S. PELOTTI[2], D. LUISELLI[3], M. TRAVERSARI[3]

[1] Department of Mathematical, Physical and Computer Sciences, University of Parma; [2] Sezione di Medicina Legale, Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna; [3] Department of Cultural Heritage, University of Bologna, Ravenna, Italy

The object of this study is the so-called "green mummy" of Bologna, a naturally mummified body that was found in the basement of an ancient mansion in Bologna in the 1920s of last century. The hard and soft tissues of the body are green for the most. They were analyzed by FTIR spectroscopy with the aim to gain information about the biochemical degradation process, to explain both the origin and the nature of the green color and to understand how it affected the body's preservation.

The corpse was found in a copper or bronze cist that, at the time of recovery, was closed with a lid but broken at the base. It is reasonable to assume that the body had undergone the processes of putrefaction, liquefaction, skeletonization and mummification within the container. Probably, the semi-fluid mass of water and decomposing substances came out from the cist through the break at its base; therefore, the not yet putrefied tissues exposed to the atmosphere of the basement, desiccated and mummified. The acidic liquids originated by the decomposition caused the container corrosion, favouring the release of copper ions. Metal ions both inhibited the enzymes responsible for chemical reactions in the early stages of the decomposition process and acted as biocides of microorganisms involved in decomposition.

FTIR analysis of soft tissues, with and without green color, showed that tissues lacking the green patina were the best preserved revealing the protein structure only minimally deteriorated, contrarily to what was observed in green areas. We have hypothesized that copper ions might have caused the decarboxylation of the RCOO group of polypeptidic backbone favoring protein degradation. Therefore, copper did not favor the mummification process, which was probably due to the environmental conditions, such as the low temperatures typical of the basement and the low availability of oxygen. Due to the known biocidal action of copper ions, we assume that copper ions might have damaged dead tissue cells in the same way they damage the cell membrane of microorganisms with which it comes into contact, causing their death. After the body decomposition liquids came out from the container, copper corrosion products precipitated as copper compounds giving the remains the green color. On the soft tissues two mineral forms of copper have been found: copper carbonate and copper phosphate called malachite and sampleite.

Also, FTIR analysis of bones revealed that copper did not contribute to tissue preservation. The measured mineralization index showed the alteration of both collagen and hydroxyapatite of colored bones unlike the colorless ones. A compound known as pseudomalachite was identified in the green bones, a form of hydroxyapatite where copper replace calcium. This substitution is responsible for the unusual green coloration of the bones.

References

Bobroff V, Chen H-H, Javerzat S, et al. *What can infrared spectroscopy do for characterizing organic remnant in fossils?* Trends in Analytical Chemistry 2016;82:443-56. https://doi.org/10.1016/j.trac.2016.07.005

Scott DA. *Copper in contact with organic materials. In Copper and Bronze in Art: Corrosion, Colorants, Conservation*. Losa Angeles: Getty Publications 2002, pp. 72-9.

Hong R, Kang TY, Michels CA, Gadura N. *Membrane Lipid Peroxidation in Copper Alloy-Mediated Contact Killing of Escherichia coli*. Appl Environ Microbiol 2012;78:1776-84. https://doi.org/10.1128/AEM.07068-11

Scott DA. *New insights on the corrosion of ancient bronzes using X-ray powder diffraction: The importance of paratacamite, sampleite, and connellite.* Studies in Conservation 2017;62:410-8. https://doi.org/10.1080/00393630.2016.1219466

Hopkinson KA, Yeats SM, Scott GR. *For whom the coin tolls: green stained teeth and jaws in medieval and post-medieval spanish burials*. Dental Anthropol 2008;21:12-7.

Paleopathological and paleoradiological investigation of the Egyptian embalmed head from the Civic Archaeological Museum of Erba (Como)

R. FUSCO[1], B. CERMESONI[2], A. RAVAGNAN[3], P. BADINO[1], O. LARENTIS[1], M. LICATA[1], C. TESI[1]

[1] Centre of Research in Osteoarchaeology and Paleopathology, Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2] Civic Museum of Erba, Como; Civic Museums of Varese, Italy; [3] International Council of Museums' Committee for Egyptology (ICOM-CIPEG)

The new paleoradiological investigations carried out on the Egyptian mummified human remains, a head, a left hand and foot, housed in the Civic Archaeological Museum of Erba (Como, northern Italy), allowed us to acquire comprehensive digital images and to study the anthropological and paleopathological data of the subjects.

The first macroscopic investigation revealed the presence of blue-glazed Faience tubes, adherent to the dorsal wrappings of the foot. This custom was particularly attested in the 26th dynasty, even if it appeared already from the 21st dynasty and seemed to continue until the Ptolemaic period.

The tomographic analysis revealed the non-compatibility of the three mummified parts to a single individual, based on the different degrees of bone development and degeneration.

The radiological investigation allowed us to acquire data on the embalming techniques applied, and on the health status and pathological conditions of the head. In particular, the parietal bones of the cranium exhibited two symmetrical areas of thinning and resorption of the outer table, which suggest a case of "biparietal thinning", also known as "biparietal osteodystrophy". The areas involved are well-circumscribed and elliptical in shape, localized between the obelion and the superior linea temporalis. Macroscopically, these areas are noted as slight depression and flattening of the outer cortical layer.

The CT imagings also revealed the mature age of the individual, which, according to several studies, is compatible with this finding.
A research in the literature, showed a prevalence of this condition in Egyptian individuals, associating it with different definitions and causes. Although the etiology of this affection is still not well-known, here the embalmed head of an elderly individual revealed the presence of biparietal affection, with thinning and resorption of the outer table, adding additional evidence of this finding in an Egyptian subject.

References

Cederlund C-G, Andrén L, Olivecrona H. *Progressive bilateral thinning of the parietal bones.* Skel Radiol 1982; 8: 29-33.
Engelbach R. *Introduction to Egyptian Archaeology.* Cairo: Institut Français d'Archéologie Orientale, 1946.
Fusco R, Larentis O, Cermesoni B, Ravagnan A, Tesi C. *The "Mummy of Erba": A study proposal for the analysis of a mummified Egyptian specimen*. Medicina Historica 2018; 2: 163-165.
Grilletto R. *Il mistero delle mummie: dall'antichità ai nostri giorni attraverso il tempo e lo spazio*. Roma: Newton & Compton, 2005.
Giuffra V, Pangoli D, Cosmacini P, Caramella D, Silvano F, Fornaciari G, Ciranni R. *Paleopathological evaluation and radiological study of 46 Egyptian mummified specimens in Italian Museums.* Egitto e Vicino Oriente 2009; 3: 121-55.
Mann RW, Kobayashi M, Schiller AL. *Biparietal thinning: accidental death by a fall from standing height.* J Forens Sci 2017; 62: 1406-1409. https://doi.org/10.1111/1556-4029.13425
Seiler R, Rühli F. *A unique case of perforated bilateral parietal bone thinning in a Third Intermediate Period Egyptian mummy (Geneva Museum D 0242).* J Egypt Archaeol 2014;100:467-74.

Diet and genomics in a human nutritional frame: the GEDEON project

T. DI CORCIA, F. DE ANGELIS, M. ROMBONI, V. VELTRE, C. MARTÍNEZ LABARGA, O. RICKARDS

Centre of Molecular Anthropology for Ancient DNA Studies, Tor Vergata University of Rome, Italy

The GEDEON project will allow us to broaden the knowledge about the human adaptation against changes in nutritional resources consumption. The main purpose of the project is to investigate the putative role of selective pressure that may have acted on specific genetic markers linked to changed dietary conditions. In order to reach this topic, the project aims to connect the scientific evidence obtained through osteological studies of the ancient remains dating across the major nutritional transitions, with the evaluation of genetic markers involved in metabolic pathways that may have been affected by nutrient bio-availability. The whole genome analysis of ancient selected skeletal specimens will be sequenced to compare the data obtained with those from extant people suffering of nutritional impairments, whose information on dietary requirements is available. Known polymorphisms that are classically referable to diet-derived homeostatic alterations will be selected, such as rs2066844, rs2066845 and rs2066847, whose association with inflammatory intestinal diseases (IBD) is well known. Other variants mapping on NOD2 gene (for example rs2066843 and rs2076756) will be focused, but they will represent only the starting point for the identification of causative molecular pathways modifiers. Furthermore, the markers with a well-known association with alterations such as celiac disease (CD) and primary hypolactasia (PH) will be also considered: PTPN2 and IL18RAP loci as well as various HLA system factors and the LCT gene will be primarily evaluated. This shortlist selection will constitute the beginning for the identification of several new markers to shed light on human genetic adaptation to the changed environmental conditions including the nutritional requirements.

References

Macciardi F, Scorrano G, Martinez-Labarga C, et al. *Sequencing the whole genomes of two Anatomically Modern Humans (AMH) from the Upper Paleolithic and the Neolithic: considerations on methods". In: Biological and cultural heritage of the Central-Southern Italian population through 30 thousand years (EPIC).* In: Rickards O, Sarti L, eds. Roma: Universitalia Editrice 2016, pp. 183-98.
Modi A, Gigli E, Vai S, Scorrano G, et al. *Complete mitochondrial genomes of three Early Neolithics from Central Italy". In: Biological and cultural heritage of the Central-Southern Italian population through 30 thousand years (EPIC).* In: Rickards O, Sarti L, eds. Roma: Universitalia Editrice 2016, pp. 199-208.
Gismondi A, Di Marco G, Canini A. *The potential role of the archaeobotany in the archaeological sciences: the case study of Neolithic Vitis vinifera L. carpological remains*. In: Rickards O, Sarti L, eds. *Biological and cultural heritage of the Central-Southern Italian population through 30 thousand years (EPIC).* Roma: Universitalia Editrice 2016, pp. 173-82.
De Angelis F, Scorrano G, Rickards O. *DNA antico principi e*

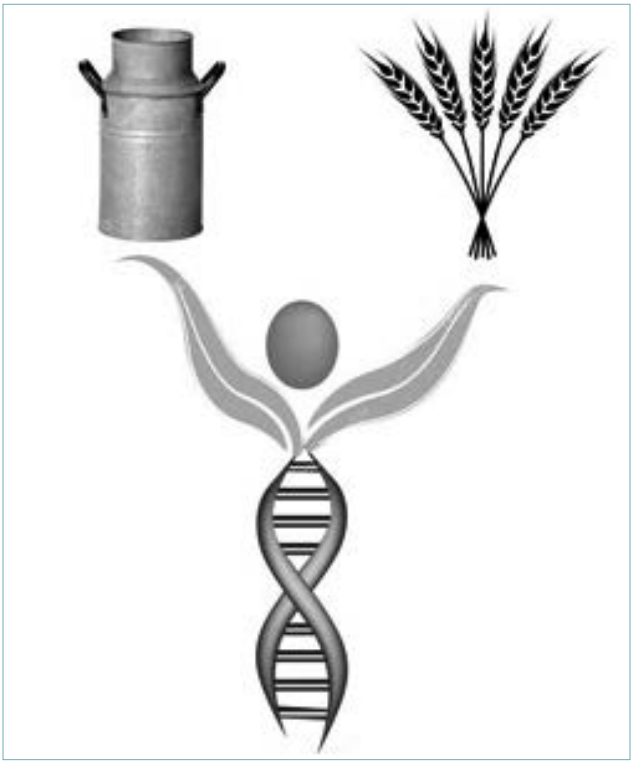


Figure.

