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PATHOLOGICA

Ospedali Galliera di Genova
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by mail addressed to:
Pathologica – pathologica@pacineditore.it

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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 .TIF or JPEG format, resolution at least 300 dpi (100 x 150 mm). Will not be accepted for publication manuscript with images of bad quality.

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

Examples of the correct format for bibliographic citations:

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

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

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Published by Pacini Editore, Pisa, Italy - June 2016
Signet-ring cell ependymoma is a rare variant of ependymoma with only seven cases described in literature. Biological behavior and prognosis of this entity are not well-known until now. We present a case of a 49-year-old female with a history of headache and gait instability. Magnetic resonance imaging showed an upper cervical tumor with cystic component and mural nodule. The patient underwent surgery. Microscopically some cells displayed an eccentric nucleus compressed to the periphery by vacuolated cytoplasm. Pervascular pseudorosettes and ependymal rosettes were seen only focally. The cells were positive for glial fibrillary acidic protein and epithelial membrane antigen. The diagnosis was ependymoma with diffuse signet-ring features, grade II according to the World Health Organization. It may be difficult to diagnose this unusual variant of ependymoma especially on small biopsies or frozen sections. A complete examination of the specimen is recommended with immunohistochemical confirmation to rule out potential morphologic mimics, such as metastatic adenocarcinomas and gliomas in the differential diagnosis.

Case reports

Cellular fibroma in the Douglas cavity, mimicking a malignant neoplasia: fibroma, fibrosarcoma or mitotically active cellular fibroma? A. Di Lorito, P. Viola, S. Rosini, G. Lattanzio

Introduction. Ovarian fibroma is a benign stromal tumour composed of spindle/ovoid fibroblastic cells producing collagen. Approximately 10% of fibromas are densely cellular with small amount of collagen. In these cases, if mild nuclear atypia is present, they are best addressed as cellular fibroma. However cellular fibroma may show a greater mitotic activity and therefore they should be referred as mitotically active cellular fibromas. Mostly benign, it is necessary to differentiate them from malignant tumours such as fibrosarcomas.

Methods. We report a case of an unusual presentation of mitotically active cellular fibroma, detected in the Douglas cavity of a young woman, with normal appearing ovaries and uterus, mimicking a malignant neoplasia clinically and on imaging. In fact abdomino-pelvic mass may be associated with acute pain, resulting in clinical emergency, really difficult to distinguish from a frank malignancy, before surgical procedure.

Results. We described the clinical, radiological and pathological characteristics of our case and we make a comparison of what previously described in literature.

Discussion. The differential diagnosis among those entities is based on the microscopic features such as atypia and the number of mitoses. However, according to their dimensions, it may be necessary to generously sample these tumours and sometimes, to perform a panel of immunohistochemical markers, in order to make a correct diagnosis, establish the best treatment and the right follow-up. In fact, the prognosis is not certain, due to the possible recurrence, especially if not completely excised.

Type II congenital pulmonary airway malformation associated with intralobar pulmonary sequestration: report of a case and review of classification criteria M.G. Mastrogiulio, A. Barone, M.G. Disanto, A. Ginori, M.R. Ambrosio, S.F. Carbone, D. Spina

Pulmonary congenital abnormalities are rare disorders including congenital pulmonary airway malformations (CPAM) and pulmonary sequestration (PS). CPAM is a lesion characterized by the presence of anomalous bronchial or acinar structures, variable in size, either cystic or not cystic. PS is generally

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Review

Health Technology Assessment: introducing a vacuum-based preservation system for biological materials in the anatomic pathology workflow

R. Saliceti, E. Nicodemo, A. Giannini, A. Cortese

Introduction. The objective of this work is to assess the implementation of a newly introduced medical equipment technology for the vacuum-based preservation of biological materials within an Anatomic Pathology service.

Methods. The approach selected for the analysis is the Health Technology Assessment (HTA), a comprehensive evaluation method based on relevant scientific evidence and designed to support healthcare decision makers in purchasing, replacing or disposing of technologies. The analysis focused on specific domains such as Technology, Organization, Safety and Economy.

Results. The study proves that the use of such technology ensures the biological specimen to be suitably preserved (up to 72 hours), both reducing the amount of fixative being employed in the diagnostic process (30% to 55%) and resulting, in the particular context under examination, in savings of 93%.

Discussion. The HTA reported no significant drawbacks related to the use of the technology being examined. Nonetheless, the workflow for managing the transfer of biological materials from the Operating Room to the Anatomic Pathology department needs to be redefined – in terms of handling, processing, storage and disposal. Other elements concerned the monitoring of storage temperature, fresh tissue handling and especially fixative amount reduction, which positively impacts on the operators’ safety with regard to chemical hazards.

Original article

Ependymoma with diffuse signet-ring features: report of a case and review of the literature

L. Cima, S. Beccari, C. Ghintenton, G. Pinna, A. Beltramello, M. Chilosi, M. Brunelli, A. Eccher

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L. Cima, S. Beccari, C. Ghintenton, G. Pinna, A. Beltramello, M. Chilosi, M. Brunelli, A. Eccher
defined as nonfunctioning lung tissue that is not in normal continuity with the tracheobronchial tree and that derives its blood supply from systemic vessels. We describe a case of a baby girl with a very rare association between CPAM type 2 and intralobar pulmonary sequestration (IPS) focusing on the cystic lesions typical of CPAM and on the lymphatic and blood vessels. The cells lining the cysts often were positive for D2-40 (oncofetal protein M2A). Lymphatic endothelial cells, positive for D2-40, were widely present in the lung parenchyma and dilated lymphatic vessels were present also in the inter-alveolar septa. Moreover, we discuss the pathogenesis of CPAM and its classification criteria.

Neuroglial heterotopia of the scalp
Heterotopic glial nodules of the scalp are non hereditary congenital malformations composed of mature brain tissue isolated from the cranial cavity. The majority of these lesions are found in the nasal region and occur rarely on the scalp. They are frequently diagnosed in newborn infants. However, they may rarely be found in adults. The pathogenesis of these lesions remains unknown. We describe the case of a temporal scalp nodule in a 50 year-old man. At the time of the excision, the mass was not associated with intracranial connection. Histological examination revealed neural tissue staining with S100-protein and the glial fibrillary acidic protein (GFAP).
2015 GIPaM Recommendations
(developed in 2013; updated December, 2014; updated December, 2015)

A DOCUMENT SHARED WITHIN GIPaM MEMBERS
(GRUPPO ITALIANO DI STUDIO DI PATOLOGIA MAMMARIA)

DOCUMENT TRANSLATED BY DAVIDE BALMATIVOLA¹ AND FRANCESCA MALETTA²
¹ Dipartimento di Scienze Mediche-Università di Torino; ² AOU Città della Salute e della Scienza di Torino

- Request form for cytological and microhistological specimens
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- In situ hybridization
  - FISH: interpretation of results
  - CISH/SISH: interpretation of results
Request form for cytological and microhistological specimens

GENERAL DATA
1. Patient identifiers
   - Name
   - Date and place of Birth
   - Gender
   - Unique identifier (i.e. health record, master index number, national insurance number)
   - Address and phone number

2. Identifiers of the authorized person requesting the exam (name, operative unit, phone number)

3. Date and time of specimen collection

RELEVANT CLINICAL DATA ON EXTRA-MAMMARY PATHOLOGIES:

SENSOLOGICAL ANAMNESIS:
- prior pathologies
- prior surgery
- recent/on-going pregnancy
- on-going breastfeeding
- menopause
- use of hormone replacement therapy (HRT) or oral contraceptive (OC)

CLINICAL DATA ON THE PRESENT BREAST LESION:

FEATURES OF THE LESION:  □ Palpable  □ Not palpable  □ Ulceration  □ Other ............

SITE OF THE LESION/S:  □ Right breast  □ Left breast
- UOQ (Q1) - UIQ (Q2) - LOQ (Q3) - LIQ (Q4) – Central-reatroareolar (Q5)  □ Nipple
- Intermediate QQ: superior – equatorial internal - inferior – equatorial external
- Axillary tail of the breast  □ Axillary lymph node

GUIDANCE USED FOR SAMPLING:
- Palpation  □ Mammography (stereotactic-guidance)  □ Ultrasound  □ MRI

TYPE OF CYTOLOGICAL SAMPLE:
- Fine needle aspiration cytology (FNAC)
- Secretion
- Scraping
- Smear (specify the number of slides) ............
- Liquid-based cytology (cytolit)

TYPE OF HISTOLOGICAL SAMPLE:
- Tru-cut (NCB)
- Vacuum-assisted (VANCB)
- Needle diameter G..........  □ Number of specimens........

PRESENCE OF MICROCALCIFICATIONS AT RX EXAMINATION OF THE BIOPSY:  □ Yes  □ No

REQUISITION TO ASSESS PROGNOSTIC/PREDICTIVE FACTORS ON BIOPSY FOR NEOADJU-VANT THERAPY:  □ Yes  □ No
INSTRUMENTAL DIAGNOSTICS PERFORMED ON THE PRESENT BREAST LESIONS
(enclose a copy of the reports and/or fill in the following form):

MAMMOGRAPHY: (exam date.............)
☐ N° of lesions: ☐ Dimensions (mm): ☐ Radiological category: R....... or BIRADS....... 

☐ TYPE OF LESION: ☐ Opacity with spiculated margins ☐ Opacity with well defined margins
☐ Parenchymal distortion ☐ Asymmetrical density

☐ MICROCALCIFICATIONS: ☐ Absent ☐ Morphology: ☐ Distribution: ☐ Extent:

☐ Diagnostic hypothesis...........................................

ULTRASONOGRAPHY: (exam date.............)
☐ N° of lesions ☐ Dimensions (mm): ☐ Ultrasound category: U………

☐ TYPE OF LESION (solid or cystic) and echogenicity: ………………………………

☐ Diagnostic hypothesis...........................................

MRI: (exam date.............)
☐ N° of lesions: ☐ Location: ☐ Dimension (mm): ☐ MRI category: ........

☐ Diagnostic hypothesis...........................................

OTHER INSTRUMENTAL DIAGNOSTICS (specify which): ………………………………

PRIOR CYTOLOGICAL/HISTOLOGICAL EXAMS

CYTOLOGICAL EXAM: ☐ Category (C): C1 (inadequate) C2(benign) C3 (atypical, probably benign) C4 (suspicious, probably malignant) C5 (malignant) (specify the identification serial number C/………. of the Department of pathology of ………………… date of execution……………)

MICROHISTOLOGICAL EXAM: ☐ Category (B): B1 (normal tissue/uninterpretable) B2 (benign) B3 (lesion of uncertain malignant potential) B4 (suspicious of malignancy) B5a (in situ carcinoma) B5b (invasive carcinoma) (specify the identification serial number B/………. of the Department of Pathology of ………………… date of execution……………)

HISTOLOGICAL EXAM: (specify the identification serial number B/………. of the Department of Pathology of ………………… date of execution……………) Type of sample: ☐ Punch ☐ Incisional biopsy/lumpectomy
☐ Cuneiform resection of ducts ☐ Excisional biopsy/lumpectomy ☐ Quadrantectomy
☐ Breast scar revision for local recurrence ☐ Other ………

Enclose a copy of prior cyto/histological exams performed in a different Institution.

Signature (clear script/legible) of the person requesting the exam
Request form for surgical specimens

GENERAL DATA
1. Patient identifiers
   • Name
   • Date and place of Birth
   • Gender
   • Unique identifier (i.e. health record, master index number, national insurance number)
   • Address and phone number

2. Identifiers of the authorized person requesting the exam (name, operative unit, phone number)

3. Date of surgery

RELEVANT CLINICAL DATA ON EXTRA-MAMMARY PATHOLOGIES:

SENOLOGICAL ANAMNESIS:
☐ prior pathologies   ☐ prior surgery   ☐ familiarity for breast neoplasms   ☐ BRCA1/BRCA2 gene mutations

CLINICAL DATA ON THE PRESENT BREAST LESION:

FEATURES OF THE LESION:   ☐ Palpable   ☐ Not palpable   ☐ Ulceration   ☐ Other .............