metodologie. Med Secoli 2013;25:51-84.
Gasbarrini G, Rickards O, Martínez-Labarga C, et al. *Origin of celiac disease: how old are predisposing haplotypes?* World J Gastroenterol 2012;18:5300-4. https://doi.org/10.3748/wjg.v18.i37.5300

Next generation sequencing as a tool for diagnostics: a case study

V. VELTRE[1], F. DE ANGELIS[1], S. VARANO[1], C. CALDARINI[2], W. PANTANO[2], P. CATALANO[3], C. MARTÍNEZ-LABARGA[1], O. RICKARDS[1]

[1] Centre of Molecular Anthropology for Ancient DNA Studies, Tor Vergata University of Rome; [2] Freelance Anthropologist; [3] Soprintendenza Speciale Archeologia Belle Arti e Paesaggio di Roma, Rome, Italy

The application of the next generation sequencing techniques to the study of ancient DNA represents an outstanding improvement for clarifying complex scenarios related to genomic-based physio-pathological conditions, whose identification in ancient remains can be tricky. Indeed, the sole presence of osteological markers could be misleading for proper diagnosis due to the non-specific nature of such lesions. A thorough molecular evaluation has been performed on a skeleton of an adult woman dating to the Roman Imperial Age. The erosive and osteolytic markers located in the tarsal bones have led to hypothesize that she probably suffered from gout. In an attempt to integrate the differential diagnosis based on osteological data, whole genome sequencing analysis was performed. The bioinformatics pipeline identified the presence of two variants in the TSC2 gene, that is known to be associated with a rare genetic disorder, the Tuberous Sclerosis Complex, featured by signs that could be shared with those due to gouty arthritis. The application of these ultimate molecular techniques surely represents a successful diagnostic tool for the identification of genetic related disorders that could be only hypothesized in ancient times.

Figure.

References

Anastasiou E, Mitchell PD. *Palaeopathology and genes: investigating the genetics of infectious diseases in excavated human skeletal remains and mummies from past populations.* Gene 2013;528:33-40. https://doi.org/10.1016/j.gene.2013.06.017
Dalbeth N, Stamp LK, Merriman TR. *The genetics of gout: towards personalised medicine?* BMC Med 2017;15:108. https://doi.org/10.1186/s12916-017-0878-5
Minozzi S, Bianchi F, Pantano W, et al. *A case of gout from Imperial Rome (1st-2nd Century AD).* J Clin Res Bioeth 2013;4:4. https://doi.org/10.4172/2155-9627.1000162
Thumfart J, Weschke B, Ringe H, et al. *Acute renal failure unmasking Lesch-Nyhan disease in a patient with tuberous sclerosis complex.* Eur J Paediatr Neurol 2016; 20: 649-51. https://doi.org/10.1016/j.ejpn.2016.04.014
Yohe S, Thyagarajan B. *Review of clinical Next-Generation Sequencing*. Arch Pathol Lab Med 2017;141:1544-57. https://doi.org/10.5858/arpa.2016-0501-RA

Session 2

Chairmen: E. Rabino Massa (Torino) e M. Licata (Varese)

Canonical exhhumation and reconnaissance of the mortal remains of the servant of God P. Raffaele da Sant'Elia a Pianisi

M. GUERRIERO[1], F. COLASURDO[2], A.M. POLLIO[1], G.V. PONZIO[3]

[1] Department of Pathology, Regional Health Authority of Molise, Antonio Cardarelli Regional Hospital, Campobasso; [2] Independent biomedical laboratory technician, Campobasso; [3] Department of Hygiene and Public Health, Regional Health Authority of Molise, Antonio Cardarelli Regional Hospital, Campobasso, Italy

Domenico Petruccelli (Raffaele da Sant'Elia a Pianisi), servant of God, was born in Sant'Elia a Pianisi (Campobasso) in 1816 in a family of honest and wealthy peasants. He lived in different convents between Puglia and Molise. Wherever he went, he won the esteem and veneration of everyone, so much so as to be called the "Holy Monk". He died in the convent of Sant'Elia a Pianisi in 1901. In the same convent another famous monk arrived in 1904 fr. Pius from Pietrelcina (better known as Padre Pio), here the young monk breathed the aura of holiness left by the Holy Monk who died a few years earlier, drawing strength and inspiration.
A first exhumation had been performed in 1934 and the skull was placed in a metal case. In 2017, 200 years after his birth, during the second exhumation and canonical recognition of the mortal remains, the skull was in good condition of conservation. It was covered in a thin layer of yellowish-white powder and traces of deteriorated fabric which formed part of the inner lining of urn.
Cleaning operations. The skull was cleaned. During this operation, we proceeded to collect in a container of numerous fragments adhered to the outer and inner surface of the skull.

Figure.

Consolidation operations. The skull was impregnated with a thin protective membrane consisting of a very thin film of an acrylic resin (Paraloid B72™) which protects it and consolidates its most delicate and fragile parts.

Paleopathological study. The alterations and lesions found are: in the left orbit, two holes (of undetermined origin) in the left parietal and temporal bones, in the right parietal bone, at the base of the skull, the jaw is absent.

Attribution of sex. We highlight a series of morphological findings characteristic of the male sex. They are: protruding glabella and sloping forehead; Rounded, wide and thick super-margin; Wide zygomatic arch that extends beyond the external acoustic meatus; Mastoid process robust, big and verticalized; Necked crest marked, wrinkled and very evident.

Diagnosis of age. Coronal, sagittal and lambdoidal sutures were considered, in accordance with the criteria of Acsàdi and Nemeskèri (1970) modified by Masset (1989). The endocranial closing index can be estimated at around 4, so the subject's age is between 50 and 80 years, according to the known age of Father Raffaele of 85 years at the time of death.

Craniometry. The craniometric assessments carried out highlighted: a small, rounded, long, narrow, medium-high skull with rounded sagittal contours, oval and angular orbits, mean interorbitary distance, narrow nasal opening. All these characteristics allow us to state that the skull belonged to a Caucasian subject.

Histological examination. The fragments examined were composed of human organic material. These are extensively necrotic and rotten tissues. An important result has been achieved with the finding in the histological findings of many fungal hyphae, better highlighted with histochemical stains PAS and Grocott.

References

Marella GL. *Elementi di Antropologia Forense.* Padova: CEDAM 2003.

Cattaneo C, Grandi M. *Antropologia e Odontologia Forense*. Milano: Monduzzi 2016.

Taylor JH. *Unwrapping a mummy.* London: British Museum Press 1995.

Borgognini Tarli S, Pacciani E. *I resti umani nello scavo archeologico. Metodiche di recupero e studio*- Bulzoni Editore 1993.

Canci A, Minozzi S. *Archelogia dei resti umani, dallo scavo al laboratorio*. Roma: Carocci 2005.

The first bishop of Forlì: Saint Mercurialis. Scientific recognition and palaeopathological analysis

M. TRAVERSARI[1], E. CILLI[1], D LUISELLI[1], G. GRUPPIONI[1], M.G. BRIDELLI[2], L. SARAGONI[3], E. PETRELLA[4]

[1] Department of Cultural Heritage, University of Bologna, Ravenna; [2] Department of Mathematical, Physical and Computer Sciences, University of Parma, Parma; [3] Department of Pathological Anatomy, AUSL Romagna, Morgagni-Pierantoni City Hospital, Forlì; [4]Department of Radiology and Nuclear Medicine, USL Romagna, Morgagni-Pierantoni City Hospital, Forlì, Italy

Historical information related to the life of St. Mercurialis is very scarce, everything we currently know, we owe to its legenda, is contained within the manuscript Casanatense 718 dating to the 12th century. The only certain historical information concerns the ordination of one of his successors, Grato, which took place in Ravenna during the 5th century. The relics of St. Mercurialis, preserved inside the homonymous abbey, in the Cathedral of Santa Croce and in the Santissima Trinità church, during 2018 were object of the sixth canonical recognition, necessary to verify the state of conservation of the bones. Preliminary studies were performed by direct anthropological and radiological analyses by CT scan, FTIR analysis, ancient DNA and radiocarbon dating. St. Mercurialis, was about 1.60 meters tall, the age at death is 45-50 years, and he was not particularly robust, even if marked by repeated musculoskeletal stress probably linked to habitual activities such as walking and weightlifting. He suffered from osteoporosis and perhaps had some discomfort with the shoulder girdle. He had a deviated nasal septum from birth, a condition that perhaps caused him disorders such as sinusitis. He did not suffer from osteoarthritis and he had no particular indicators about deficiencies suffered during the first and last period of life. Analyses did not reveal indicators due to traumatic events and probably did not die by strangulation, as the hyoid bone was intact. FTIR analysis was carried out on the brown substance that partly covered the lower skeleton district, shows the

typical spectrum of clayey materials. The characteristic bands reveal the presence of aluminum and silicon in greater quantities, and of other elements in smaller quantities. The clays constituting the soil can therefore be considered essentially illites containing kaolinite, smectite and quartz. This aspect confirms the numerous historical information concerning the floods suffered by the abbey. A patina that covered some bones was also detected. The obtained spectrum presents the typical absorptions of the vibrations of the hydrocarbon radicals CH2 and CH3, in addition to the intense absorption typical of the C-O-C group characteristic of carbohydrates. The characteristics of the spectrum therefore seem to be typical of a methylcellulose preservative. Radiocarbon dating and accelerometry mass spectrometry (AMS) dated the relics to the I-III century AD, an interesting date that is chronologically before the only historical indication we have about the life of St. Mercurialis and which collocates his episcopate into the first stages of evangelization of the Emilia Romagna Region. The preliminary analyses of ancient DNA were targeted on the hypervariable region 1 (HVR-1) of the mitochondrial DNA (mtDNA) and on Short Tandem Repeats (STRs) of the Y-chromosome and highlighted a rather pronounced diagenesis of the DNA. The subsequent analyses will be targeted to the capture of the entire mtDNA, coupled with next generation sequencing.

Figure. The artificial mummy of Andrea da Montereale.