NEOADJUVANT THERAPY:   ☐ No
   ☐ Yes: Prior typization:   ☐ ER   ☐ PgR   ☐ Ki-67   ☐ HER2

Clinical-instrumental response:   ☐ Complete   ☐ Partial   ☐ Absent

SITE OF THE LESION/S:   ☐ Right breast   ☐ Left breast
☐ UOQ (Q1) - UIQ (Q2) - LOQ (Q3) - LIQ (Q4) – Central-reatroareolar (Q5)   ☐ Nipple
☐ Intermediate QQ: superior – equatorial internal - inferior – equatorial external
☐ Axillary tail of breast   ☐ Axillary lymph node

TYPE OF SAMPLE:
• Incisional biopsy
• Excisional biopsy (lumpectomy)
• Duct excision/Microdochectomy
• Large resection:   ☐ with pectoral fascia   ☐ without pectoral fascia
   ☐ with skin   ☐ without skin
• Quadrantectomy   ☐ with pectoral fascia   ☐ without pectoral fascia
   ☐ with skin   ☐ without skin
• Simple mastectomy
• Skin sparing mastectomy
• Nipple sparing mastectomy
• Radical mastectomy
• Axillary lymph nodes dissection
• Sentinel lymph node
• Axillary lymph nodes dissection (after sentinel lymph node biopsy)
• Second/additional surgery (specify the orientation/new margin: .............)
• Breast parenchyma from reduction mammoplasty (specify if it is an aesthetic mammoplasty, or it has been performed to match with the other breast/oncoplastic breast reduction)
• Radicalization mastectomy (specify the histological number of the report of the prior surgery and provide a copy, if surgery was carried out in another institution)
• Retro-areolar disc
• Other (specify) 

MARGIN MARKERS ON THE SURGICAL SPECIMEN (specify the type of marker):
• Superior-medial margin: ...........................................
• Inferior-lateral margin: ...........................................
• Deep margin/toward the fascia: .................................. 
• Superficial margin/toward the skin: ............................
• Retro-areolar margin/toward the nipple: ....................... 

Note: If the skin is not present, use at least three markers

INSTRUMENTAL DIAGNOSTICS PERFORMED ON THE PRESENT BREAST LESION
(enclose a copy of the reports and/or fill in the following form):

MAMMOGRAPHY: (exam date.............)
☐ N° of lesions: ☐ Dimensions (mm): ☐ Radiological category: R....... or BIRADS....... 

☐ TYPE OF LESION: ☐ Opacity with spiculated margins ☐ Opacity with well-defined margins
☐ Parenchymal distortion ☐ Asymmetrical density

☐ MICROCALCIFICATIONS: ☐ Absent ☐ Morphology: ☐ Distribution: ☐ Extent: 
☐ Diagnostic hypothesis..........................

ULTRASONOGRAPHY: (exam date.............)
☐ N° of lesions ☐ Dimensions (mm): ☐ Ultrasound category: U………..

☐ TYPE OF LESION (solid or cystic) and echogenicity: ……………………………
☐ Diagnostic hypothesis..........................

MRI: (exam date.............)
☐ N° of lesions: ☐ Location: ☐ Dimensions (mm): ☐ MRI category: ........
☐ Diagnostic hypothesis..........................

OTHER INSTRUMENTAL DIAGNOSTICS (specify which): ....................................

PRIOR CYTOLOGICAL/HISTOLOGICAL EXAMS

CYTOLOGICAL EXAM: ☐ Category (C): C1 (inadequate) C2(benign) C3 (atypical, probably benign) C4 (suspicious, probably malignant) C5 (malignant) (specify the identification serial number C/………. of the Department of Pathology of .......... date of execution...........)

MICROHISTOLOGICAL EXAM: ☐ Category (B): B1 (normal tissue/uninterpretable) B2 (benign) B3 (lesion of uncertain malignant potential) B4 (suspicious of malignancy) B5a (in situ carcinoma) B5b (invasive carcinoma) (specify the identification serial number B/........ of the Department of pathology of ................. date of execution..........)

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☐ Cuneiform resection of ducts ☐ Excisional biopsy/lumpectomy ☐ Quadrantectomy
☐ Breast scar revision for local recurrence ☐ Other ......
Enclose a copy of prior cyto/histological exams performed in a different institution.

Surgery (clamping of arterial vessels: beginning of warm ischemia) started at ..........  
The complete removal of the surgical specimen (end of warm ischemia/beginning of cold ischemia) ended at ..........  
After removal, the surgical specimen was  
☐ immediately fixed in 10% neutral buffered formalin (end of cold ischemia)  
☐ submitted as “fresh” tissue to the pathology laboratory, where it arrived at .................

Signature (clear script/legible) of surgeon

Figure to be used for schematic representation of the site of the lesion, the type of surgical intervention and the site of markers (if any)

For a better localization of the lesion, please specify:  
The hour on the face of a clock corresponding to the site of the lesion: hour ..............  
Distance from the nipple: cm ............  
Depth from the skin (distance between the skin and the surface of the lesion): mm .......
How to submit fresh tissue samples to the Pathology Laboratory

In order to allow a correct morphological, immunophenotypical and molecular evaluation (which are necessary to define the therapeutic protocol), surgical samples must be immediately, accurately and completely fixed. The best way to obtain is to transfer as quickly as possible (maximum 30 min after the sampling) the fresh-tissue samples, or the formalin-fixed samples, to the pathology laboratory. Operators involved in the management of neoplastic samples must reach an agreement on how to guarantee a correct and immediate fixation, also through possible variation of the time of execution of biopsies and surgical sampling. Surgical samples must be intact and well oriented (with sutures, clips, etc). In absence of cutis, the presence of at least three markers, one of which for the nipple, is required.

When a surgical sample is incidentally or necessarily torn, its reconstruction with a suture is mandatory, and the problem should be reported to the pathologist, in order to allow the optimal evaluation of surgical margin. When the immediate delivery of the samples to the pathology laboratory is not possible, these must be quickly (in maximum 30 minutes) fixed in 10% neutral buffered formalin. Samples must be totally absorbed by formalin, in order to obtain an optimal fixation, also performing parallel cuts from the deep margin to the skin (maximum thickness of 0.5 cm), which may involve neoplastic nodes too.

Alternatively to formalin, samples can be vacuum-packed and maintained this way for a maximum time span of 24-48 hours at the temperature of 4°C. This system is available for samples of any dimension, especially when the diameter is larger than 2 cm. This procedure manages to preserve original tissue features and allows samples for tissue-banking and other scientific purposes. Remember to enclose radiological reports (MX, US, MRI), cytological and histological reports and, when possible, the RX of the surgical sample, including the evaluation of resection margins. In case of not palpable lesions, always enclose RX of the surgical samples, in order to facilitate a correct sampling.

Macroscopical examination and sampling of surgical breast specimens

The target of macroscopical examination and sampling of surgical specimens is the identification of the pathological lesions and their histological evaluation. An incorrect macroscopical analysis and wrong or incomplete sampling can crucially condition the final diagnosis, with unavoidable and potentially serious prognostic, therapeutic and legal repercussions.

In these phases, the presence of medical staff with proven expertise in breast surgical management is highly recommended.

MACROSCOPICAL EVALUATION

The macroscopical evaluation may comprise a detailed description of surgical sample and its sampling. It is composed by:

A - External evaluation

Description should include:
the three dimensions of surgical sample;
the two dimensions of cutis (if present) and the possible presence of the nipple;
weight (optional);
the presence of surgical markers for orientation;
the consistency, i.e. the presence of palpable nodules.

B - Inking of surgical margins

Surgical margins in breast-conservative surgery must be inked with Indian Ink or acrylic in different colours for their correct identification and histological evaluation (see Appendix 1).

For mastectomy samples, inking can be useful in case of macroscopic lesion close to the deep margin (e.g.

References

2 Linee guida referetazione citologia mammaria propose da SIAPEC-IAP. Pathol 2012;104:172.
pectoralis fascia, pectoralis muscle) or skin (in cases of mastectomy without removal of the skin overlying the lesion).

C - Dissection
It should be performed as early as possible after arrival in Pathology Laboratory (whether specimens arrive fresh, or formalin-fixed or vacuum-packed).

Samples are dissected and immediately absorbed in 10% neutral buffered formalin (pH around 7) to allow a correct penetration of the fixative, without altering the shape or the orientation (see Appendix 1). Fixation must be carried out in formalin for no less than 24 hours for all surgical samples.

A pathologist will then examine macroscopically each section to evaluate the presence of lesions and tissutal characteristics. It is mandatory to note:

• Number, size and location (quadrant/quadrants in case of mastectomy; distance from margins) of identified lesions.

• Distance of the lesion from the skin and deep margin (pectoralis fascia in case of mastectomy) or from the closest margin of excision (in case of breast-conservative surgery) (optional).

• Appearance of the lesion: consistence, color, margins, macroscopic presence of necrosis, hemorrhage and calcifications.

• Identification and description of bioptic site, in case of previous agobioptic procedures, if possible.

• Photo of each section and related lesions, if possible.

D - X-ray examination of the surgical material
If the sample size doesn’t allow a complete inclusion of the specimen, the execution of radiograms of each macrosections is recommended (in particular, for non-palpable lesions and calcifications), in order to select those areas corresponding to radiological abnormalities. Alternatively, for non-palpable lesions and calcifications, the sample should be positioned on a radiological grid, in order to take samples in areas of radiological interest.

The sampling for histological examination can be performed on fresh material or after formalin-fixation.

The sampling technique and the number of required samples and inclusions vary for each case according to:

• Size of surgical sample.

• Dimension and clinical-radiological features of nodes: palpable lesions (opacity, thickening, distortion) versus non-palpable tumours/calcifications.

Regardless of the intrinsic variables in each sampling procedure, the minimal absolute goals are:

• An accurate measurement of the maximum diameter of the lesion;

• A detailed examination of the state of the margins of resection and, when possible, the microscopic distance of the lesion from them.

A prerequisite for achieving these objectives is the entirety of the surgical sample and its correct orientation through the use of markers. It is clear that, in presence of multiple samples or single sample dissected and not reconstructable, the evaluation of the margins may be inaccurate or even impossible, and the pathological dimensions (pT) or the unifocality/multifocality (in case of invasive carcinoma) could not be accurately defined (e.g. for tumour present in more than one sample).

A - General considerations

• For samples of limited size (approximately up to 5-7 cm on major axis) or in the presence of not-palpable/not macroscopically detectable lesions (e.g. calcifications) the total inclusion in blocks through ordinary sequential sampling, or alternatively in “large blocks” (macro-sections) is preferable (see Appendix 2).

• For larger samples, see paragraph “Specific recommendations”.

• Surgical margins can be sampled and evaluated in various ways (see Appendix 2):
  - Perpendicular sampling in ordinary blocks;
  - Perpendicular sampling in “large blocks” (macro-sections);
  - “Shaved” sampling (peeling);
  - Separate biopsies from surgical cavity/surgical bed.

• In case of multiple and macroscopically suspicious lesions, each of them should be sampled; as a general rule, normal interposed tissue should be sampled as well, in order to check whether the lesions are truly separate.

• When present, the nipple should be entirely included in two or more fragments (through longitudinal cuts, perpendicular to the skin) together with the sampling of a retroaerolar disk (through two sections parallel to the skin), which will enable the analysis of the region of the lactiferous ducts.

• Sampling of axillary lymph nodes: all nodes should be collected and included in toto for histological examination. Their optimal sampling is described in Appendix 3.

B - Specific recommendations related to different types of surgical specimens:

• Nodulectomies / surgical “diagnostic” biopsies;

• Therapeutic excisions / quadrantectomies (breast-conservative surgery)
  - As already indicated (see above), when dimensions allow it, or in presence of not-palpable/not macroscopically visible lesions (e.g. calcifications), the optimal procedure for processing the samples is their entire inclusion in sequential blocks or in “large blocks” (macrosections). In case of sequential
sampling in ordinary blocks, the extension of the lesion is defined by multiplying the number of consecutive levels that include the lesion with the thickness of a slice.

When complete inclusion is not possible, it is highly recommended to produce a radiogram of the sample and/or of the macro-slices obtained after cutting and to sample selectively the areas corresponding to mammographic abnormalities. For a correct spatial reconstruction of the lesion and evaluation of its extension, it is advisable that the sampling of selected areas is made with ordinary sequential technique in blocks or “large blocks” (macrosections).

- In case of preoperative diagnosis of DCIS or suspected DCIS (calcifications) - in which radiological dimensions often “underestimate” the real extension of the lesion - sampling should include the borders (e.g. proximal and distal) of the radiological target and the apparently healthy surrounding tissue; for a useful radiological-histological correlation, sampling sites can be found on the RX using colour markers, or other markers. For the assessment of resection margins, sampling will necessarily include the tissue corresponding to the shorter distance between mammographic abnormality and resection margin. Additional targeted sampling of the remaining surface of resection is highly recommended. When microcalcifications cannot be recognized at microscopic examination, the paraffin blocks can be X-rayed to confirm the presence of the radiological target and to cut further deeper sections.

- In case of not-palpable / not-macroscopically detectable lesions (in particular, DCIS), when sampling takes place with non-sequential technique or without macrosection the extension of the tumour can only be approximated by taking into account the number of blocks and inclusions involved.

- In case of macroscopically detectable or palpable lesions, sampling can be targeted and made through sampling in “radial blocks” according to orthogonal planes of space (superior-inferior, medial-lateral, surface-deep). In case of small excisions it is possible to include the lesion and the margins of resection in one inclusion only; for larger samples, the use of more inclusions is necessary, with separate sampling of the various margins. For each breast cancer, when the size allows it, at least 3 inclusions should be obtained, including in some sampling the interface with non-neoplastic parenchyma.

- When more surgical samples are separately sent, the real size of the lesion(s) will be rebuilt only if the pieces are mutually oriented to each other. In any case, it is a good practice to measure the lesion/s in each separate sample.

- In case of conservative interventions, after diagnosis through needle biopsy or surgical biopsy, if total inclusion is not possible, it is essential to sample in a targeted manner (preferably using ordinary sequential samples or macro-blocks) the site of the previous biopsy and the surrounding tissue. Additional samples will be carried out on the parenchyma at a distance from the neoplasia and on the margins of excision.

Second surgery (re-excisions)

- The surgical specimen of an additional surgery or a re-excision for positive margins must be oriented to allow the analysis of the margin corresponding to the margin previously found positive, and to study the new margins of re-excision.

- If tumoral tissue is found in the additional surgical specimen as well, the reconstruction of the real size of the lesion is difficult. However, the separate sampling of tissue adjacent to the previous surgical excision and at a distance is a good practice. This will enable at least a partial reconstruction of the size of the lesion and a proper recognition of unifocal versus multifocal lesion.

Mastectomy

- Each neoplastic lesion, when its size allows it, should be sampled with at least 3 inclusions, comprising in some sampling the interface with non-neoplastic parenchyma. Any relationship of the tumour with skin, nipple and fascia/pectoralis muscle should be evaluated with targeted sampling. All macroscopically suspicious lesions must also be sampled, specifying their topography. Random sampling of each quadrant, even in case of apparently normal tissue, is a good practice.

- As for conservative surgery of large dimension (see above), in case of mastectomies performed for not-palpable/not-detectable lesions (usually DCIS) a selective sampling of the areas corresponding to the mammographic abnormalities (usually calcifications), identified by radiograms of the slices from macroscopic sectioning, is recommended. When this is not possible, it is necessary to have at least the reports of previous radiological investigations, on the basis of which topographical areas can be selected to target the sampling.

- In case of a macroscopically evident lesion that is close to the deep margin (e.g. pectoralis fascia/muscle) or the surface (towards the skin in cases of skin-sparing mastectomy, without removal of the skin overlying the lesion) it is advisable to mark and sample these margins.

Areolar margin in “nipple sparing” mastectomy

For histological (intraoperative and/or definitive) examination of retroareolar parenchyma, the surgeon should send separately a retroareolar disc sampled directly from
the surgical specimen. This fragment (0.5–1 cm thick) should be marked on the side towards the nipple (“true margin”) with surgical thread or metal clip. The examination of the retroareolar parenchyma can be performed during surgery as intraoperative examination (intraoperative frozen section) or after surgery as a definitive exam. The retroareolar disk, usually single, is measured (diameter and thickness) and the side towards the nipple (“true margin”) must be inked.

Histopathological examination may be performed through:

- Coronal sections (perpendicular to the nipple axis) obtained by sectioning the discoid fragment from the breast as “a dish” (with recovery of the “true margin” through additional sections, almost until the exhaustion of the fragment).
- Sagittal sections (parallel to the nipple axis) from the nipple to the breast: the entire disk is sectioned into slices, 3–5 mm thick, which should be totally included.

The areolar disc analysis may provide 3 sections during intraoperative examination at levels of 200-300 microns and an additional section during definitive exam, or in alternative 4 sections cutted every 200-300 microns for the definitive exam.

Examination of the breast

The breast (without the retroareolar disk, if it was already sent for intraoperative examination by frozen section), is sent to the pathology laboratory with markers (suture threads) to allow the orientation: 1 suture thread marks the site where the retroareolar disc was removed, 2 suture threads mark the axilla; one marker is in the site of the lesion, in its projection on the surface of the gland, to allow the assessment of the relationship of the lesion with the overlying subcutaneous tissue, the deep plan and the remaining retroareolar parenchyma after removal of the disk. It is important that the request of histological examination exactly specifies the site (quadrant) of the lesion, or the sites in case of multiple lesions (possibly by attaching a diagram/draw).

* Taken from “Consensus Document on Nipple Sparing Mastectomy” http://www.senologia.it/rivista/pdf/59/59forum.pdf

References


APPENDIX 1: Marking of surgical margins and specimen sectioning

MARKING OF SURGICAL MARGINS

Before the ink is applied, the surface of the specimen should be thoroughly dried with blotting paper/tissue paper, so that the ink better adheres to the surface of the sample. Subsequently, the ink is distributed with a brush on the surface and the specimen is plunged (or covered) into Bouin’s fixative, or alternately in 10% acetic acid solution or in absolute ethyl alcohol, in order to fix definitely the ink. Finally, the specimen should be dried again with blotting paper to ensure that the ink remains coated on the surface.

In the technique of inking margins with acrylic or tempera paints, different colours can be used to mark the different margins. Standardization in the choice of colours is suggested, so that the same colour always corresponds to the same margin. The colours are applied and lied with a brush covering the areas as indicated by the anatomical markers designated by the surgeon, and taking care to keep net margins between adjacent colours.

SPECIMEN SECTIONING

Cutting and sectioning methods can vary in relation to the various methodologies adopted for sampling. As a general rule, for samples from small to medium size (diagnostic biopsy or conservative therapeutic surgery) the specimen should be dissected with serial sections at 3 to 5 mm intervals.

Sections should be conducted perpendicular to one of the spatial axes (e.g. medial-lateral; cranial-caudal) or in parallel to the direction nipple – pectoral fascia. The cuts can be partial (thus maintaining the orientation of the specimen) or complete at full thickness (in this case the sections obtained should be fixed on a support with labels affixed on it, with the orientation mark written in pencil).

For mastectomy specimens, the breast should be placed on a cutting board with the skin surface down and the deep fascial plane (pectoral fascia) facing upward. Parallel sections with a medial-lateral direction, perpendicular to the skin, should be produced. These cuts should not go all the way through the specimen, but should
leave the sections attached together by a rim of unsectioned breast or skin; the cuts should possibly be directed along the major axis of the lesion as shown in the reports of instrumental exams (ultrasound or mammography). By adopting this procedure, it will be possible to fix the entire breast in a single box; attention should be paid in putting between one slide and the other a few sheets of gauze that will help the formaldehyde to penetrate and will prevent the tissue slices from collapsing.

APPENDIX 2: Sampling and evaluation of surgical resection margins

Sampling may be performed in perpendicular tissue blocks, obtained on separate and distinct sequential sections (levels): each section/layer may comprise one or more blocks in relation to the size of the sample. This method enables a precise topographical location of the single block and the reconstruction (at least in two dimensions) of the sampled lesion. Alternatively, it is possible to use the technique in “large blocks” (macro-sections), which allows the examination of larger areas of the sample by keeping the topographical relationships between the lesion and the surrounding structures, or between different anatomical lesions.

Concerning the resection margins, both procedures allow a correct evaluation, by providing an accurate microscopic measurement of the distance between the surface of excision and the lesion. By using the technique in “large blocks”, the advantages are the possibility to investigate the margins in their entirety (at least for the plan taken into exam) and to define more easily the size of the lesion (in particular for DCIS), and the lower number of inclusions needed.

A further possible method for the study of the margins is the “shaved margins” technique (peeling): after inking the margins, the pathologist performs sections parallel/tangential to the margins; sections thus obtained are included by the same side of the margin (side marked with ink). This technique allows the examination of the entire surface of the margins with a smaller number of inclusions than with ordinary blocks; nevertheless, part of the true margin is lost during the process of trimming the paraffin blocks to an optimal cutting surface. Moreover, and most importantly, by using this method it is not possible to measure the distance between the margin and the lesion.

APPENDIX 3: Sampling of axillary lymph nodes

During macroscopic examination of the surgical specimen, attention should be paid to the search of lymph nodes, which can be isolated both from fresh tissue or after fixation (in this case, the search is usually easier). It is important to eliminate as much fat tissue surrounding the lymph node as possible, in order to facilitate fixation and processing.

Each macroscopically “negative” node must be included in its entirety. Sampling must ensure recognition of all macrometastases (> 2 mm). When the size allows it, each node must therefore be dissected with thin cuts (approximately 2 mm) along the major axis. Small lymph nodes can be included into a single block. The inclusion in a single block of more than one lymph node, or of parts of a single node, should be described in order to assess the effective number of lymph nodes by microscopic examination.

In macroscopically positive (metastatic) lymph nodes, it is recommended the sampling of areas suspicious for extra-nodal infiltration.

APPENDIX 4: Diagnostic protocol for sentinel lymph node biopsy

INTRODUCTION AND OBJECTIVES

The intraoperative examination of sentinel lymph node (SLN) should not be requested when the surgical treatment is provided in two times.

The probability to diagnose micro-metastases and isolated tumour cells (ITC) increases with the increase of the number of sections examined (Weaver et al. Am J Surg Pathol 2009;33:1583-1589), and the use of immunohistochemistry (IHC) for cytokeratins.

The “European guidelines for quality assurance in breast cancer screening and diagnosis” provide the following information:

a. Minimal processing method: identification of metastases > 2 mm.
b. Optimal processing method: detection of micrometastases
c. Processing method for the identification of ITC: “multistep sectioning” and immunohistochemistry.

SENDING METHOD

Every SLN should be placed in a suitable container, labelled and immediately sent to the Pathology Laboratory. If it is not possible, the sample should be stored in an appropriate amount of 10% neutral buffered formalin.

MACROSCOPIC GROSSING

Lymph nodes with a diameter larger than 5 mm should
be sectioned along the minor axis, at intervals of about 1-2 mm, in order to obtain a more comprehensive evaluation of the capsule and the marginal sinus (preferential site for ITC localization), and included totally, in a single tissue-block, if possible; sections thus obtained should be placed in the block always in the same direction (with the aid of sponges for embedding cassettes) and included by technicians following the orientation provided by the pathologist (Weaver et al., Modern Pathology 2010;23:S26-S32).

The lymph nodes with a diameter smaller than 5 mm should be cut in half along the major axis and entirely included. In order to make the cutting of the lymph node easier, we suggest letting the lymph node fix for about 2 hours after the elimination of the peripheral fat tissue (taking care not to tear the capsule away). We recommend to place the sections of the lymph node on sponges for embedding cassettes.

METHODS FOR EXAMINATION

There are four methods for the examination of the lymph nodes:

1. **Examination in paraffin only**: the SLN is fixed in formalin and embedded in paraffin.
2. **Examination exclusively with cryostatic sections**: the SLN is exhausted during the intraoperative examination.
3. **Hybrid examination**: part of the lymph node is examined with cryostatic sections, and part is fixed in formalin and embedded in paraffin.
4. **Molecular analysis**.

1. EXAMINATION IN PARAFFIN ONLY

Each SLN must be sampled separately for microscopic examination.

In case of metastatic SLN at macroscopic examination, a single section stained with H&E is sufficient, without the need of “multistep sectioning” and immunohistochemistry (IHC).

Immunohistochemistry for cytokeratin is optional, as suggested by the European Guidelines.

If no metastatic lesion is identified using H&E, it is advisable to examine at least 3 sections, cut at different levels, using IHC with broad-spectrum cytokeratin antibodies.

The immunohistochemistry for cytokeratins, rarely needed for the identification of macro-metastases, is useful for the diagnosis of micro-metastases and ITC; in case of metastases from lobular carcinomas, it can also assist in the identification of macro-metastases.

If a concomitant different disease possibly affecting the lymph node is suspected, the above-mentioned protocol should be abandoned.

2. EXAMINATION EXCLUSIVELY WITH CRYOSTATIC SECTIONS

SLN must be evaluated macroscopically first (visually and by palpation). The firm consistency and the variation of colour on the cut surface may be the result of a non-metastatic process (e.g. fibrosis or lymphoma). Adipose tissue in excess should be removed carefully and safeguarding the capsule.

Intraoperative examination of SLN with diameter < 3 mm should be discouraged.

In case of intraoperative examination, the lymph node should be dissected with the same protocols adopted for samples embedded in paraffin.

The intraoperative examination, using cryostatic sections and/or cytology obtained from imprinting, has a low risk of false positive results but a high risk of false negatives, with a sensitivity varying from 66% to 100% for the cryostatic examination and from 65% to 94% for the cytological examination with imprint. The choice between the two methods depends on the preferences and the experience of each centre.