References

Biblioteca Casanatense, Ms.718, Roma.

Bridelli MG, Dell'Anna A, Stani C, et al. *FTIR spectroscopy and microspectroscopy of ancient Egyptian embalmed heads from the Museum of Anthropology and Ethnography of the University of Turin*. Yearbook of mummy studies 2014;2:167-74.

Canci A, Minozzi S, *Archeologia dei resti umani, dallo scavo al laboratorio*. Roma: Carocci 2005.

Petrella E, Piciucchi S, Feletti F, et al. *CT Scan of Thirteen Natural Mummies Dating Back to the XVI-XVIII Centuries: An Emerging Tool to Investigate Living Conditions and Diseases in History*. PLoS One 2016;11:1-18. https://doi.org/10.1371/journal.pone.0154349

Zagnini F. *San Mercuriale: il santo e il suo monastero*. Forlì 2015.

The blessed Andrea da Montereale (1397?-1479). A retrospective survey on canonical recognitions of his body

L. VENTURA[1,2], M. RIPANI[3]

[1]UOC di Anatomia Patologica, Ospedale San Salvatore, L'Aquila; [2]Sacra Lipsanoteca, Arcidiocesi Metropolitana, L'Aquila; [3]Dipartimento di Scienze Motorie, Umane e della Salute, Università degli Studi di Roma "Foro Italico"

Andrea was a Catholic priest and a member of the Order of Saint Augustine. He was born to a modest family in the village of Mascioni (northern borders of L'Aquila province) and as a child he worked as a shepherd. Around 1417 he met Augustine from Terni, Prior of the Augustinian convent in the near Montereale, and asked to enter their ranks to be ordered as a priest at the age of 25. He earned a bachelor and master's degree in theology, becoming professor in Siena (1443), and Provincial Prior of the Umbria region. He also served as a travelling preacher in Italy and France, reforming several Augustinian monasteries in Umbria. He died on 18 april 1479 in Montereale and, according to hagiographies, his body was exposed without balsams in the conventual church for 30 days, giving off sweet odor and performing miracles. Subsequently, it was placed in the Choir until 1568, when it was displayed beneath the main altar. His beatification was celebrated by Pope Clement XIII on 18 february 1764. In 1787 the body was translated into a newly built repository, inside the new chapel dedicated to him.
Documented Canonical Recogntions took place in August 1786, July 1943, June 1961, and between June and July 1989. The last was performed by one of us (MR) and the late professor Giulio Marinozzi.
External examination of the body allowed to recognize a partially skeletonized mummy belonging to an old male subject (more than 70 years of age at death) and measuring 164 cm in length. The face was almost entirely covered by mummified skin, with traces of hair in the perioral region, chin, cheeks and anterior neck, according to devotional representations of the Blessed as a bearded elder. Soft tissues of forearms,

hands, legs and feet appeared in a good preservation state. A large bone defect was observed in the occipital squama. Large skin cuts were observed in the anterior neck and left hemithorax. The ventral portions of the left ribs appeared cut and displaced within the thoracic cavity. Cut marks were also found on the left margin of the sternum body and on anterior branches of the pelvis. Preserved skin was observed only in the right hemithorax. No traces of internal organs were found in thorax, abdomen and pelvis. Moderate-marked osteoarthritis of the spine was noted. Unfortunately, a radiologic investigation of the body was not performed. After external examination, conservative treatment was performed.

The body of Andrea revealed indisputable evidence of artificial mummification, representing the eleventh described case of an embalmed Saint or Blessed in Catholic Religion. Nine of these artificial mummies were created in central Italy (Umbria, Toscana, Abruzzo, Lazio) between the XIII and XV century. The employed evisceration procedures appear somewhat rough, without the complexity observed in other examples. It is worth to note that Montereale is located not far from L'Aquila and on the main route towards Cascia and Spoleto. The embalming of the Blessed Andrea took place only 35 years after the death and embalming of Saint Bernardino da Siena in L'Aquila and represents the second case in Abruzzo region.

References

Beato Andrea da Montereale. *1397-1997: VI Centenario della nascita*. L'Aquila: IRRSAE 1997.

Murri F. *Santi, Beati, Servi di Dio e morti in concetto di santità dell'Arcidiocesi Aquilana*. L'Aquila: ISSRA, 1993;167-76.

Cotta G. *Vita prodigiosa del B. Andrea da Montereale Maestro in teologia, e missionario dell'Ordin'Eremitano di Sant'Agostino*. Perugia 1726.

Tomassini M. *Il pastorello di Mascioni. Il Beato Andrea da Montereale dell'Ordine Eremitano di Sant'Agostino*. Roma, 1952.

Trinchieri R. *L'Ordine di Sant'Agostino nell'Abruzzo Aquilano*. Bollettino della Deputazione Abruzzese di Storia Patria, 1941-1943;32-34:115-201.

Exhumation and anthropological study of the skeletal remains attributed to Liutprand, king of the Longobards (690 ca ad-744 ad)

R. GAETA[1], S. MINOZZI[1], A. FORNACIARI[1], V. GIUFFRA[1], G. RICCOMI[1], S. LOMARTIRE[2], G. FORNACIARI[1]

[1]Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa; [2]Department of Humanistic Studies, University of Eastern Piedmont 'Amedeo Avogadro', Vercelli, Italy

Liutprand, one of the greatest Longobard sovereign, was born in the early 90s of the 7th century and died in 744 at the age of about 55 years. According to the Historia Longobardorum of Paolo Diacono, he was King of Longobards from 712 to 744.

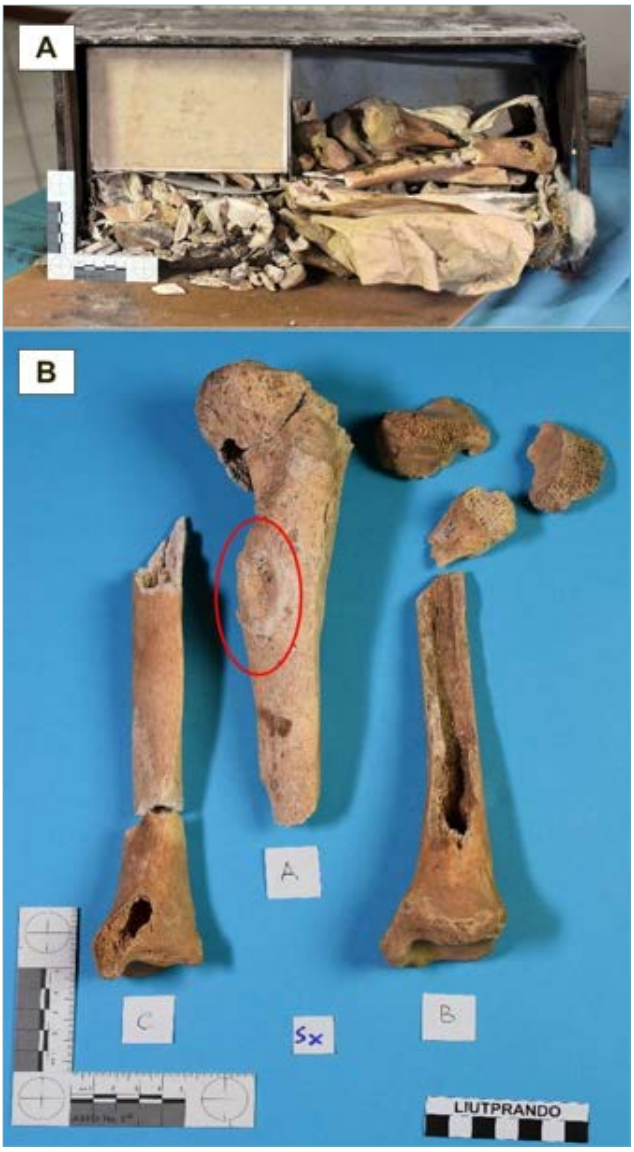


Figure 1. A) The fragmented skeletal remains as they appeared once opened the wooden box. B) The three left tibiae. The central one, signed with the letter 'A', showed enlarged diaphysis due to osteomyelitis; a circular depression on the bone surface is highlighted within the red circle.

The remains of King Liutprand suffered from many translations in the centuries and this is the main problem for the validation of the authenticity of the bone remains. The first grave was in the chapel of Sant'Adriano in the Longobard cemetery of Santa Maria in Pertica (Pavia). Later, in the second half of 12th century, the body of Liutprand was translated in the Basilica of San Pietro in Ciel d'Oro and located in a monumental mausoleum. New translations took place after the Council of Trento and in 1895, when the bones were placed in a niche in the floor of the church where they were found in January 2018.

The bones, contained in a wooden box, appeared extremely fragmented and in a poor state of preservation. Anthropological examination highlighted the presence of bones attributable to three individuals. Most of the remains belongs to a robust male individual with strong muscular insertions, with an age at death between 40 and 50 years. There is also a second older male with strong muscle insertions, and a third adult individual of similar size. In fact, there are a pathological left tibia and some fragments of its right controlateral, and other fragments attributable to two other left tibiae. The tibia with pathological alterations presents the upper third of the diaphysis completely altered and enlarged by bone thickening due to a severe form of osteomyelitis. Bone repair is evident and the presence of a circular depression with a diameter of about 10 mm at the point of maximum thickening could represent the trace left by a pointed object that caused the perforation of the bone and the subsequent infection with osteomyelitis. The reparative process has however led to the healing of the lesion before death, which should have occurred not far from the event (maximum 2 years). Imaging studies (CT and radiological) on the skeletal remains were performed at the "San Matteo" General Hospital in Pavia.

^{14}C dating provide a range from 430 to 640 for the first subject, 600-770 for the second and 530-670 for the last male.

Isotopic data show a rather high nutritional status for the time, with a varied diet rich of meat.

In conclusion, currently it is not possible to accurately define the identity of the three individuals for lack of archaeological data and for the fragmentary nature of the bones. The age of the subjects, the robust constitution and the nutritional data suggest a belonging to a high social class perhaps devoted to war activity. Future molecular studies may perhaps reveal a possible degree of kinship between the individuals and clarify the identity of the subjects.

References

Berto LA. *Liutprando, re dei longobardi.* In: Dizionario Biografico degli Italiani - Volume 65, Istituto della Enciclopedia Italiana, Roma 1965.

Diacono P. *Historia Longobardorum*. Milano: Mondadori, 1992.

Majocchi R. *Le ossa di re Liutprando scoperte in San Pietro in Ciel d'Oro di Pavia*. Milano: Tipografia commerciale lombarda 1890.

A preliminary survey on the mummy of the blessed Jean Bassand from Besançon (c. 1360-1445)

L. VENTURA[1,2], R. GAETA[3], L. PICCHIONI[4], M. IOANNUCCI[2]

[1]Division of Pathology, San Salvatore Hospital, L'Aquila; [2]Sacred Lipsanotheca of the Metropolitan Archbishopry of L'Aquila; [3]Division of Paleopathology, Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa; [4]Artist, Montereale, L'Aquila, Italy

Figure. The face of Jean Bassand's mummy.

Born to one of the leading families in Besançon, Jean Bassand was a French Christian monk. After his initial profession in the Augustinian house of Saint Paul, he joined the Celestines (a branch of the Benedictine Order) in Paris, and subsequently became prior in the city of Amiens. The Celestine monks of France were a self-governing province of an Italian Benedictine reform of the late XIII century that no longer exists. They had a great influence, representing one of the most prominent observant groups in France, and an inspiration for reform movements across multiple orders.

Jean Bassand represented the most important figure in the French Celestine congregation between XIV and XV century, being elected provincial prior on five occasions. He made great efforts to establish new Houses in the French province as well as abroad. The English King Henry V invited him to found and direct a friary in Isleworth near Sheen (now Richmond, London), whereas Martin I of Aragon asked him to establish the congregation in Barcelona.

In 1443, he went to L'Aquila by order of the Pope Eugenius IV, to reform the monastery of Santa Maria di Collemaggio. He had troubles in this task and retreated to Rome arguing that the Aquilans were "difficult men", but the Pope sent him back until his mission was accomplished. He died in L'Aquila on

26 August 1445. His body, covered with lime to be displayed, was found intact 18 years after. Since his death the mummy of Jean Bassand used to be kept in the Basilica of Collemaggio.
After the major earthquake that struck down the city in 2009, his remains were recovered from the church to be kept in a secret location. Recently, an inspection of the body took place as a preliminary step of a forthcoming Canonical Recognition. The mummy appeared still fully dressed, with face and hands uncovered. The skin surface was extremely well preserved, and oblique illumination disclosed multiple, round, well-circumscribed plaques on the forehead, cheeks, and upper lip. Careful examination of the digital pictures enabled us to recognize at least 19 lesions.
From a modern clinical viewpoint, the facial skin eruption of Jean Bassand meets most of the diagnostic criteria for multiple seborrheic keratoses. The age at death and the lifestyle of the Blessed, with frequent, long-distance travel under severe conditions, are fully compatible with this diagnosis. It is well known that male sex, increased age and sun-exposure may predispose individuals towards the development of these lesions.
This case might represent the first ancient seborrheic keratosis described in the literature, although further analyses (external examination, computed tomography, histology) are needed to confirm the diagnosis. Advanced investigation methods might also enable us to understand if an ancient case harbours the same genetic mutations detected in modern patients. In this particular case, the treatment of the corpse with lime referred to in the ancient literature may have preserved the skin lesions by dehydration.

References

Gaeta R, Ventura L, Fornaciari G. The cutaneous cancer of Ferdinando Orsini, 5th Duke of Gravina. JAMA Dermatol 2017; 153:643. https://doi.org/10.1001/jamadermatol.2017.1847
Shaw RLJ. The Celestine Monks of France, c. 1350-1450. Observant reform in an age of schism, council and war. Amsterdam: University Press 2018.
Dunod de Charnage F-I. Histoire de l'èglise, ville et diocese de Besançon. Besançon: Daclin and Charmet 1750.
Ventura L, Gaeta R, Ioannucci M, et al. Facial skin eruption in the mummy of the Blessed Jean Bassand (c. 1360-1445). A report from the preliminary survey. Paleopathology Newsl 2019;185: 29-32.
Heidenreich B, Denisova E, Rachakonda S, et al. Genetic alterations in seborrheic keratosis. Oncotarget 2017; 8: 36639-36649. https://doi.org/10.18632/oncotarget.16698

Scurvy among 22 juvenile burials from an early italian medieval anthropological series that may have also been affected by tuberculosis

S. PANELLA[1], I. MICARELLI[1,2], R.R. PAINE[1], M.A. TAFURI[1], G. MANZI[1]

[1]Dipartimento di Biologia Ambientale, Sapienza Università di Roma; [2]Dipartimento di Scienze dell'Antichità, Sapienza Università di Roma, Italy

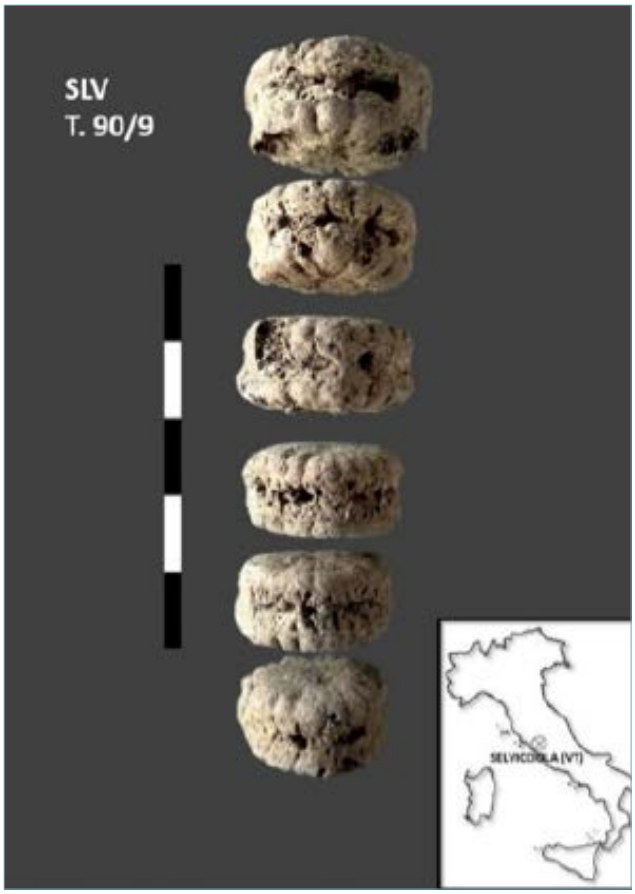


Figure. Vertebral bodies with bone tissue resorption of the anterolateral surface from a 4-5 years old subadult from the necropolis of Selvicciola.

This study presents the skeletal evidence for scurvy among 22 juveniles from the Longobard necropolis of Selvicciola, Italy (VII-VIII centuries AD). The paleopathological analysis revealed a combination of bilateral porous and proliferative bone lesions affecting the orbital roof (i.e., cribra orbitalia), the cranial vault (i.e., porotic hyperostosis) and some specific areas of the entire skeleton (i.e., sphenoid, hard palate, and scapula). This pattern is typical of infantile scurvy (Geber & Murphy, 2012). The investigation also revealed a bone tissue resorption of the anterior and lateral surface of thoracic and lumbar vertebral bodies and rib lesions in 17 of the 22 juveniles that showed signs of scurvy. This kind of skeletal manifestation associated with endocranial alteration and diffuse periosteal new bone formation (PNBF) is often linked to atypical or early-stage tuberculosis (Spekker et al., 2012). Our assessment suggests that the diet in Selvicciola was mainly deficient in vitamin C, causing scurvy. Among other problems, this might indicate poor sanitary conditions and further factors related to

local environment and general state of health. Consequently, scurvy may have reduced the immune resilience of the juveniles leading to the development of TB in many of them (Miladinović-Radmilović & Vulović, 2015). Additional assessment via isotopic studies of the Selvicciola burial collection (Tafuri et al., 2018) indicates that the consumption of animal proteins in their diet was quite high. Hence, TB may have originated as Mycobacterium bovis (Roberts & Buikstra, 2003), coming from dietary consumption of infected animals. The preliminary macroscopic investigation of these subadult skeletons reveals lesions that suggest a complicated relationship among several factors influencing the health of these children. Diet, infection and lack of vitamins may have contributed to the poor health and death of these juveniles. Hence, showing a complicated situation in which, these children lived and dead in. This anthropological work illustrates how paleopathology can be used to interpret the health status of individuals of past communities even when the lesion evidence suggests multiple causal factors associated with death.

References

Geber J, Murphy E. *Scurvy in the Great Irish Famine: Evidence of vitamin C deficiency from a mid-19th century skeletal population*. Am J Phys Anthropol 2012;148:512-24. https://doi.org/10.1002/ajpa.22066

Spekker O. *New cases of probable skeletal tuberculosis from the Neolithic period in Hungary: a morphological study*. Acta Biologica Szegediensis 2012;56:115-23.

Miladinović-Radmilović N, Vulović D. *The case of scurvy from Singidunum*, Starinar 2015;65:183-95.

Tafuri MA, Goude G, Manzi G. *Isotopic evidence of diet variation at the transition between classical and post-classical times in Central Italy*. J Archaeol Sci Rep 2018;21:496-503.

Roberts CA, Buikstra JE. *The bioarchaeology of tuberculosis: a global perspective on a re-emerging disease*. University Press of Florida 2003.

Case report of a post-partum death due to partial retention of placenta in a rachitic individual from the ancient autoptic register of Genoa

F. BUFFELLI[1,2], A. BONSIGNORE[3], R. CILIBERTI[3], V.G. VELLONE[2], E. FULCHERI[1,2]

[1]Fetal and Perinatal Pathology Unit, IRCCS Istituto Giannina Gaslini, Genova; [2]Pathology, DISC, University of Genova; [3]Department of Health Sciences (DISSAL) - Section of Legal and Forensic Medicine and Bioethics, University of Genova, Italy

The Institute of Clinical Pathology currently keeps the autopsy registers from 1891 to the present; the oldest one consists of some volumes in which the protocols and the related diagnoses were written by hand; for each case, a succinct clinical history preceded the external examination.

The case presented here concerns obstetric pathology related to maternal post-partum death due to uncontrollable bleeding. The autopsy was carried out in 1892 on a woman suffering from rickets with scant

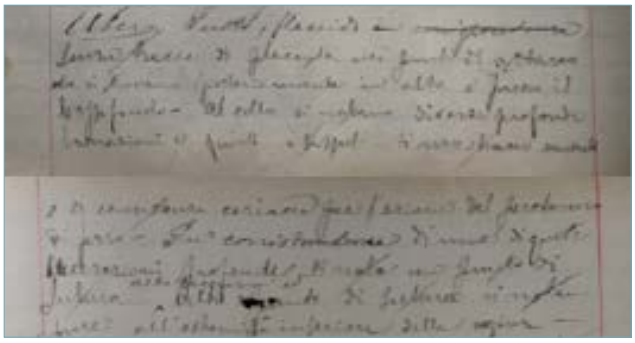


Figure.

muscular masses; the autopsy was performed 37 hours after the patient's death.