Sensitivity and positive predictive value of intraoperative examination of SLN may be enhanced by special techniques, such as the rapid immunohistochemistry for cytokeratin.

Rapid immunohistochemistry is useful mainly in cases of invasive lobular carcinoma, providing a better and more accurate assessment of the size of the metastatic deposit and the identification and sub-classification of ITC, micro-metastases or macro-metastases.

3. HYBRID EXAMINATION

Part of the lymph node is examined with cryostatic sections and part is fixed in formalin and embedded in paraffin. The SLN, received at the Pathology Laboratory immediately after the removal and not fixed, is dissected following the above-mentioned procedures and frozen in toto. After the intraoperative exam, the remaining material is fixed in 10% neutral buffered formalin, embedded in paraffin and analysed according to the above-mentioned procedure.

It should also be clearly stated that a final and definitive diagnosis would follow after the completion of the procedure.

If the SLN is found to be metastatic during intraoperative examination, a single H&E section would suffice for the definitive diagnosis.

4. MOLECULAR ANALYSIS

Centres equipped to perform this type of examination should strictly follow the procedural recommendations of the manufacturers.

One-step nucleic acid amplification (OSNA®) is considered the technique of choice, because it has a high level of accuracy and can be performed intraoperatively.
The evaluation of the lymph node with molecular techniques excludes the comparative histological evaluation; for this reason, it is recommended the execution of an imprint cytological slide in order to exclude potential pathologies (other than metastasis) in the lymph node.

APPENDIX 5: Neoadjuvant chemotherapy

MACROSCOPIC ASSESSMENT AND SAMPLING

The identification of a tumour after neoadjuvant chemotherapy can be very difficult in case of complete clinical and instrumental response to treatment. Therefore, it is highly recommended to mark the lesion (with metal clip or skin tattoo) before therapy starts, in order to allow the localization of the lesion after treatment. Without a marker, radiological data (mainly MR) are essential. When taking into account the type of response to therapy, the macroscopic examination and sampling procedures do not differ in substance from those used for the usual quadrantectomy/wide excision or mastectomy (see above).

If the tumour is still detectable (absent pathological response), sampling follows the usual indications for malignant lesions.

In cases of clinical and instrumental partial response the residual disease can appear nodular, partially sclerotic, or composed by multiple foci that surround an oedematous and/or sclerotic area. On palpation the residual tumour has a soft consistency.

All macroscopically evident lesions should be described, measured and sampled: if the residual lesion is less than 3 cm in the maximum diameter, it should be included entirely; if it is greater than 3 cm an extensive sampling is recommended (with complete inclusion of the suspect area, if possible). It is always necessary to report the distance from the surgical margins of resection.

In case of clinical and instrumental complete response, the identification of the tumoral bed can be difficult. Nodules are usually not present, while it is often present an area with ill-defined contours and a centrally oedematous and/or fibrous appearance. It is therefore necessary the sampling of the whole area with contiguous sections of 3-5 mm, after its measurement in two dimensions.

Similarly, if a marker is present, all of the adjacent area should be systematically sampled. It is useful to remember that microcalcifications associated with the neoplasm do not disappear after chemotherapy; therefore, radiography exam can facilitate the recognition of the area to be sampled. Without a marker it is highly recommended to sample the specimen with the aid of radiographs (in case of calcifications) or on the basis of pre- and post-therapy MR data.

For multifocal lesions the procedure of sampling must be performed on all the identified areas. In all cases, margins between residual lesion and adjacent parenchyma must be sampled. If present, the skin overlying the tumoral bed (in case of complete clinical and instrumental response) or overlying the tumour (in case of absent pathological response or partial clinical and instrumental response) should be sampled, performing sections in continuity with the tumour, if possible.

Check list for the reporting of microscopical diagnosis of invasive breast carcinoma

• Histotype (according to WHO 2012):
  - Histological grade (according to Elston et al. 1991):
    - Mitosis: #/10 HPF score # (field diameter #)
    - Nuclear pleomorphism: score #
    - Formation of tubules: score #

• Peritumoral vascular invasion (not evident, present):

• Massive peritumoral vascular invasion (OPTIONAL):

• Peritumoral perineural invasion (OPTIONAL):

• Multiple foci of invasive carcinoma (distinct foci separated by healthy parenchyma):

• Peritumoral carcinoma in situ*: (% histological type, nuclear grade):
  * indicate the presence of extensive intraductal component (when DCIS is > 25%)

• Presence or absence of necrosis (OPTIONAL) (absent; present central necrosis “comedo type”; present focal necrosis):

• Intratumoral carcinoma in situ (OPTIONAL):

• Size of the microscopic invasive component:

• Overall dimensions (invasive component plus in situ component):

• Location:

• Nipple, retroareolar parenchyma, skin and chest wall:

• Microcalcifications (stromal / endoluminal):

• Evaluation of microscopic resection margins using the following definitions:
1. POSITIVE MARGIN (presence of ink on the lesion), specifying:
a) which edge/s is/are involved; b) if it is a single focus or multiple foci of invasion; c) the size of the linear extent of the involvement of the margin expressed in mm; d) the presence of in situ component on the margin.

2. FREE FROM INVASION MARGIN/S (there is no ink on the lesion), specifying the extent of the distance from the margins of the sampled lesion, if it is less than 1 cm (including the distance from eventual in situ component).

- Parenchyma free from neoplasm:
- Staging (pT according to AJCC 2010, seventh edition)

Check list for the reporting of microscopical diagnosis of in situ breast carcinoma

- Type:
- DIN-classification according to Tavassoli (OPTIONAL):
- Site:
- Main histotype:
- Microscopically-evaluated calcifications:
- Necrosis (absent; present central necrosis “comedo-type”; present focal necrosis):
- Maximum extension (microscopically measured): .......... mm
- Evaluation of microscopic resection margins with the following definitions:

  1. POSITIVE MARGIN (presence of ink on the lesion), specifying: a) which edge/s is/are involved; b) if there is a single focus, or multiple foci of invasion; c) the size of the linear extent of the involvement of the margin expressed in mm; d) the presence of in situ component on the margin.

  2. FREE FROM INVASION MARGIN/S (there is no ink on the lesion), specifying the extent of the distance from the margins of the sampled lesion, if it is less than 1 cm.

- Microinvasion (< or = 1 mm): absent/present, single or multiple foci

- Nuclear grading (according to European Guidelines 2006):
- Other lesions:

**Reporting of prognostic/predictive factors determined by immunohistochemistry**

Estrogen receptor (ER) (clone #, company #): #% positive neoplastic cells

Progesterone receptors (PgR) (clone #, company #): #% positive neoplastic cells

Ki-67 (clone #, company #): #% positive neoplastic cells
c-erbB2 oncoprotein (clone #, company #):

1. POSITIVE (score 3+)

Complete and intense staining of the cell membrane, circumferential in > 10% of invasive carcinoma cells (specify the percentage).

2. EQUIVOCAL (score 2+; FISH evaluation will follow)

- Complete staining of the cell membrane, with weak or moderate intensity, circumferential in >10% of invasive carcinoma cells.
- Incomplete (basal-lateral or lateral) staining of the cell membrane, with moderate/intense staining in > 10% of invasive carcinoma cells (rare, usually on micropapillary invasive carcinoma)*.
- Complete and intense staining of the cell membrane, circumferential in 10% or less of invasive carcinoma cells (rare)*.

* These kinds of immunohistochemical results are rare and have to be prudentially considered as score 2+, and tested with ISH analyses.

3. NEGATIVE (score 1+)

Incomplete staining of the cell membrane, with low intensity, in >10 % of invasive carcinoma cells (specify the percentage).

4. NEGATIVE (score 0)

Absence of cell membrane staining of invasive carcinoma cells, or incomplete and low intensity membrane staining in 10% or less of invasive carcinoma cells.
5. INDETERMINATE

[ASCO/CAP 2013 recommendations; AIOM-SIAPEC 2014 Consensus; Best possible care in Breast Cancer (BICE); GIPaM 2014].

Specify if the immunohistochemical evaluation is performed using:

- Image analysis techniques.
- Controls (of high intensity, low intensity and negative) of the proteins’ expression on the slides.

The evaluation of hormone receptors (ER and PgR) in ductal carcinoma in situ is optional.

References


SENTINEL LYMPH NODE

According to SIAPEC-GIPaM Protocol, “sentinel” lymph node (SNL) is examined on paraffin sections conducted at intervals of 200 microns until depletion of the tissue, with microscopic examination conducted on 10 H&E-stained sections and by immunostaining (OPTIONAL) [n. #] with anti-pancytokeratin antibodies (clone #).

If the macroscopic reduction is conducted with 2 mm deep sections, for each section 10 cut layers will be obtained. At this point, two sections in parallel can be collected, one for H&E stain, the other as reserve for any further immunohistochemical staining (especially in case of lobular carcinoma, in doubtful cases or in case of unexpected lymphomas). On the whole, 10 H&E and 10 unstained sections will be obtained, with exhausted material. It is also possible to increase the number of unstained sections for each level.

The microscopic report should include:

- The total number of SNL received and examined.
- Macroscopic metastases, if present.
- The number of lymph nodes with metastatic disease.
- The amount of the metastatic lesion expressed in mm, particularly in case of micrometastases, and adopting the pN categories of AJCC 2010, VII edition.
- If several metastatic foci are found within a lymph node, the larger one should be taken into account. Using the categories of AJCC 2010, VII edition, the suffix (sn) should be used in case the nodal status is determined on the basis of the biopsy of the SNL only (that is, without axillary dissection).
- Specify the adopted protocol and whether the positivity was assessed only on the basis of the H&E, or using immunohistochemistry with antibodies anti-pancytokeratin.
- If present, isolated tumour cells (ITC) should be reported, but their systematic search is not recommended.

AXYLLARY LYMPH NODES

- Total number of examined lymph nodes: #
- Number of metastatic lymph nodes: #
- Extracapsular extension: #
- Lymph node Staging (pN according to AJCC 2010, VII edition): #

Reporting of microscopical diagnosis after neoadjuvant chemotherapy or primary systemic therapy (PST)

On the needle biopsy, before neoadjuvant chemotherapy or PST, the report should specify:

1. Number of diagnostic frustules
2. Histological typing
3. Histological grade (or, if it is not possible, the nuclear grade)
4. Presence or absence of vascular invasion
5. Presence of in situ carcinoma
6. ER, PgR, HER2 and Ki67
7. If a skin sample is present in the frustules, specify the
possible presence of infiltration, ulceration, dermal vascular invasion.

For surgical specimens after neoadjuvant chemotherapy or primary systemic therapy (PST) the diagnostic microscopic checklist is comparable to that for invasive carcinoma, with the following additions and indications:

**Residual tumour size**

In case of absence of pathological response to therapy, the size of the tumour and the histology result almost unchanged.

In case of partial pathologic response, histological changes may be of different degrees; most carcinomas show a reduced cellularity, with nests of neoplastic cells (more or less cellular) scattered in the tumoral bed. It often happens that the only residual tumour can be found in the lymph-vascular spaces, and this is an important finding, since it is associated with an increased risk of recurrence.

In case of a pathologic complete response the morphological picture is characterized by an oedematous connective tissue and high vascularization, with chronic inflammation and macrophage infiltration. In difficult cases, the use of immunohistochemical staining with anti-pancytokeratin antibodies is useful in differentiating histiocytes from any residual malignant epithelial cells. Usually, carcinoma *in situ* appears more resistant to therapy, and foci can be encountered even in the absence of an invasive component.

If there are multiple invasive residual foci separated by loose fibro-elastotic or myxoid stroma with any presence of foci of necrosis, the size of the entire area affected by the residual neoplastic foci should be reported. If there are no residual foci of invasive carcinoma, the area of fibrous regression must be measured taking into account also the possible preoperative placement of markers. The use of anti-pancytokeratin antibodies can be useful to confirm the absence of residual tumour and a pathologic complete response (pCR).

**Cellularity of the residual tumour**

It is expressed as the percentage of neoplastic cells compared with fibrous tissue and it is evaluated by comparing the surgical post-PST sample with pre-PST pre-surgical biopsies.

If pre-PST biopsies are not available, cellularity can be expressed as a percentage of neoplastic cells compared to fibro-myxoid tissue evaluated in the surgical post-PST specimen.

For a detailed description and for the calculation of the residual cellularity, refer to the website: http://www.mdanderson.org/breastcancer_RCB

**Nodal status**

Lymph nodes usually show large areas of fibrosis, which may be associated with foci of necrosis and abundant macrophage infiltrate. These histological changes are interpreted as the response of the metastatic disease, induced by therapy. However, it may happen that complete response in a metastatic lymph node doesn’t leave any histological evidence of neoplasm.