At that time (specifically from 1886 to 1905) the Director of the Unit of Clinical Pathology was Professor Vincenzo Brigidi and the autopsy room was situated near Pammatone Hospital, which was built in the district of Portoria and almost five centuries earlier, in the fifteenth century. This was the main hospital in Genoa, and played a fundamental role in local public health; the whole institute consisted of a single autopsy room.

Full-blown or paucisymptomatic rickets was common in the Italian population of the nineteenth century and the first half of the twentieth century.

The high incidence of this disease was due to deficiency factors or chronic nutritional stress, which led to forms of pseudo-rickets or latent rickets; this general situation required strict supervision of pregnant patients in order to avoid fatal intra-partum accidents both in the mother and in the foetus.

Moreover, it is known that the skeletal lesions typical of rickets can seriously worsen the classical pattern of common brachipelvization, resulting in more serious pathologies.

Anthropologically, brachipelvization and the evolution to the erect position constitute a peculiarity of our species. Over the centuries, obstetrics has developed complex studies for the evaluation of the pelvis and in particular, for the study of planes, axes and obstetric conjugates.

In the nineteenth century and in the first decades of the twentieth century, pelvimetry was carefully practiced in obstetrics to monitor the pathological conditions of the pelvis. The management of postpartum haemorrhage was less theoretically developed and, in obstetric practice, was also represented a frequent cause of maternal death.

In the case presented, therefore, obstetric procedures, such as sutures of the cervix of the vagina and the use of the so-called iron perchloride as a haemostatic cauterant, were used to stop bleeding.

Ferric chloride is an iron salt (hence it is wrong to call it acid, as it is wrong to call it perchloride). The haemostatic action of the latter has been known for a very long time, but owing to its caustic action, which deeply manifests itself in the tissues, it has been absolutely abandoned in modern obstetric practice.
Indeed, the report reveals that ferric chloride gave the tissues inside the uterus a leathery consistency, without - however - managing to save the mother's life.

References

Melis M. *La storia dell'insegnamento dell'Anatomia Patologica in Genova*. Pisa: Giardini 1961.
Testut L, Latarjet A. *Trattato di anatomia umana*. Vol VI, Apparato Uro Genitale Peritoneo. Traduzione di Ciardi-Duprè G. Torino: UTET 1972.
Comby GM. Il rachitismo piccola biblioteca medica (prima serie) pubblicata sotto la direzione di Charcot-Debove, Torino 1894. Traduzione italiana arricchita di aggiunte sotto la direzione del Dott. Prof. B. Silva
Fulcheri E. *Alcune considerazioni e riflessioni sulla paleonutrizione, paleopatologia e paleo demografia di popolazione*. Alba pompeia 1999;20.

Session 3

Chairmen: M. Traversari (Forlì) e R. Gaeta (Pisa)

A case of trepanation and something more: the early medieval Domus Mariae site in Trieste

P. SACCHERI, L. TRAVAN

Laboratory of Osteology, Department of Medicine, University of Udine, Italy

The typical modern native from Trieste is tall - they are the tallest among Italians -, over 65 years old - Trieste has the highest seniority index in Italy - and loves spending time at the seaside or eating out with friends (no statistic data are available but you can witness that with a day trip to the city!).
A group of 41 individuals who lived in the Early Middle Ages in Tergeste (the modern Trieste), seems to have a lot in common with the modern inhabitant of Trieste: they were tall (average Trotter and Gleser stature for male and female are 174.2 cm and 163.2 cm, respectively) and most of them were over 55 yeas old at death (more than 50% of adult males and 50% of adult females). Moreover, some of them spent a significant amount of time in the sea, which can be seen by the auditory exostosis in males and high frequency of third distal tibiae and fibulae periostitis in adult mature females – findings suggestive of a long time spent in the sea water looking for clams. Medieval archaeological layers in Trieste show plenty of shells. These people also loved eating: the four eldest male skeletons show marks of DISH, a pathology clearly associated with metabolic disorders. One of them may have died of suffocation caused by a small-size herbivore distal humerus epiphysis showing clear signs of slicing – a morsel of stew – found on C5/C6. Talking of skeletal remains, this diagnosis can be only a suggestion; nevertheless, dysphagia leading to suffocation in the elderly is a classic.
These people were buried out of the walled circle of the ancient Tergeste and not so far from the Madonna del Mare, an early Christian Basilica built in the V/VI century, on the site called Domus Mariae. In 3 out of a total of 21 tombs (the number is low because of the overlapping of different bodies), we find a skull placed in a ritual position close to the left ankle of the body. The following are the combinations found and the age at death in each of these burials: female skeleton aged 30-40/skull of child aged 4; male skeleton aged 50-60/skull of female aged more than 50; male skeleton aged 30-45/skull of male aged more than 50. In the last mentioned tomb, besides the skull to the left of the ankle there are two other skulls: one between the femora, belonging to a man aged about 20-25, and the other one to the left of his skull, belonging to a child aged about 9. The skull connected to the buried skeleton presents a trepanation probably made by scarnification: an oval hole of about 17 mm x 12 mm, on the left parietal bone. The lesion edges clearly demonstrate survival after trepanation, possibly not longer than 1 or 2 months. In fact, in the inner table, near the hole, some coral-like new bone lesions reveal a meningeal involvement. Neither the skull nor the other bones reveal traumatic lesions; we found only a sternal foramen, the lack of fusion of the transverse foramens of C2 and a osteochondritis dissecans of the right capitellum.

References

Eerkens JW, Nichols RV, Murray GGR, et al. *A probable prehistoric case of meningococcal disease from San Francisco Bay: Next generation sequencing of Neisseria meningitidis from dental calculus and osteological evidence*. Int J Paleopathol 2018;22:173-80. https://doi.org/10.1016/j.ijpp.2018.05.001
Paredes J, Ferreira MT, Wasterlain SN. *Early illness: a possible case of meningitis in a modern child from the wheel of Santa Casa da Misericórdia (Faro, Portugal)*. Cadernos do GEEvH 2014;2:40-6.
Van der Merve AE, Maat GJ, Watt I. *Diffuse idiopathic skeletal hyperostosis: diagnosis in a palaeopathological context*. Homo 2012;63:202-15. https://doi.org/10.1016/j.jchb.2012.03.005

Two medico-legal reports at the dawn of legal medicine

G.M. GOTTARDI

Independent Researcher

In the Middle Ages few examples of forensic medicine had appeared, even if imperfect and sporadic, in the field of healthcare art. In the Renaissance they took on greater consistency and framed themselves better in more defined limits. The two reports presented here are part of a trial against Jews in the city of

Trento in 1475. This work takes into consideration what happened, the historical period, the trial and the sentences, and the subsequent historical revision. The study of these reports also analyzes the guidelines available at that time.

References

Bonelli B. *Dissertazione apologetica sul martirio del beato Simone da Trento nell'anno MCCCCLXXV dagli ebrei ucciso*. Trento: Parone, Giovanni Battista stampator vescovile, 1747.

Chemelli A. *Trento nelle sue prime testimonianze a stampa*. Trento: tipolitografia TEMI, 1975.

Divina G. *Storia del Beato Simone da Trento*.Trento: Artigianelli, 1902.

Toaff A. *Pasque di sangue, Ebrei d'Europa e omicidi rituali*. Società editrice Il Mulino, 2007.

Figure.

"Lament not the absence of the name of any disease". Diseases and names from antiquity to the early modern period

A. FOSCATI[1], S. FORTUNA[2]

[1]SISMEL, Società Internazionale per lo Studio del Medioevo Latino, Florence; [2]Department of Clinical and Molecular Science, Polytechnic University of Marches, Ancona, Italy

To understand a disease of the past, historians need to distance themselves from today's epistemological paradigm. The disease must lie within the epistemological limits in which it is explained. In Western medical texts of the past, from Hippocrates and Galen onwards, it was the sign on the patient that led to diagnosis through a deductive process. A debatable diagnosis by our criteria. It follows that the names attributed to diseases bore several meanings, also due to translations over time from one major language to another (Greek, Arabic, Latin) and moreover in relation to the authors describing the disease – physicians, authors of literary texts, chronicles, hagiographies – representatives of an inhomogeneous medical culture. This led to an acknowledgement of the limits of retrospective diagnosis with reference to the lexicon of the textual sources. A significant example regards erysipelas, which today indicates a precise bacterial disease: this term is first found in various points of the Corpus Hippocraticum, including book III of the Epidemics, in a story subsequently commented on by Galen. From the description of the signs the term is linked to different symptoms or diseases, if interpreted with our criteria. Transliterated into Latin, in mediaeval texts the term is placed under the category of the apostemata – exceedingly complex diseases or disturbances – especially after the translation of Avicenna's Canon.

The association with ignis sacer, an expression of Latin origin, in the De Medicina of Cassius Felix (5th C.) led in some cases to a semantic change, borne out by non-medical sources. Ignis sacer in fact, independently of its oldest meaning, first came into use in chronicle sources from the 11th century, to indicate "burning" epidemics, in which ergotism and gangrene in general may be recognised.

Renaissance medical sources, following direct translations of Greek medical texts without mediation of the Arabic, tended in part to recover the meaning of the term erysipelas indicated by Galen.

In Hoffman's Dissertatio of 1729, albeit with due precautions, we may recognise the symptoms of present day erysipelas, although the physician's association with Rosa and with fuoco selvatico begs consideration of the polysemy of two nosographic expressions found in "popular" culture in many areas of Europe.

The aim of this presentation is to analyse the polysemy of a nosographic term that has come down to us from the Hippocratic tradition, together with its changes in meaning over time and in accordance with contexts. A paradigmatic case for highlighting the difficulty facing historians wishing to carry out a retrospective diagnosis by means of the medical lexicon.

References

Fortuna S. *Il metodo della diagnosi in Galeno (De Locis affectis, VIII 1-452 K.)*. Elenchos, 2001;22.2:281-304.

Foscati A. *Ignis sacer. Una storia culturale del 'fuoco sacro' dall'antichità al Settecento*. Florence: SISMEL 2013.

Grmek M D. *Nuove prospettive per la storia delle malattie antiche.* Napoli: Guida 1988.

Hippocrates, Epidemiae, III, in Opera Omnia, ed. and French translation by E. Littré. Paris: Chez J. B. Baillière, 1839-1861, vol. III: 70-76.

Hoffmann F. *Dissertatio inauguralis medica. De febre erysipelacea von der Rose*. Magdeburg: Hilligerus, 1729.

The petrification by Gorini: histological investigations on the preservation status of the skin

C. ROSSETTI[1], M. LICATA[1], O. LARENTIS[1], A. CARLI[2], D. PIOMBINO-MASCALI[3], J.M. BIRKHOFF[1], S. CISINI[1], E. FULCHERI[4]

[1]Centre for Research in Osteoarchaeology and Paleopathology, Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2]Department of Humanities, Education and Social Sciences, University of Molise, Campobasso, Italy; [3]Faculty of Medicine, Vilnius University, Lithuania; [4]Section of Pathological Anatomy and Histology (DICMI), University of Genoa, Italy

Paolo Gorini (1813-1881) was one of the first scientists who experimentedwith the"petrification" of corpses, a particular technique used to obtain an artificial preservation of bodies, which found wide application in Italy in the 19th century. This technique allowed the exact features of the deceased to be maintained and for tissue, internal organs and hair to be preserved, mostly in a state of stone hardness. This specific mechanism was based on the replacement of biological liquids with chemical preservatives obtained through intravascular injections. Paolo Gorini performed "petrification" on hundreds of specimens, onentire cadavers as well as on parts of corpses, most of which are now housed in the Paolo Gorini Anatomical Collection of Lodi. Lodi is also home to the manuscript with the two formulas used by Gorini to petrify corpses: "a sulfuric acid solution in the proportion of ten percent or an alcohol-saturated solution of mercuric bichloride and muriate of calcium in the proportion that the volume of the first is ten times that of the second".

The aim of our work is to verify the preservation status of skin that was subjected to "petrification" by Gorini. Our study was carried out on an entirely petrified body of an unknown individual held in the aforementioned collectionat Lodi. The man had been affected by a widespread bulbous-bullous infection, possibly smallpox or pellagra. A superficial fragment of skin, free of lesions, was biopsied from the lateroplantar region of the right foot. The analysis was performed using microscopic slides following the inclusion of the samples in epoxy resin, as well as by a stain with hematoxylin-eosin and Masson's trichrome. Other sections were stained via immunohistochemical technique with anti-cytokeratin antibodies (AE1, AE3) and vimentin.

The histological investigations revealed discretely preserved epithelial tissue, with a structure that is still recognizable on the tangential sections. It is possible to distinguishan easily detachable epithelium of the stratum corneum and deeper, more cohesive, layers (stratum granulosum and spinosum) in which the shadows of nuclei are still recognizable. Histochemical investigations revealed positivity for cytokeratins and negativity for vimentin.

In contrast to natural or embalmed mummified bodies, historic petrified specimens have never been histologically analyzed. This first study demonstrates that the "petrification" method performed by Gorini guaranteed good skin preservation, allowing its histological, histo-chemical, metachromatic and antigenic characteristics to be maintained.

References

Carli A, Piombino-Mascali D. *I preparati anatomici lombardi tra Otto- e Novecento. Paolo Gorini e Giuseppe Paravicini*. Med Secoli 2015;27:413-25.

Carli A. *Storia di uno scienziato. La collezione anatomica Paolo Gorini*. Azzano San Paolo, Italy: Bolis, 2005.

Fulcheri E, Boano R, Grilletto R, et al. *Ancient Egyptian mummies: histological examinations to assess the presence of contaminations or pollutants*. In: Lynnerup N, Andreasen C, Berglund J, eds. *Mummies in a New Millennium*. Nuuk, Copenhagen: Greenland National Museum and Archives and Danish Polar Center 2003:89-92.

Places of culture. Museums and collections of pathological anatomy as vectors of new social relations

R. BOANO[1], R. CILIBERTI[2], F. DE STEFANO[2], A. BONSIGNORE[2] E. FULCHERI[3, 4], E. RABINO[1]

[1]Department of Life Sciences and Systems Biology, University of Turin; [2]Department of Health Sciences (DISSAL) - Section of History of Medicine and Bioethics, University of Genova; [3]Fetal and Perinatal Pathology Unit, IRCCS Istituto Giannina Gaslini, Genoa; [4]Department of Surgical Sciences and Integrated Diagnostics (DISC) - Pathology Division of Anatomic Pathology, University of Genova, Italy

Far from representing a sample of evidence without any current interest, the anatomical and pathological assemblages stored in academic structures are still a valuable scientific and cultural resource for museum collections. In fact, these findings are able to provide, through their precise historical contextualization, important data on epidemiological aspects and medical knowledge over the time.

The lack of suitable places to store them and the absence of human and financial resources together with cultural and emotional barriers regarding the death, damn the biological items to the obscurity, despite attempts to enhance them through systematic activities of cataloguing, restoration, conservation and exhibition.

The promotion of a newsensitivity towards these collections as well as the developmentof a network system among academic structures may promote the recovery of this biological heritage. These actions could increase the scientific value of the items as well as the the memory of the past and could consign to museums a new role of "places for the scientific reflection and the epistemological revision".

The public exhibition of the biological findings, in

accordance with human dignity as well as ethical values, could be a valuable teaching resource towards the knowledge of the human body and also to promote the health awareness. The exposure of healthy organs and pathological ones –in reflecting of unhealthy behaviours and lifestyles or catastrophic natural events – may encourage a critical reflection on the culture of life. At the same time, past human stories, albeit incomplete and fragmentary, may also be an instrument of education in the culture of death and the values of solidarity.

In conclusion, we suggest Museums and collections as vectors of new social relations to be shared with the "community of the living", in order to promote acts of the highest moral value, through awareness campaigns, on the donation of the post-mortem body for study and research purposes.

References

Suarez AV, Tsutsui ND. *The Value of Museum Collections for Research and Society*. BioScience 2004;54:66-74.

Monza F, Licata M. *Anatomical preparations in museums a special category of cultural heritage*. Med Secoli 2015;27:615-28.

Ciliberti R, Monza F, De Stefano F, et al. *The trial of the skull studied by the founder of Criminal Anthropology: The war of the Lombroso Museum*. J Forensic Leg Med 2018;59:13-15. https://doi.org/10.1016/j.jflm.2018.07.011

Ciliberti R, Armocida G, Licata M. Re*bury the "Atavistic Skull" Studied by Lombroso?* Am J Forensic Med Pathol 2019. https://doi.org/10.1097/PAF.0000000000000460

Licata M, Monza F. *Ethical issues in paleopathological and anthropological research experiences*. Acta Biomed 2017;88: 315-8. https://doi.org/10.23750/abm.v88i3.5653

Ancient DNA evaluation from wet specimens in the pathology museum of Turin: a strategic approach

L. FERRARI[1, 2], D. PULCINELLI[3], D. RULLO[4], A. LOMBARDI[3], J. METOVIC[2], F. PAPOLA[3], L. VENTURA[4]

[1]S.C. Anatomia Patologica, Ospedale Cardinal Massaja, Asti; [2]Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Torino; [3]Centro Regionale Immunoematologia e Tipizzazione Tissutale, Ospedale San Salvatore, L'Aquila; [4]U.O.C. Anatomia ed Istologia Patologica, Ospedale San Salvatore, L'Aquila, Italy

Pathology Museums house ancient specimens obtained during autopsies and generally used for educational purposes. The collections usually consist of dry and wet specimens showing diseases that no longer exist or with their natural course unmodified by therapies. The preservation of the macroscopic features due to the storage fluid has a great historical and paleopathological interest. In recent years, increasing attention is being paid to the study of the wet specimens by modern techniques. Ancient DNA (aDNA) has been investigated in different specimens from natural history museums, but the experience with human material is still limited. The time elapsed between death and fixation, and the chemical composition of fixation and storage fluid may irreversibly damage the DNA, thus routine techniques may result ineffective. We propose a simple and reliable approach to aDNA collection and extraction from museum wet specimens.

Ten wet specimens were selected from the Pathology Collection of Turin and submitted to histopathologic re-evaluation. As the chemical composition of the storage fluids is currently unknown, pH value was measured in each specimen. Four cases representative of different classes of pH were submitted to DNA analysis by conservative sampling.

Tiny fragments of tissue were frozen at -20°C to obtain sixty 10 mm-thick sections, collected in microtubes containing 1 ml of digestion solution (75 mM NaCl, 10 mM tris, 0.5 mM EDTA, pH 8.0) and 100 ml of proteinase K solution (18 mg/ml). The samples were incubated at 56°C for 48 h and 50 ml fresh of proteinase K solution were added for 72 h. 400 ml of solution were extracted with magnetic beads using a Roche MAGNA PURE COMPACT instrument.

DNA quantity and quality were evaluated using the full absorption spectrum (220/340 nm) obtained by the Nanophotometer P 300 spectrophotometer. DNA concentration in ng/ml and absorbance ratio at 260/280 nm were calculated from 4 ml samples. The quality of DNA was also observed by electrophoretic run in 1.3% of agarose gel. In order to verify DNA integrity, short tandem repeat (STR) analysis was performed using the PowerPlex 16 HS system (PROMEGA) employed for personal identification.

The cases were originally diagnosed as lymphosarcoma, uterine myosarcoma, esophageal, gastric, and rectal cancers, pancreas tumor, lung cancer, and pleural sarcoma. The range of pH values was comprised between 1.46 and 4.65. The pH value of the specimens submitted to DNA analysis was 2.56, 3.15, 4.45, and 4.65 and the revised diagnoses were necrotic lung carcinoma, uterine leiomyosarcoma, lung metastases from squamous carcinoma of unknown primary, and from uterine leiomyosarcoma.

The first two samples gave negative results on both spectrophotometer and electrophoretic runs. The other two showed a low quantity of DNA (6 ng/ml; 7 ng/ml) with an absorbance ratio of 1.53 and 1.50 at the spectrophotometric analysis. The electrophoretic analysis showed a light band of DNA with molecular weight around 1000 bp in both samples. STR analysis displayed DNA fragmentation, evidenced by ladderization of the electropherograms result. The amplification of amelogenin STRs of chromosome X allowed the precise identification of one patient.

It is well known that DNA is better preserved in alkaline medium, but its quantity and quality may be acceptable also in specimens preserved at pH around 4.5. Museum wet specimens may represent a valid source of aDNA to investigate genetic molecular fea-

tures of ancient diseases. The measurement of pH value of the storage fluid may be useful as a screening method for aDNA preservation.

References

Ferrari L, Coda R, Fulcheri E, Bussolati G. *The role of Pathological Anatomy Museum: past glory, present crisis and future prospects*. Pathologica 2001;93:196-200.

Schaeffer S, Zachos FE, Koblmueller S. *Opening the treasure chest: a DNA-barcoding primer set for most higher taxa of Central European birds and mammals from museum collections*. PLOS One 2017; 12: e0174449. https://doi.org/10.1371/journal.pone.0174449

Hühns M, Röpenack, Erbersdobler A. *Molecular and immunohistochemical characterization of historical long-term preserved fixed tissues from different human organs*. PLOS One 2015;10: e0135297. https://doi.org/10.1371/journal.pone.0135297

Göbbel L, Schultzka R, Klunker R, et al. *Nuchal cystic hygroma in five fetuses from 1819 to 1826 in the Meckel – Anatomical Collections at the University of Halle, Germany*. Am J Med Genet Part A 2007;143A:119-28.