Both the metastatic lymph nodes and those with areas of fibrosis or foci of necrosis, along with lymph nodes in which the two findings coexist, should be described and quantified. In cases where there is no evidence of residual neoplastic cells on the H&E slides, the use of anti-cytokeratin antibodies (AE1/AE3 or CAM 5.2) can disclose micrometastases or residual isolated tumour cells.

**ER, PgR and Ki67 expression and HER2 status**

Since no univocal scientific data are available on the modification in the expression of ER, PgR and Ki67 and on HER2 status after PST, these parameters must be determined again on residual tumour in the surgical sample after PST in case of partial response (pPR) or no response (pNR).

**STAGING**

The pathological report should be completed with the pathological staging according to the AJCC 2010, VII edition, adding the prefix “y” to pT and pN.

**REPORT ON TUMOUR RESPONSE**

Various grading systems to assess tumour response to neoadjuvant therapy or PST have been published, and there is currently no consensus on which has the best prognostic impact.

It is here reported the system proposed by Pinder et al. (Pinder et al., Histopathology 2007; 50:409-417), which has been adopted by the 2012 European Guidelines.

**Tumour response**

1. **Complete pathological response** to therapy, further divided into:
   i) absence of residual carcinoma;
   ii) absence of residual infiltrating carcinoma, but presence of *in situ* carcinoma.

2. **Partial response** to therapy, further divided into:
   i) minimal residual disease / near total effect (e.g. <10% of tumour remaining);
   ii) evidence of response to therapy, but residual disease equal to 10-50% of tumour remaining;
   iii) > 50% of tumour cellularity remains evident with areas of fibrosis, inflammation and macrophages with hemosiderin.
3. **No evidence of response** to therapy.

**Response in the lymph nodes**

1. No evidence of metastatic disease and no evidence of changes in the lymph node parenchyma.

2. No evidence of metastasis but evidence of response (fibrosis, inflammation, etc.) that indicates a down-staging linked to neo-adjuvant chemotherapy.


**References**


**IN SITU HYBRIDIZATION**

**BASED ON ASCO/CAP 2013 RECOMMENDATIONS**

Currently licensed ISH techniques are: FISH, CISH and SISH with double probe (HER2 gene and CEP17) or single probe (HER2 gene only). Pathologist has to guarantee the correspondence of all morphological, immunohistochemical and in situ hybridization data. It is mandatory to participate in external quality control programs.

**ISH: interpretation of results**

Careful examination of the whole ISH sample to evaluate if different populations of cells, with different number of HER2/nucleus signals, are present; otherwise, a selection of potentially positive areas can be performed referring to immunohistochemical samples. If ISH samples are homogenous, at least 20 cells for field need to be evaluated, on at least 2 fields of the invasive component of the carcinoma previously identified on H&E stained slides.

If two different populations of cells are identified, one of which showing an increased number of HER2/nucleus signals and being representative of more than 10% of the entire sample, a separate evaluation has to be performed, counting at least 20 cells of the population showing an increased number of signals. In case of heterogeneous neoplastic population, ISH testing has to be considered positive if the percentage of amplified cells is >10% of the neoplastic cells present on the examined slide. Data of both populations of cells, with percentages, must be reported.

**POSITIVE HER2 ISH CASES (DOUBLE PROBE):**

- **HER2/CEP17 ratio ≥ 2.0**
  - with mean HER2/nucleus signals ≥ 4.0
  - or
  - **HER2/CEP17 ratio < 2.0**
  - with mean HER2/nucleus signals ≥ 6.0
  - or
  - Heterogeneous case in which the amplified population (nuclei with number of HER2 signals ≥ 6.0) is >10% of the neoplastic cells present on the examined slide

**POSITIVE HER2 ISH CASES (SINGLE PROBE):**

- **mean HER2/nucleus signals ≥ 6.0**
- or
- Heterogeneous case in which the amplified population (nuclei with number of HER2 signals ≥ 6.0) is >10% of the neoplastic cells present on the examined slide

**NEGATIVE HER2 ISH CASES (DOUBLE PROBE):**

- **HER2/CEP17 ratio < 2.0**
  - with mean HER2/nucleus signals < 4.0

**NEGATIVE HER2 ISH CASES (SINGLE PROBE):**

- **mean HER2/nucleus signals < 4.0**

**EQUIVOCAL HER2 ISH CASES (DOUBLE PROBE):**

- **HER2/CEP17 ratio < 2.0**
  - with mean HER2/nucleus signals ≥ 4.0 and < 6.0
EQUIVOCAL HER2 ISH CASES (SINGLE PROBE)

- mean HER2/nucleus signals $\geq 4.0$ and $< 6.0$

NOTE:
In case of equivocal HER2 ISH cases, both using double and single probe, it is necessary to perform further examinations:
- Reflex test 1: on the same sample, to perform ISH evaluation using alternative probes on chromosome 17.
- Reflex test 2: on the same sample, to perform HER2 IHC reactions, if they have not been previously performed.

OR

- New tests: if available, to perform ISH and/or IHC evaluations on a different sample (core biopsy, metastatic lymph node and/or metastasis sample) of the same patient.

If the additional ISH and/or IHC examinations are not useful to clarify the HER2 status, the case must be reported as EQUIVOCAL.

Equivocal cases, both at IHC and ISH examination, are the most critical: in this situation, oncologist is allowed to consider the use of anti-HER2 therapy. The final clinical decision has to be personalized on the basis of the characteristics of each patient and disease, and must be discussed and shared with every single patient.

INDETERMINATE HER2 ISH CASES:

If it is not possible to evaluate as positive, negative or equivocal one or both of the tests (IHC and or ISH) performed on a tumoral sample, due to technical problems (inadequate fixation and/or sample processation, presence of crush artefacts or artefacts on the borders) or to the failure of analytic testing, such a case should be reported as “indeterminate”.

References


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Health Technology Assessment: introducing a vacuum-based preservation system for biological materials in the anatomic pathology workflow

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Key words
Health Technology Assessment • Histopathology • Under-vacuum sealing • Tissue specimens • Fixatives

Summary

Introduction. The objective of this work is to assess the implementation of a newly introduced medical equipment technology for the vacuum-based preservation of biological materials within an Anatomic Pathology service.

Methods. The approach selected for the analysis is the Health Technology Assessment (HTA), a comprehensive evaluation method based on relevant scientific evidence and designed to support healthcare decision makers in purchasing, replacing or disposing of technologies. The analysis focused on specific domains such as Technology, Organization, Safety and Economy.

Results. The study proves that the use of such technology ensures the biological specimen to be suitably preserved (up to 72 hours), both reducing the amount of fixative being employed in the diagnostic process (30% to 55%) and resulting, in the particular context under examination, in savings of 93%.

Discussion. The HTA reported no significant drawbacks related to the use of the technology being examined. Nonetheless, the workflow for managing the transfer of biological materials from the Operating Room to the Anatomic Pathology department needs to be redefined – in terms of handling, processing, storage and disposal. Other elements concerned the monitoring of storage temperature, fresh tissue handling and especially fixative amount reduction, which positively impacts on the operators’ safety with regard to chemical hazards.

Reference context

Immunological and molecular diagnostic techniques have become increasingly effective. As a result, histological surveys have improved over the years, allowing for enhanced morphological diagnoses at subcellular level. In order to ensure the accuracy, standardization and reproducibility of diagnostic tests, it has thus become necessary to perform the best possible preservation of protein components and nucleic acids, which is obtained by means of fixatives of various compositions working as stabilizers.

However, special attention should be paid to some of the critical elements which can be highlighted in the anatomic pathology workflow, namely tissue storage times and methods prior to fixation, fixation duration and specimen transfer conditions on the way to the Anatomic Pathology laboratory.

Moreover, the carcinogenicity and/or mutagenicity of the fixatives that are generally in use constitute a risk factor for the operators. These considerations lead the scientific community to search for alternative solutions, both aimed at minimizing any molecular alterations and morphological modifications of biological materials due to hypo- or hyper-fixation and ensuring improved operational safety.

Within this framework, a HTA study has been conducted in collaboration with the Anatomy and Histopathology Department of the “Nuovo Ospedale di Prato”, run by USL 4 of Prato. Its objective was to assess possible implications for the introduction of a vacuum-based transport and preservation system for biological specimens as an alternative to the current procedure, which provides for immersion in a glyoxal-based fixative.

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The HTA model

The introduction of a vacuum-based, temperature-controlled preservation system for biological materials has implications of different nature which encompass multiple aspects. HTA was identified as the most appropriate tool to thoroughly review the many outlooks offered by the use of the technology. Each result was supported by scientific evidence.\textsuperscript{7-9}

This study was conducted through a preliminary planning phase, directly involving the decision maker, where the specific needs and the peculiarities of the technology’s new context were to be identified. Particular attention was paid to the analysis of Prato’s histopathological workflow and to determining volumes of activity (number of specimens/year), as well as annual fixative consumption and its related costs, both with respect to purchase and disposal.

The policy question constituting the central idea of the whole evaluation process was formulated as follows: “Assessing the potential impact of the introduction of a vacuum-based preservation system for biological materials, followed by an automated insertion of formalin, in an anatomic pathology service workflow”. The policy question is substantiated by research questions as specified in Table I.

In order to identify and select the scientific evidence answering these questions, a research protocol has been developed by determining a priori which strategy should be adopted to review the literature and the study enrollment from databases and topic-specific search engines (HTA-Engine, PubMed, NICE, The Cochrane Library, NHS-EED, CRD and so on). The technology has been recently introduced in the medical equipment industry, so its diffusion is still quite limited among healthcare services and diagnostic processes. Assuming a low num-

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<th>Tab. I. Research questions.</th>
<th>HTA domain</th>
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<td>What scientific evidence is available about the effectiveness of the procedure for transferring biological materials from the Operating Room to Anatomic Pathology within fixative-filled containers, compared to a low-temperature, vacuum-based transport?</td>
<td>TECHNOLOGY</td>
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<td>What is the impact on organization in the anatomic pathology workflow?</td>
<td>ORGANIZATION</td>
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<td>What is the impact on the operators’ safety?</td>
<td>SAFETY</td>
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<td>What is the economic impact of using the technology compared to the standard procedure?</td>
<td>ECONOMY</td>
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<th>Tab. II. Research terms.</th>
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<td>Under-vacuum Sealing</td>
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<th>Tab. III. Study Enrollment.</th>
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<td><strong>Title</strong></td>
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<td>Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin</td>
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<td>Histologic validation of vacuum sealed formalin-free tissue preservation and transport system</td>
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<td>Evaluation of tissue preservation using a vacuum-based refrigeration system for specimen transfer from theatre to laboratory</td>
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<td>Analyse qualitative et quantitative d’échantillons tissulaires par le procédé TissueSafe® en vue d’analyses morphologiques et moléculaires. Evaluation sur une série de 10 cas</td>
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ber of supporting scientific evidence, no tight restrictions were set to include publications in the review. Table II shows the key terms used for research, which has been conducted continuously during six months. The selected studies (Tab. III) provide suitable answers to the research questions and constitute the baseline for analyzing HTA domains.

**HTA domain assessment**

**The Technology domain**

**Technology**

Stemming from the food industry, the technology pertains to the field of in vitro diagnostic medical devices (Directive 98/79/CE). A market analysis has been conducted to identify the most relevant manufacturers of such systems worldwide: Milestone S.r.l., Kaltek S.r.l. and Rivac S.r.l. For this purpose, the technical and commercial materials made available on public channels by manufacturers, suppliers and institutional research centers has been taken as reference. Therefore, the main system manufacturers and suppliers were contacted. The key elements of the technology can be summarized as follows:

- vacuum type;
- specimen container type;
- temperature monitoring system;
- automated filling with fixative;
- traceability system.

As regards the vacuum type, the market offers systems that can completely extract air from the specimen container (Tissue Safe and Seal Safe by Milestone S.r.l., Tissue Vacuum by Kaltek S.r.l. and VM-VAC Med Series by Rivac S.r.l.) as well as protective atmosphere solutions, which can compensate oxygen with an inert gas – generally nitrogen (Tissue Vacuum Plus by Kaltek S.r.l. and T-Filler by Rivac S.r.l.). In the former case (Figs. 1-3), bags are used for preserving biological materials, available in different sizes and made of gas-impermeable materials. Their vacuum level can be selected and suited to a specific biological tissue type from the control panel, so that the chance of causing damage or shocks, which might make the tissue useless for diagnostic purposes, will be reduced. In the latter case (Figs. 4-5), the containers intended for preservation are quite similar to those used in traditional procedure, where nitrogen proves to be a proper filler as it prevents the container from imploding and therefore the contained materials from being squeezed.
Clinical effectiveness

The analysis of scientific evidence relied on a constant comparison with the traditional procedure. In terms of diagnostic effectiveness, this was found to be substantially equivalent to vacuum-based preservation. According to reference bibliography, the assessment focused on biopsies that were bigger than 2 cm – to which the technology being examined fully applies – omitting the histopathological transfer of small tissue specimens (< 2 cm), which are collected into containers previously filled with fixative.