From time to time: multiple healed trauma seen in an early medieval burial of a senile man from La Selvicciola (Italy)

I. MICARELLI[1,2], R.R. PAINE[2], M.A. TAFURI[2], G. MANZI[2]

[1]Dipartimento di Scienze dell'Antichità, Sapienza Università di Roma; [2]Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Italy

Investigating multiple traumas observed in an individual or among members of an entire historic community has always been an area of great interest for paleopathologists and bioarchaeologists. One task faced by paleopathologists is related to the nature of the traumatic event. Such violent events can be accidental or intentional in origin. Intentional violence might be self-inflicted or an act from another person. Once this has been determined, discriminating between multiple injury events and a single event (with multiple fractures) is challenging. While assessing the skeletal collection from the post-classical necropolis of Selvicciola (Viterbo, Latium, Italy; 4th-8th centuries AD), an adult male burial revealed a unique pattern of healed injuries. This male (T 90/5) was buried without grave goods. His tomb is located far from the church, which is the centre of the necropolis. T 90/5 is part of a specific burial group of Longobards situated in South-Eastern funerary area (dated to later period of the 7th century AD). Of these elements we located 6 fractures. This included a well healed nasal fracture, right clavicle fracture, a right scapula fractured with healing along the entire superior body (glenoid fossa to vertebral border), left scapula with an acromion process fracture healed but unfused, a healed rib right fracture and a left femoral neck fracture (unfused). This last facture appears to have happened a few months before his death. The lower edge of the fracture on the femur is well healed with a line of 2 mm of new bone formation. Moreover, the periosteal surface under the neck shows eburnation compatible with the eburnation of the inner part of the head of the femur. The inner surface of the femoral head shows polished remodelled trabeculae lesion. Its creation can likely suggest that a pseudo-articulation between the edge of the diaphysis and the head of the femur was formed as a result of movement of the joint area after the fracture occurred. Also related to the health status of the male is the considerable state of DJD of long bones, as well as the significant evidence of vertebral OA and Schmorl's nodes. Towards the end of his life this individual was suffering from a number of chronic problems, which produced skeletal lesions specific to biomechanics and old age. Some of this might have been influenced by the trauma experienced earlier in life. Likely, at the end his life his last fracture might have been due to osteoporosis of the femoral neck. The survival of this man testifies to community care and a high value given to human life. The variety of implications in this case-study inform us the care for this individual, that for most part of his life was a disable. Not only the injuries, but also the pain suffered had to affect his daily life. In the end, the protocol of cares was realized by the community at least two times; for the first pattern of trauma (nose, shoulders, clavicle and rib) and for the femur break.

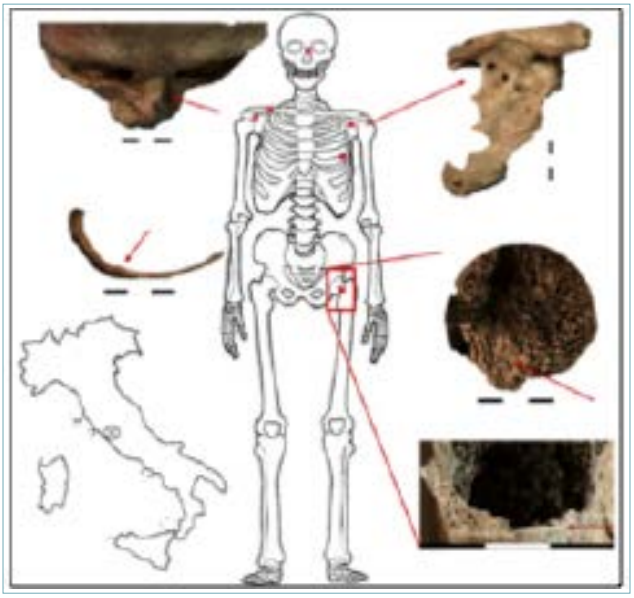


Figure. Diagram of skeleton t 90/5 with the healed fractures in red. On left: the nasal bones (up), and the right rib (centre). Right: the right scapula (up), the head and the edge of the shaft of the right femur (centre and bottom). On left bottom the site of Selvicciola, VT.

References

Buikstra JE, Cook DC, Bolhofner KL. *Introduction: Scientific rigor in paleopathology*. Int J Paleopathol 2017;19:80-7. https://doi.

org/10.1016/j.ijpp.2017.08.005
Gazzetti G. *La villa romana in località Selvicciola (Ischia di Castro)*. Oxbow Monograph 1995;297-302.
Paine RR, Mancinelli D, Ruggieri M, et al. *Cranial trauma in Iron Age Samnite agriculturists, Alfedena, Italy: implications for biocultural and economic stress*. Am J Phys Anthropol 2007;132:48-58. https://doi.org/10.1002/ajpa.20461
Tilley L. *Theory and practice in the bioarchaeology of care*. New York: Springer, 2015.

Session 4

Chairmen: L. Ferrari (Asti), E. Fulcheri (Genova)

A new medieval case of rheumatoid arthritis-like polyarthropathy from the cemetery of San Biagio in Cittiglio (Northern Italy)

C. TESI[1], V. GIUFFRA[2], G. FORNACIARI[3], O. LARENTIS[1], M. LICATA[1]

[1]Centre of Research in Osteoarchaeology and Paleopathology, Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2]Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa; [3]Department of Civilizations and Forms of Knowledge, University of Pisa, Italy

Rheumatoid arthritis is a chronic, systemic, inflammatory condition that starts from a synovitis, leading to diffuse erosions in the marginal area of joints and finally conducting to articular deformity and destruction of bone ends. The aetiology of the disease is unknown but multiple genetic and environmental factors have been linked to its development. According to clinical studies, 10-30% of cases undergo healing of lesions and spontaneous remission of the disease.

Today's approach to inflammatory diseases is conditioned by the early diagnosis, thanks to the evolution of the diagnostic methods and by the mitigation of drug therapies. In the past, the remission was entrusted only to the individual's immune resistance.

A case of erosive polyarthropathy has been discovered in an elderly male individual recovered from the medieval cemetery of San Biagio in Cittiglio (northern Italy). The well preserved skeleton was unearthed in the external area in front of the church access and, according to the archaeological stratigraphy, it dates back to a period between the 12th and the 13th century. The bone elements, following macroscopic and microscopic analysis, exhibit several erosive lesions with symmetrical distribution, affecting firstly the appendicular skeleton of the little joints of hands and feet and other larger joints, such as the shoulder, elbow and hip. The bony tissue involved by the erosions is the so-called "bare area", in the marginal region of the joints, where the synovium membrane-lined bone is found.

The diagnosis of this erosive polyarthritis is complicated by the mild expression of the lesions and by the presence of a subtle sclerotic border to some erosions radiographically observed. Next, a careful differential diagnosis was necessary to clarify the aetiology of the polyarthropathy; the skeletal distribution of the lesions and their macroscopic and radiological appearance are suggestive of a case of rheumatoid arthritis-like polyarthropathy. A hypothetical remission phase of the disease, as demonstrated by the frequent presence of smoothed borders and sclerosed margins on radiographic images, is also suggested. Co-existence of diffuse marginal lipping, joint degeneration and severe areas of eburnation, is also recorded, suggesting a co-morbidity of the erosive condition with osteoarthritis, which is compatible with the advanced age of the individual.

With this medieval case, we present new evidence of the existence of erosive arthritis and, specifically, of rheumatoid arthritis-like polyarthropathy in Europe before the discovery of the Americas, entering into the long debate about the antiquity of the disease that, firstly, was considered as originating in the New World and subsequently spread to the Old World.

On the basis of this and other already published cases, rheumatoid arthritis seems to have been present in Europe more anciently than was previously thought.

References

Deane KD, Demoruelle MK, Kelmenson LB, et al. *Genetic and environmental risk factors for rheumatoid arthritis*. Best Pract Res Clin Rheumatol 2017;31:3-18. https://doi.org/10.1016/j.berh.2017.08.003
Fontecchio G, Fioroni MA, Azzarone R, et al. *Genetic predisposition to rheumatoid arthritis in a Tuscan (Italy) ancient human remain*. Int J Immunopathol Pharmacol 2007;20:103-9. https://doi.org/10.1177/039463200702000112
Rothschild BM, Woods RJ, Ortel W. *Rheumatoid arthritis "in the buff": Erosive arthritis in defleshed bones*. Am J Phys Anthropol 1990;82:441-9. https://doi.org/10.1002/ajpa.1330820406
Waldron T, Rogers. *An epidemiologic study of sacroiliac fusion in some human skeletal remains*. Am J Phys Anthropol 1990;83:123-7. https://doi.org/10.1002/ajpa.1330830114

The medieval cemetery of via Orfeo (Bologna): four possible cases of venereal treponematosis

A. PIETROBELLI[1], V. MARIOTTI[1], S. FUSARI[1], M. BETTUZZI[2], M.P. MORIGI[2], M.G. BELCASTRO[1]

[1]Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna; [2]Department of Physics and Astronomy, Alma Mater Studiorum University of Bologna, Italy

Between 2012-2014 the Superintendence of Archaeology, Fine Arts and Landscape of Emilia-Romagna, has conducted archaeological fieldworks on a large Late Medieval cemetery (14th-16th century), which archaeological and documentary sources attribute to a Jewish context. The Laboratory of Bioarchaeol-

ogy and Forensic Osteology of University of Bologna conducted an anthropological study on a sample of 130 individuals. This contribution aims to present four possible cases of venereal treponematoses (TT. 91, 136, 170, 187).
Human skeletal remains of graves 91, 136, 170, and 187 were examined to reconstruct the biological profiles and to conduct paleopathological and tomographic analyses, given the presence of lesions on several anatomical districts. Cranial lesions were present on individuals from TT.91 (M, 25-35 years), 170 (M, 25-35 years) and 136 (M, 15-18 years), in which simultaneous destructive and proliferative processes (caries sicca) with focal destruction and remodeling of the external surface and diploe are denoted. Long bones of these three individuals also present osseous alteration such as gummatous osteoperiostitis, with an increased bone density and non-uniform thickening. Individual of T. 187 (11-12 years) presents a hole (3 cm Ø) located on frontal bone, whose margins are remodeled with proliferative processes both on ectocranial and endocranial sides. These lesions are likely linked to treponematosis (bacterial infection by Treponema), interpreted as venereal syphilis. After differential diagnoses, we suppose the individuals of TT. 91 and 170 were likely affected by an advanced stage of the infection, while individual T. 136 seems to have been affected by a tardive congenital form of the disease. Lesions of individual of T.187 suggest an infective origin, but poor bone preservation prevents a clearer interpretation. These cases of treponematosis, possibly linked to venereal syphilis, are relevant for paleoepidemic aspects, as well as contributing to only few Italian osteological cases dating to the same period.

A probable case of spinal tuberculosis. The 18th-20th century concealed body of Azzio, Varese, Northwestern Italy

O. LARENTIS[1], C. ROSSETTI[1], E. TONINA[2], C. TESI[1], R. FUSCO[1], M. LICATA[1]

[1]Centre of Research in Ostearchaeology and Paleopathology, Department of biotechnologies and life Sciences, University of Insubria, Varese; [2]B. Bagolini Laboratory, Department of Humanities, University of Trento, Italy

Spinal tuberculosis (STB) is a well-known disease in paleopathology. Paleopathologists have highlighted in the last decades some morphological criteria for its diagnosis. Commonly, we are witnessing the destruction of the intervertebral disc space and the adjoining vertebral bodies, the collapse of the vertebrae and the anterior wedging which lead to a structural kyphosis classifiable in gibbus deformity. Here we present the probable STB case of a male subject, 55 years, concealed between the 18th and the first half of the 20th century in the Franciskcan monastery of Azzio, Varese, Italy. The skeleton was found both in an optimal state of preservation and representation. Anthropological analysis was performed according to Buikstra and Ubelaker standards. Paleopathological diagnosis was conducted thanks to macroscopic, microscopic and radiographic analysis, also in order to perform the differential diagnosis. Even if STB was widely present in the last centuries in northwestern Italy, only few paleopathological cases was directly studied.

References

Fornaciari G, Giuffra V. Lezioni di paleopatologia. Genova: ECIG, 2009.

Licata M, Rossetti C. The Skeleton of the Azzio's Crypt (Northern Italy): A Forensic Case in an Archaeological Context. Am J Forensic Med Pathol 2017;38:272-4. https://doi.org/10.1097/PAF.0000000000000327

Spekker O, Hunt DR, Váradi OA, et al. Rare manifestations of spinal tuberculosis in the Robert J. Terry Anatomical Skeletal Collection (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA). Int J Osteoarchaeol 2018; 28: 343-353.

Cystic echinococcosis of 13th century from the abbey of Badia Pozzeveri, Lucca

A. FORNACIARI[1], A. AMARO[1], L. CAVALLINI1, F. COSCHINO[1], R. GAETA[1], C. TESI[2], G. VERCELLOTTI[3], M. DE SANCTIS[4], R. ISHAK[4], G. ARINGHIERI[5], F. BRUSCHI[6], M. MANNINO[7], G. FORNACIARI[1], V. GIUFFRA[1]

[1]Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa; [2]Centre of Research in Osteoarchaeology and Paleopathology, Department of Biotechnology and Life Sciences, University of Insubria, Italy; [3] Department of Anthropology, The Ohio State University, USA; [4]Department of Civil and Industrial Engineering, University of Pisa; [5]Diagnostic and Interventional Radiology, University of Pisa; [6]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy; [7]Department of Archaeology and Heritage Studies, Aarhus University, Denmark

Cystic echinococcosis (CE) is a zoonosis caused by Echinococcus granulosus. The life cycle of the parasite develops in the canids, which house the adult tapeworm in the intestine, and in the intermediate mammal hosts. Humans are occasional dead-end hosts, infected by eggs ingestion via fecal-oral route. The larvae from the digested eggs penetrate the human gut wall and are disseminated throughout the body by the blood. The soft tissues involved at the level of the capillaries may host the larvae, and the hydatid cyst can develop in different organs. The liver is the first organ that the larvae encounter through the blood stream and consequently it is the most frequently involved; it is followed by the lungs and then other organs in frequency.
The hydatid cyst is a fluid-filled formation that grows

Figure. Hydatid cyst from a 13th century burial of the Abbey of Badia Pozzeveri, Lucca.

centrifugally and that can survive in the intermediate host for years. In 10 years, it can growth to a diameter of 15-20 cm. Inside hyaline outer membrane, a cellular germinating layer produces microcystic structures that develop scolices. The scolices pouring out of the cyst develop one or more cysts that can reach every tissue. The life cycle is completed when the definitive host feeds on organs of the intermediate host that contain fertile metacestodes. Death of the germinating layer within the metacestode produces calcification of the cyst wall in the intermediate host.

Calcified hydatid cysts found as archaeological finds are generally associated with skeletal remains in the thoraco-abdominal site. In archaeological records, the presence of echinococcosis is underestimated, and the find is relatively rare for different reasons: 1) difficulty of recognition by archaeologists; 2) need of accuracy in excavation and recovery of osteoarcheological remains; 3) fragility of calcified formation in the soil. Furthermore, the taphonomic alterations can cause the translation from the original site of the calcified formation and undermine the recognition of the organ affected.

In Italy there are only two archaeological samples of calcified formation, most probably of echinococcosis origin, described in the paleopathological literature: one from Siena (13th-14th centuries) and one from Abruzzo (early 20th century). In this report we describe another calcified formation found in the archaeological excavation of the monastic site of Badia Pozzeveri, near Lucca, Tuscany, for which we propose a diagnosis of CE.

This finding comes from a privileged lithic coffin built on the northern side of the monastic Church of San Pietro. The grave was used in the 13th century as collective burial by the same laical family group. Calcification, associated with a female individual of about 35-45 years, was discovered in the thoraco-abdominal region. We propose the diagnosis of hydatid cyst from Echinococcus granulosus based on gross morphology, micro-morphology, and a multicomponent approach with cone beam computed tomography, SEM/EDS and stable isotope analysis.

References

Bruschi F. *Helminth Infections and their Impact on Global Public Health*. Wien: Springer-Verlag 2014.

D'Anastasio R, Vitullo G, Paolucci A, et al. *A paleopathological case of Echinococcus cyst*. J Paleopathol 2008;20:67-73.

Fornaciari G, Tornaboni D, Pollina L, et al. *Nota paleopatologica: un caso di cisti da echinococco*. In: Boldrini E, Parenti R. *Santa Maria della Scala. Archeologia e edilizia sulla piazza dello Spedale*. Firenze: Edizioni "All'Insegna del Giglio" 1991.

Fornaciari A, Francesco C, Cariboni A, et al. *Badia Pozzeveri (LU). Lo scavo bioarcheologico di un monastero lungo la via Francigen.*, Notiziario della Soprintendenza Archeologica della Toscana 2016;11:123-35.

Reinhard KJ. *Parasitology as an interpretive tool in archaeology.* Am Antiquity 1992;57:231-45.

A possible case of biparietal osteodystrophy from the medieval church of Sant'agostino, Caravate, Varese (Northwestern Italy)

E. TONINA[1], C. PANGRAZZI[1], M. LICATA[2], I. GORINI[2], O. LARENTIS[2]

[1]B. Bagolini Laboratory, Department of Humanities, University of Trento; [2]Centre of Research in Ostearchaeology and Paleopathology, Department of Biotechnologies and life Sciences, University of Insubria, Varese, Italy

Biparietal osteodystrophy (BO), with symmetrical and bilateral thinning of the parietal bones, is a condition rarely discussed in the paleopathological literature. In the past, it has been described as a non-metric trait, anatomical variation and development anomaly. Even though the aetiology is still unknown, today it is described as a pathological condition. In many individuals it appears to be age-related, therefore it has been attributed to osteoporosis, postmenopausal and senile atrophy. Other causes could be congenital and hereditary transmission. Here, we presented a possible case of BO, detected on the remains of a 50-years-old female subject (Tomb 8) excavated in the cemeterial area of the medieval church of Sant'Agostino in Caravate (Varese). Anthropological analysis was performed in accordance with the standards proposed by Buikstra and Ubelaker. Pa-

leopathological conditions were evaluated macroscopically and microscopically. Moreover, CT scan was carried out to investigate pathological evidences. Even if the cause of this uncommon condition is not yet well understood, the present case is highly significant as it enters into the debate upon the aetiology of the disease.

References

Cederlund CG, Andrén L, Olivecrona H. *Progressive bilateral thinning of the parietal bones*. Skel Radiol 1982;8:29.

Epstein BS. *The Concurrence of Parietal Thinness with Postmenopausal, Senile, or Idiopathic Osteoporosis*. Radiology 1953;60: 29-35.

Mann RW, Kobayashi M, Schiller AL. *Biparietal thinning: accidental death by a fall from standing height*. J Forensic Sci 2017;62: 1406-9. https://doi.org/10.1111/1556-4029.13425

Atherosclerosis in the skeletal remains of a 15th century man

R. BOANO[1], V.G. VELLONE[2,3], M. PAUDICE[2], C.M. BIATTA[2], L. PEÑUELA[4], A. CINTI[1], E. FULCHERI[2,4]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino; [2]Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate, Università di Genova; [3]U.O. Anatomia Patologica Universitaria, IRCCS Ospedale Policlinico San Martino, Genova; [4]U.O.S.D. Patologia Feto-Placentare, IRCCS Istituto Giannina Gaslini, Genova, Italy

Atherosclerosis and its complications represent an important health problem throughout the modern world, although it seems to have accompanied humanity since its beginnings. Important documents have been reported in mummified remains. Atheromas may undergo extensive deposition of calcium and bone metaplasia (Monckeberg's arteriosclerosis) and persist to the disintegration of the soft tissues.

A complete 45-55-years-old male skeleton, 165 cm tall, from bishop's Palace in Ivrea (Turin) was discovered during archaeological excavation in 2016. Radiocarbon analysis dated the skeleton to 1400-1600 AD.

During skeletal preparation in laboratory, an ectopic biological calcification tubular shaped (19 mm in length and 7 mm in diameter) were detected among pelvic bones.

At the macroscopic examination, the finding appears as an irregular tubular calcification; in cross section, the mineralized deposits span the entire volume of the lumen and some bony trabeculae in the central space are well defined.

Severe calcification of the blood vessel is supposed. Calcification along the expected course of an artery/vessel was considered to be probable atherosclerosis. In relation to the anatomical localization it is supposed to be an atherosclerotic calcification of iliac or femoral artery.

The case study reports an uncommon finding of arterial/vessel calcification detected on skeletal remains of an adult male from the post-medieval period. Since the age plays an important role in atherosclerosis, we do not exclude that vascular calcifications affected arteries in many regions of the body. The presented paleopathological specimens suggest that our knowledge of risk factors and the etiology of atherosclerosis are incomplete. A chronic inflammatory burden may have played a greater role than previously considered in ancient cultures and population including upper classes of Italian Renaissance. While increasingly prevalent with age in ancient and modern cultures, a strong gene-environmental interplay is established in the development of atherosclerosis across the lifespan. While genes create the vulnerability, the environment determines when and if atherosclerosis becomes manifest clinically.