The introduction of the vacuum-based preservation technique allows fresh tissues to be properly preserved in the Operating Room in respect of morphological and biomolecular properties. Scientific literature provides evidence of an optimal preservation of tissues for a time span varying from a few hours to 72 hours, if vacuum-sealed at 4°C. In doing so, the next fixation step can be optimized, since the anatomic pathologist is the only one in charge of monitoring times and standardizing methods besides having biological materials available for tissue banking or molecular surveys. Nevertheless, in this context it should be considered that operators may initially object to handling a higher amount of fresh tissues, which have a different consistency compared to fixated biological materials.

As concerns those systems relying on a protective atmosphere, there has been no evidence from publications. Probably, this is because they have recently been introduced in the market and some dedicated studies have likely been started or will be carried out in the near future. Furthermore, the reviewed literature provided no evidence that under-vacuum sealing and the preservation in a protective atmosphere share an equivalent clinical effectiveness.

Temperature monitoring

Reviewing the literature has stressed another significant topic: vacuum prevents tissues from drying, thus facilitating their cooling, delays autolysis processes owing to the lack of air insulation, as well as facilitates transport and optimizes storage. However, it should not be deemed to be an alternative to cooling as a preservation method for histological tissues. This being said, it becomes increasingly important to monitor and maintain the preservation temperature of histological materials at constant values, i.e., about 4°C, so as to ensure adequate preservation. For instance, this requirement becomes essential in the event of a prolonged transfer into cool bags, with the Operating Room and the Anatomic Pathology laboratory being reasonably far apart.

The solutions found on the market range from using temperature sensors (Kaltek S.r.l., Rivac S.r.l.) to data loggers based on RFID technology (Milestone S.r.l.). Recorded data can be transmitted to a computer where the management software is installed, then they can be used to create reports summarizing the most relevant information. In this way, it is possible to monitor and furnish detailed proof of the transport conditions of tissue materials, which will constitute the baseline for a standardizable and reproducible fixation protocol.

Automated filling with fixative

Some of the systems observed throughout the market analysis phase provide for the automated filling of specimen containers with fixative. Such systems are able to utilize the right amount of fixative liquid, which is calculated by suitable sensors on the basis of the weight or volume of tissue specimens.

Systems such as T-Filler by Rivac S.r.l. and Seal Safe by Milestone S.r.l. give the opportunity to select the most appropriate program to meet specific needs, so that is can be used with a high degree of flexibility in different workflow steps.

Seal Safe allows for the vacuum-sealing of specimen bags. Moreover, it adds the advantages of vacuum to the
automated filling with fixative: by creating a microenvironment saturated with fixative, for which tissue penetration is facilitated, vacuum assures preservation and uniform fixation. Specifically, it is possible to opt for four preset programs which differ according to their specimen weight/fixative weight ratio (1:1, 1:2, 1:2.5, 1:3). As a result, fixative consumption is significantly reduced.

The T-Filler system provides the opportunity to perform an automated formalin-filling cycle into the specimen container, thus optimizing the amounts needed for proper fixation (volume ratio 1:10). Besides, the specimen containers can be packed following, if need be, an inert gas empty and/or fill cycle. The Tissue Filling Plus system produced by Kaltek S.r.l. is currently being placed on the market. Hence, no proper assessment has been made due to a lack of relevant information.

The introduction of such a system in Prato’s context has a major impact on the fixative type being used and provides the basis for replacing glyoxal with formalin throughout the histopathological routine. Nonetheless, methods need to be validated and all different working protocols, as well as the detection systems of histopathological and immunohistochemical surveys, require further examination. Such a change is perfectly consistent with the needs expressed by the decision maker: the use of a fixative that is well-established within most international contexts appears to be an important requisite in a field such as Anatomic Pathology, where prospective quality control, second opinion and external advice are mostly essential. Along with the proven potential of vacuum-based preservation for molecular pathology, this could set the ground for performing a broader test panel at Prato’s Anatomy and Histopathology Department, where biomolecular trials would provide useful diagnostic insights.

The Organization domain

Workflows redefinition

Over the years the histopathological workflow phase concerned by the technology has experienced no significant modifications and the human factor still plays a crucial role. Thus, any change needs to be taken into account and assessed by considering its potential impact on the organizational context and workflows, as well as according to the staff being involved.

As far as operators are concerned, scientific studies give evidence of a high degree of satisfaction with the use of the technology, both in terms of occupational ergonomics and safety. By introducing specimen bags, transport operations have been optimized owing to volume and weight reduction. On the other hand, the transport method is partially optimized when containers are used for protective atmosphere preservation (Modified Atmosphere), as only weight is reduced – yet not the overall size. Such aspects are more or less relevant depending on the hospital ward structure and the distance between the Operating Rooms and the Anatomic Pathology laboratory, which might cause trouble in transferring containers filled with fixative liquid.

The storage of unprocessed materials, which are designed to be disposed of after reporting, could also benefit from utilizing bags with a vacuum-based packing system. Particularly, those tissue specimens that are already fixated can be stored under vacuum, if need be with a low fixative amount: this can produce about 50% of storage space reduction. The impact on waste management is also significant: the collection and disposal procedures can become easier as separating biological materials from fixative is no longer needed.

The manufacturers commit to identifying various solutions and flexible configurations so as to enable the decision maker to opt for the best arrangement for the specific context. Once the most appropriate configuration was found, the workflow – as shown in Figure 6 – was therefore proposed to and shared with Prato’s Hospital, in order to define the work streams and the division of roles and activities.

Traceability

To ensure the traceability of biological materials, it is essential to assign a univocal identification code from the Operating Room, which allows to follow them throughout the whole process and track their position. In this connection, the solutions available on the market (Rivac S.r.l., Milestone S.r.l.) are equipped with the following elements:

- barcode printer;
- barcode optical scanner;
- data management software.

To implement such a traceability system, the information system of the Operating Room should be integrated with the management software used at the Anatomic pathology laboratory. In doing so, information would be shared both effectively and efficiently.

The Safety domain

This domain was assessed by paying special attention to the implications of the technology use and those concerning the safety of the operators involved, so the analysis was focused on the chemical hazard related to the use of fixatives.

In Prato’s histopathological practice, the first contribution in terms of safety consists in replacing glyoxal-based fixative (mutagenic) with formalin (carcinogenic). Moreover, scientific literature provides evidence of the positive impact of the vacuum-based procedure. As regards risk assessment, adverse events are remarkably less likely to occur as the use of fixative is limited to histopathological laboratories, where it is safely handled under exhaust hoods. In fact, its use in Operating Rooms has strongly decreased (up to 70-90%),


\[ b \] The International Agency for Research on Cancer (IARC) classifies formalin as carcinogen Group 1.
thus allowing to reduce exposure related to the manual filling of containers. As a result, accidental fixative spillages during transfer operations are also reduced, as well as the formation of toxic vapors when opening histological containers in reduction rooms or storing post-sampling residual materials. Furthermore, an additional reduction of chemical hazard is made possible by the automated filling of specimen containers with fixative, because operators are less likely to be exposed to the source of danger.

Conversely, biological hazard requires some considerations: with the introduction of vacuum-based technology, most surgical biotic specimens are fresh rather than submerged in fixative liquid when they reach the Anatomic Pathology laboratory, which is likely to increase such hazard. However, literature analysis did not offer any information concerning the increase/decrease of the biological risk related to the use of the new procedure. Indeed it is true that, according to the colloquial evidence provided by clinicians, operators’ safety is
properly ensured by the adoption of adequate preventive measures as well as personal and collective protective equipment – which are normally used in laboratories.

**THE ECONOMY DOMAIN**

Being duly contextualized in Prato’s framework, the economic assessment rests on a comparison between the traditional management of biological materials (current scenario) and their vacuum-based management.

As regards market supply, offering systems that are designed to be used in Operating Rooms and Anatomic Pathology, the two following scenarios (A and B) have been postulated and developed for the comparison:

- **current scenario** – immersion of the biological specimen in glyoxal-based fixative, immediately after being taken in the Operating Room;
- **scenario A** – introduction of the technology in the Operating Room: transfer of fresh, under vacuum biological specimen, then fixation in formalin by the traditional procedure;
- **scenario B** – similar to the previous one, with the vacuum-based technology being introduced in Anatomic Pathology, too: transfer of fresh, under vacuum biological specimen, then fixation in formalin by using the technology.

Scientific evidence has shown that the introduction of vacuum-based technology in the Operating Room (Scenario A) would engender a fall in fixative consumption – implying a reduction of its purchase and disposal costs – which varies between 30% and 50%. A further consumption reduction would add to these values (25-30%) by utilizing tools in the Anatomic Pathology department that combine vacuum with automated filling with fixative: this situation (Scenario B) would determine a total reduction varying from about 55% to 80%.

Another significant feature of the economic analysis deals with using a fixative other than the one in use in Prato today (current situation), in case the technology is adopted (Scenario A or Scenario B): the purchase cost of glyoxal is about ten times higher than that of formalin. As far as the disposal cost is concerned, then, choosing a different fixative does not affect the expenditure as this hinges on the waste amount that needs to be managed.

In order to link these percentage values to an actual economic value, the annual fixative consumption was calculated for the considered scenarios (Fig. 7). As regards scenarios A and B, the amounts were estimated by considering the worst case, which entails a fixative consumption reduction of 30% and 55%, respectively.

It was calculated that the use of a new fixative would produce a lower annual expenditure (around 84%), which is due to both purchase and disposal costs. After integrating the results obtained from previous analyses, the estimated total savings vary from 89% to 93%, which correspond to scenario A and scenario B, respectively (Fig. 8).

Nevertheless, emerging costs should be also taken into account, namely new costs that would arise following the decision of a technological renewal (e.g. equipment purchase, technical assistance, staff training, installation operations).

A number of expenditure items that are able to generate savings should be also taken into account as they can modify the above-mentioned percentage values, although they are hardly quantifiable. Vacuum-based technology allows to strongly reduce the risk of accidental fixative spillages and therefore any costs related to the management of emergency situations which might occur (e.g. closing an Operating Room, cleaning a fixative spillage). Moreover, with an extremely low amount of formalin, the vacuum-sealing of post-sampling residual materials would imply a limitation of fixative-generated vapors inside the ventilated cabinets, allowing to change filters less frequently. Other savings should be also taken into consideration in respect of space optimization and storage volume reduction: a limited overall size involves a lower expenditure for archive management. Lastly, an easier waste management of post-sampling residual materials would certainly constitute another cost-saving solution.

An approximate assessment was made to determine a possible way to recover the investment costs, so as to identify the years needed to attain economic balance with regard to the initial costs that were covered to purchase the technology. In particular, for the two scenarios...
being considered the investment will be recovered from the first half and the second half of the fifth year, respectively, since the purchase of the technology.

Conclusions

The HTA study allowed to analyze the impact of introducing the technology for the preservation and the vacuum-sealed transfer of biological materials throughout the workflow of the Anatomy and Histopathological Department of the “Nuovo Ospedale di Prato”, run by the USL 4 of Prato. The assessment was formulated from a multidisciplinary perspective, going into a variety of aspects which might be influenced by the technology implementation and emphasizing its potential as an alternative to the gold standard, i.e. immersion in fixative. Generally speaking, the results obtained show a good degree of consistency, in terms of morphological and biomolecular preservation, between the traditional management and transport procedure applied to biological specimens longer than 2 cm and their transfer under vacuum at low temperatures.

On one side, some tools are able to add the automated filling with fixative to the benefits of vacuum-sealing, which allows to reduce the use of fixative liquids in the Operating Room and to obtain safer handling operations in the Anatomic Pathology department. On the other side, in Prato’s specific context, it implies that glyoxal is replaced with formalin. This appears to be a favorable change as it would foster the interaction between the laboratory and regional/national services, allowing also to participate in international quality control programs. Nonetheless, such a choice would require further examination of the working protocols of histopathological and immunohistochemical surveys. Beside the benefits of diagnostic effectiveness, from an organizational point of view some interesting prospects also arise with respect to the whole workflow traceability and the optimization of the transport, storage and disposal of post-sampling residual materials. In economic terms this is highly cost-saving, considering the reduction of fixation consumption related to the introduction of the technology. The main critical elements can be attributed to the necessary adaptation – or redefinition, if need be – of safety procedures and the organizational setting of the Anatomic Pathology workflow following the introduction of the technology. Other short- or long-term benefits might foreshadow a scenario that is consistent with the hypothetical increase in analytical activities, resulting in a richer test panel performed through molecular pathology procedures. Moreover, a role redefinition of the Anatomic Pathology service could be envisaged, since the technology would allow to evolve to reorganization models that are moving towards a higher service integration.

References

Ependymoma with diffuse signet-ring features: report of a case and review of the literature

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Key words
Ependymoma • Signet-ring cell • Metastatic adenocarcinoma • Frozen sections • Small biopsies

Summary
Signet-ring cell ependymoma is a rare variant of ependymoma with only seven cases described in literature. Biological behavior and prognosis of this entity are not well-known until now. We present a case of a 49-year-old female with a history of headache and gait instability. Magnetic resonance imaging showed an upper cervical tumor with cystic component and mural nodule. The patient underwent surgery. Microscopically some cells displayed an eccentric nucleus compressed to the periphery by vacuolated cytoplasm. Perivascular pseudorosettes and ependymal rosettes were seen only focally. The cells were positive for glial fibrillary acidic protein and epithelial membrane antigen. The diagnosis was ependymoma with diffuse signet-ring features, grade II according to the World Health Organization. It may be difficult to diagnose this unusual variant of ependymoma especially on small biopsies or frozen sections. A complete examination of the specimen is recommended with immunohistochemical confirmation to rule out potential morphologic mimics, such as metastatic adenocarcinomas and gliomas in the differential diagnosis.

Introduction
Ependymomas are tumors with uncertain malignant potential arising from the cells that line the ventricles and central canal of the spinal cord. They account for 6% to 9% of primary central nervous system (CNS) neoplasms1,2. The World Health Organization (WHO) 2007 classification of central nervous system tumors recognizes in this group rare histological variants: ependymoma with lipomatous differentiation, giant cell ependymoma, ependymoma with extensive tumor cell vacuolization, melanotic ependymoma, signet-ring cell ependymoma, ovarian ependymoma, ependymoma with neuropil-like islands2. Other proposed variants are: ependymoma with condroid metaplasia, ependymosarcoma, epithelioid ependymoma, ependymoma with “granular cell” features and oncocytic ependymoma3-7. Diagnosis of these rare variants is often difficult in the absence of the typical histological features of ependymoma (perivascular pseudorosettes, ependymal rosettes and canals)2. We report a case of an intramedullary upper cervical cord ependymoma rich in signet-ring cells. Issues related to diagnostic problems are discussed with a review of signet-ring cell ependymomas previously described in literature.

Clinical history
A 49-year-old female was referred to the Department of Neurosurgery for a history of recurrent headache. Neurological examination revealed uncoordinated movements, gait instability, speech impairment and difficulty with eye movements. Magnetic resonance imaging (MRI) showed an intramedullary tumor of the upper cervical cord with cystic component and mural nodule, T1-isointense and T2-hyperintense, suggesting the diagnosis of pilocytic astrocytoma (Fig. 1)8,9. The patient underwent surgery for total tumor excision via sub-occipital craniotomy.
Materials and methods

Surgical specimen obtained was fixed in 10% formalin and embedded in paraffin. Five-micrometer sections were stained using hematoxylin-eosin, periodic acid-Schiff (PAS) and immunohistochemistry. Immunohistochemical staining were performed with GFAP (clone 6F2, dako), EMA (clone E29, dako), GATA-3 (clone L50-823, zeta corporation), TTF-1 (clone 8G7G3/1, dako), CK AE1/AE3 (clone AE1/AE3, dako), cytokeratin CAM 5.2 (clone Cam 5.2, ventana) and Ki67 (clone MIB-1, dako). All samples were processed using a “Bond Polymer Refine” detection system in an automated bond immunostainer (Vision Biosystem, Menarini, Florence, Italy). Ki67 immunoexpression was considered as low (< 1% of cells), intermediate (1-5%) and high (> 5%).

Results

Macroscopically the tumor was a soft, whitish nodule, 20 millimeter diameter. At microscopic examination, in four out of five sections the majority (> 50%) of tumor cells showed an eccentrically located nucleus compressed by clear vacuolated cytoplasm (Fig. 2A-B-C) in a glial fibrillary stroma, admixed with round cells (Fig. 2D). On special stain many vacuoles were PAS positive. Perivascular pseudorosettes and ependymal rosettes were focally seen (Fig. 2D). Necrosis and vascular proliferation were absent, with a Ki-67 labeling index of 2-3%. Immunohistochemical analysis of neoplastic cells showed an intracytoplasmic dot-like pattern positivity for EMA (Fig. 3A). About one third of the cells expressed GFAP, with minor intensity in signet-ring cell areas (Fig. 3B). No cytokeratins nor GATA-3 and TTF-1 expression was revealed. The final diagnosis was “ependymoma with signet-ring cell features”, grade II according to WHO classification 2007.

Discussion

Signet-ring cell ependymoma is a rare entity with seven cases reported in literature 10-15. Six cases out of seven occurred in women, with an age range between 2 and 58 years. The most commonly involved sites were the parieto-occipital region and the 4th ventricle. In almost all cases the preoperative radiologic workup was performed with MRI and the common finding was a nodule with solido-cystic pattern, with a ring contrast enhancement, T1-isointense and T2-hyperintense. These radiological features are however not specific and reported in different tumors such as pilocytic astrocytoma, gliomas, metastatic tumors, gangliogliomas, hemangioblastomas and cystic meningiomas 8-9 16-19. Available clinico-pathological features of the reported cases are summarized in Table I and Table II. All cases were classified as ependymoma grade II according to the 2007 WHO classification. As shown in Table I two cases were submitted to radical resection and exhibited an indolent behavior with no recurrence. Complete surgical resection represents the standard treatment for ependymomas, and should be carried out whenever possible 20 21. Adjuvant radiotherapy improves local control as well as overall survival, it is commonly used after complete resection of localized high-grade ependymoma and indicated for low- and high grade lesions where residual disease is suspected after surgery 21 22. Chemotherapy has been employed at the time of relapse and platinum-based regimens are considered the best available option 23. In two cases a subtotal resection followed by gamma-knife radiosurgery was performed. In the case reported by Hirato et al. the tumor recurred after one year, leading to a total resection, while in the case presented by Cenacchi et al. there was no recurrence 11 13. Follow-up information were available in two out of seven patients and the longest follow-up period was one year with no evidence of recurrence. Ependymomas are histologically characterized by the presence of perivascular pseudorosettes, ependymal rosettes and canals. The first consist of neoplastic ependymal cells with tappered processes radiating around a wall of a centrally placed vessel, in a spoke-wheel arrangement; the last two are tubule-like structures of neoplastic cells surrounding an empty lumen 24. These typical features easily allow to distinguish ependymomas from other
Fig. 2. Morphological features. (A, B, C) Signet-ring cells (D) Round cells with perivascular pseudorosettes.

Fig. 3. Immunohistochemical features. (A) EMA with intracytoplasmic dot-like pattern (B) GFAP with fibrillary pattern.
### Tab. I. Summary of clinical features of reported cases of signet-ring ependymoma.

<table>
<thead>
<tr>
<th>Case No./authors</th>
<th>Sex/age</th>
<th>Symptoms/signs</th>
<th>Site</th>
<th>Imaging</th>
<th>Treatment/recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Zuppan et al.</td>
<td>F/12</td>
<td>NA*</td>
<td>Left parieto-occipital region</td>
<td>NA*</td>
<td>NA*</td>
</tr>
<tr>
<td>2) Zuppan et al.</td>
<td>F/44</td>
<td>NA*</td>
<td>Fourth ventricle</td>
<td>NA*</td>
<td>NA*</td>
</tr>
</tbody>
</table>
| 3) Hirato et al. | F/2     | Tonic-clonic seizure disorder | Left parieto-occipital region | 1) Solid mass  
2) Cyst, homogeneous enhancement | OP\(^{1,1}\): subtotal resection → gkrs\(^{1}\) recurrence 
OP\(^{2}\): radical resection → no recurrence |
| 4) Vajtai et al. | M/64    | Headache, dizzy spells, gait problems, cerebellar disorders | Posterior fossa | Cyst with mural nodule, T2-h\(^{1}\) ring enhancement | OP\(^{2}\): radical resection → no recurrence |
| 5) Cenacchi et al. | F/5   | Vomit, headache, gait problems, endocranial hypertension | Posterior fossa, fourth ventricle | Cyst, T2-h\(^{1}\) ring enhancement | OP\(^{1,2}\): ventriculocisternostomies → ND \(^{1}\) OP\(^{3}\): subtotal resection → gkr \(^{1}\) → no recurrence |
| 6) Mizuno et al. | F/58    | Respiratory distress, absent gag reflex, movement/sensory disorders | Medulla oblongata | Cyst, T2-h\(^{1}\) ring enhancement presence of hemorrhage | OP\(^{1}\): subtotal resection after IOFSA\(^{4}\) revised diagnosis on definitive histology |
| 7) Ertan et al. | F/35    | Headache, nausea, bilateral mild papilla edema | Foramen magnum, fourth ventricle | Solid mass, T2-h\(^{1}\) heterogeneous enhancement | OP\(^{2}\): radical resection → no recurrence |

* not available; \(^{1}\) T2-h yperintense; \(^{2}\) operation; \(^{3}\) gamma-knife radiosurgery; \(^{4}\) non diagnostic; \(^{5}\) intraoperative frozen section analysis.

### Tab. II. Summary of pathological features of reported cases of signet-ring ependymoma.

<table>
<thead>
<tr>
<th>Case No./authors</th>
<th>IOFSA*</th>
<th>H&amp;E</th>
<th>EMA</th>
<th>GFAP</th>
<th>Ki67</th>
<th>Necrosis/mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Zuppan et al.</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
</tr>
<tr>
<td>2) Zuppan et al.</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
<td>NA(^{1})</td>
</tr>
</tbody>
</table>
| 3) Hirato et al. | NO    | SP\(^{1}\): signet-ring cells, clear cells  
SP\(^{2}\): signet-ring cells, clear cells, smaller cells, fibrillary stroma, perivascular pseudorosettes | Focally positive | Positive | SP\(^{1}\): 0,1%  
SP\(^{2}\): 0,66% | Not found |
| 4) Vajtai et al. | NO    | Signet-ring cells, clear cells, rudimentary perivascular pseudorosettes | Positive | Positive | < 1\% | Not found |
| 5) Cenacchi et al. | NO    | Signet-ring cells, clear cells, smaller cells, perivascular pseudorosettes | Focally positive | Positive | 3\% | Occasional mitosis necrosis absent |
| 6) Mizuno et al. | YES   | FS\(^{1}\): signet-ring cells with atypical nuclei  
SP\(^{1}\): signet-ring cells, smaller cells, perivascular pseudorosettes | Focally positive | Intensely positive | NA\(^{1}\) | Low mitotic count necrosis absent |
| 7) Ertan et al. | NO    | Signet-ring cells, clear cells, pigmented cells, focal perivascular pseudorosettes, ependymal rosettes, rosenthal fibers | Positive | Positive | 1\% | Not found |

* intraoperative frozen section analysis; \(^{1}\) not available; \(^{2}\) specimen; \(^{3}\) frozen section
primary brain malignancies and metastatic neoplasms, but a correct diagnosis of rare histological ependymoma may be challenging in insufficient material or when the presence of typical features are lacking. In our case, the microscopic examination showed many aggregates of signet-ring cells intermixed with areas composed of round cells. Signet-ring features could be identified as a minor component in classical ependymomas. It may be predominant, leading to diagnostic pitfalls when small biopsies, representing just part of the lesion, are evaluated by the pathologist. In the case described by Mizuno et al. at intraoperative analysis, the tumor was first considered a metastatic adenocarcinoma with a signet-ring configuration leading to a subtotal removal of the mass. Afterwards, on permanent sections, the diagnosis was revised to a signet-ring cell ependymoma with a subsequent complete surgical tumor excision. It is therefore important to assess the whole tumor mass for a correct diagnosis looking for typical histological features and an associated immunohistochemical panel. The most important neoplasms to be differentiated from signet-ring cell ependymoma are metastatic adenocarcinomas and other primary brain tumors with a signet-ring cell pattern (glioblastoma, astroblastoma, oligodendroglioma) GFAP and EMA are extremely helpful in this setting. Various investigators showed two distinct patterns of GFAP immunoreactivity in ependymomas: one was fibrillary and related to perivascular pseudorosettes, the other was a diffuse cytoplasmic pattern. GFAP is constantly expressed in glioblastomas and variably expressed in oligodendrogliomas and astroblastomas, while no expression was found in signet-ring adenocarcinomas. EMA is a sensitive and specific marker of ependymal differentiation. Immunoreactivity pattern may vary depending on the subtypes and on neoplastic cell differentiation: ring-like membrane reactivity, dot-like intracytoplasmic staining or packed cytoplasmic pattern may all be detected. EMA staining has also been described in meningioma, chordoid glioma, rhabdoid tumor, chordoma and signet-ring adenocarcinoma.

Conclusions

We reported an unusual case of ependymoma with signet-ring cell features. Although the reported cases are relatively few and long-term follow-up is not available for all, the cases treated with total surgical resection show a favorable prognosis like the counterpart of classical ependymoma. Signet-ring cell ependymoma may mimic signet-ring cell adenocarcinoma and other brain tumors, with the risk of a misdiagnosis, especially on small biopsies or frozen sections.

The preliminary abstract of this work has been accepted as presentation in form of poster at the ECP 2014 London meeting (August 30- September 3, 2014).

References


Signet-ring ependymoma

Case report

Cellular fibroma in the Douglas cavity, mimicking a malignant neoplasia: fibroma, fibrosarcoma or mitotically active cellular fibroma?

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Key words

Cellular fibroma • Fibrosarcoma • Mitotically active cellular fibroma

Summary

Introduction. Ovarian fibroma is a benign stromal tumour composed of spindle/ovoid fibroblastic cells producing collagen. Approximately 10% of fibromas are densely cellular with small amount of collagen. In these cases, if mild nuclear atypia is present, they are best addressed as cellular fibroma. However cellular fibroma may show a greater mitotic activity and therefore they should be referred as mitotically active cellular fibromas. Mostly benign, it is necessary to differentiate them from malignant tumours such as fibrosarcomas.

Methods. We report a case of an unusual presentation of mitotically active cellular fibroma, detected in the Douglas cavity of a young woman, with normal appearing ovaries and uterus, mimicking a malignant neoplasia clinically and on imaging. In fact abdominal mass may be associated with acute pain, resulting in clinical emergency, really difficult to distinguish from a frank malignancy, before surgical procedure.

Results. We described the clinical, radiological and pathological characteristics of our case and we make a comparison of what previously described in literature.

Discussion. The differential diagnosis among those entities is based on the microscopic features such as atypia and the number of mitoses. However, according to their dimensions, it may be necessary to generously sample these tumours and sometimes, to perform a panel of immunohistochemical markers, in order to make a correct diagnosis, establish the best treatment and the right follow-up. In fact, the prognosis is not certain, due to the possible recurrence, especially if not completely excised.

Introduction

Ovarian fibroma is a benign sex cord-stromal tumor, accounting for 4-5% of all ovarian neoplasms. Mostly occurring during peri and postmenopause, the median age is about 52 years, very rare in children. Lesions tend to be asymptomatic. If symptoms are present, the most common is abdominal pain. Clinically, acute pain due to an abdomino-pelvic mass, is a common clinical emergency and difficult to differentiate from malignant lesion. Diagnosis is usually made by ultrasonography showing a solid ovarian lesion, or, on some occasions, mixed tumors with solid and cystic components. On gross pathology, they are firm and white or dark in color. On microscopic examination, ovarian stromal tumors composed of a pure proliferation of fibroblastic cells are fibromas, cellular fibromas (CFs), and rarely, fibrosarcomas. CFs are characterized by higher cellularity however, increased mitotic activity and sometimes nuclear atypia may be present raising the necessity to differentiate it from fibrosarcomas. To better classify these lesions in 1981 Prat proposed histologic criteria for the distinction of CFs from fibrosarcomas. According to Prat, CFs were characterized by cellular proliferation of fibroblasts with mild to moderate nuclear atypia and mitotic count of ≤ 3 mitosis/10 HPF, and a low malignant potential. In contrast, fibrosarcomas were usually associated with severe nuclear atypia, ≥ 4 MFS/10 HPFs, and an aggressive clinical course.

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Nevertheless, recent studies demonstrated that patients with ovarian fibroma with high mitotic rate had a good clinical outcome and therefore cellular fibromatous neoplasms with weak nuclear atypia should be subclassified in CFs or mitotically active cellular fibroma (MACFs), based on the number of mitotic figures. We report a challenging case of a pelvic mass detected in a young woman with normal appearing ovaries and uterus, mimicking a malignant neoplasia.

Case presentation

A 40 years old nulliparous woman came to clinic for menstrual irregularities, abdominal distention and acute pelvic pain. Clinical examination raised the suspicious for a large mass mainly solid, with minimal mobility. Ultrasonography revealed the presence of a voluminous solid pelvic mass, with some cystic areas and magnetic resonance imaging (MRI) confirmed the finding of a large lesion measuring 20 cm in maximum diameter. No obvious connections between the lesion, the uterus or the gastrointestinal tract were identified. No lymph node swelling or distant metastases were found. Tumor marker levels in the serum were within the normal range. Upon laparotomy, a solid mass was found in the Douglas cavity, minimally connected to the right ovary. However, both ovaries and the uterus were normal and there was no sign of peritoneal implants. Gross pathology revealed a tumor weighting 550 gr and measuring 23 x 11.7 x 5.8 cm. The external surface was yellow and the tumor was partially covered by a serosal surface and surrounded by a thin capsule, without any sign of rupture. Serially sliced, the tumor has a variegated appearance with solid and cystic areas, filled with a gelatinous and mucinous material. There was no clear evidence of necrosis.

Frozen sections was performed showing non-atypical spindle cells, arranged in storiform pattern with no evidence of high mitotic rate or atypical mitotic figures. The diagnosis was fibrothecoma and, in agreement with surgeons, uterus and ovaries were not removed to preserve patient fertility.

The remaining material was fixed in formalin, processed and stained with hematoxylin-eosin. To adequately sample the lesion 30 blocks were obtained (approximately one block for every cm of maximum dimension). Microscopic examination showed a spindle cell tumor within which focal highly cellular areas, with mild nuclear atypia, were identified (Fig. 1). Cystic degeneration was present, 4 mitosis in 10 HPFs were counted but there was no evidence of necrosis (Fig. 2). Immunohistochemical analysis were performed to further characterize the lesion. The tumor cells show positive staining for vimentin, CD34, CD56 and PR (Fig. 3). They were negative for pankeratins, calretinin, S100, smooth muscle actin (AML), CD 10, synaptophysin and chromogranin (Fig. 4). MIB-1 was positive in 5% of the cells (Fig. 5).

Discussion

Abdominal pain with an associated pelvic mass is a common emergency. Ovarian tumours and uterine miyomas are the most common lesions found in the female pelvis. However, only with clinical evaluation, it may be really difficult, to establish if the tumor is benign or malignant. In certain cases, cystic degeneration of fibromas has been reported to lead pre-operative misdiagnosis of malignant ovarian tumors. In a previous paper, Adad et al. described a similar case of a woman with a final diagnosis of cellular fibromas who came to clinicians for gastrointestinal disease. Our patient was admitted to the accident and emergency department for the acute abdominal pain. After radiologic evaluation, she was referred to gynecologic department for a suspicious volu-
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ominous pelvic mass detected on imaging. However onco-
logic markers (CEA, Ca 19,9 and Ca 15,3) were negative
and there was no evidence of serous effusions.

In contrast to our case, Adad et coll. described a cellular
fibroma with a prominent multicystic component filled
with mucinous-content. The tumor that we describe, in-
stead, is mainly solid with only small cystic components
filled with gelatinous material. However, in both cases the
radiological suspect was of an ovarian, mucinous tumor.
On frozen section the diagnosis was of fibrothecoma,
but of course only some sections of the solid compo-
nent were examined. The patient was treated according
to her age, clinical and surgical evaluation, gross pathol-
ogy and frozen section result. Therefore to preserve her
fertility, only the mass was removed and adjacent organs
left in situ.

Regarding the prognosis, cellular fibroma has an uncer-
tain malignant potential, capable of aggressive growth,
especially if incompletely excised. The mitotic count is
considered the most useful predictive marker, but in oth-
er studies, the completeness of excision has been con-
sidered important as well as the mitotic activity.

The main differential diagnosis is fibrosarcoma, a ma-
lignant mesenchymal tumor derived from connective
tissue. It is characterized by the presence of spindle cells
in a storiform pattern with different degree of differen-
tiation: low, intermediate and high grade. Depending on
the grade of differentiation, tumor cells may resemble
fibroblast or being anaplastic cells with severe atypia.

According to literature data, the mitotic activities, the
presence of necrosis and the grade of cellular atypia are
considered the main criteria for a diagnosis of malign-
cy 5. In our case, we did not find any necrosis, mild
nuclear atypia was detected only in focal highly cellular
areas and we counted 4 mitoses in 10 HPF. However,
we also performed the Ki67 (MIB-1) immunostaining,
in order to evaluate the proliferation index. Usually, cel-

dustrial fibroma shows less than 3% of MIB-1 positivity.

In our case, 5% of the tumor cells were positive. Some
authors described that mitotic activity and MIB-1 posi-
tivity were considered important prognostic factors.

In particular, Haung et al. in a retrospective study, con-
cluded that cellular fibromas are characterized by low
rate of mitosis (maximum 3 mitoses/10 HPF) and MIB-
1 (2-3%) as well as the presence of cellularity and no
evidence of rupture or aggressive clinical behavior.

In this case, we found 4 mitosis in 10 HPF, but the MIB-
1 staining was strong and higher than 3%. We did not
have sign of rupture of the tumor or adhesions and we
found no more than mildly cellular atypia, in focal high-
ly cellular areas.

In a large retrospective study, Irving et al. reviewed the
characteristics of fibroma and fibrosarcoma, describ-
ing also another entity: “mitotically active fibroma”
(MACF). In their series, cellular fibromatous neoplasms
with bland cytology and elevated mitotic count (more
than 4 mitoses in 10 HPF) are associated with good out-
come. This entity should be referred as MACF. How-
ever, they suggested a long-term follow-up for these
patients in order to exclude recurrences.

In the present case, 30 months after surgery, patient is still alive with-
out any recurrences.

A panel of immunohistochemical markers have been
performed to better characterise the lesion. The tumor
cells stained positive for vimentin, CD34, CD56 and PR.
They were negative for the following markers: pankera-
tins, calretinin, S100, smooth muscle actin, CD 10, sin-
aptophysin and chromogranin. So that, we could exclude
mesothelial, neural, smooth muscle, neuroendocrine and
dometrial stromal differentiations. The positivity for
CD56 and PR suggested the probably ovarian origin of
the mass, in fact it was removed from the Douglas
cavity and it did not show any connection with the sur-
rounding structures.

According to the morphology, the mitotic rate and the

Fig. 5. A. The tumor had scattered, atypical mitoses (H&E 40 X). B.
MIB-1 index was 5% (X 10).
immuno profile, we concluded that it was a MACF and a prolonged follow-up advised. In conclusion, it is difficult to make a right diagnosis of a pelvic mass with clinical evaluation only. In particular, in young women, the nature of the tumor is important to choose the type of surgery (ie. ovary-preserving surgery). However, sometimes, only after generously sampling of the tumor, and a wide immunohistochemical profile, it is possible to assess the best treatment and follow-up for each patient.

References


**Type II congenital pulmonary airway malformation associated with intralobar pulmonary sequestration: report of a case and review of classification criteria**

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**Key words**
Congenital cystic adenomatoid malformation • Congenital pulmonary airway malformation • Congenital thoracic malformations • Endoderm/mesoderm interaction • Lung cysts • Pulmonary sequestration

**Summary**
Pulmonary congenital abnormalities are rare disorders including congenital pulmonary airway malformations (CPAM) and pulmonary sequestration (PS). CPAM is a lesion characterized by the presence of anomalous bronchiolar or acinar structures, variable in size, either cystic or not cystic. PS is generally defined as nonfunctioning lung tissue that is not in normal continuity with the tracheobronchial tree and that derives its blood supply from systemic vessels. We describe a case of a baby girl with a very rare association between CPAM type 2 and intralobar pulmonary sequestration (IPS) focusing on the cystic lesions typical of CPAM and on the lymphatic and blood vessels. The cells lining the cysts often were positive for D2-40 (oncofetal protein M2A). Lymphatic endothelial cells, positive for D2-40, were widely present in the lung parenchyma and dilated lymphatic vessels were present also in the inter-alveolar septa. Moreover, we discuss the pathogenesis of CPAM and its classification criteria.

**Introduction**
Congenital pulmonary airway malformations (CPAM) and pulmonary sequestration (PS) are lesions included in the spectrum of the “congenital thoracic malformations” (CTMs) 1. CPAM, called also congenital cystic adenomatoid malformation (CCAM), is a lesion, assumed to be hamartomatous, characterized by the presence of anomalous bronchiolar or acinar structures, variable in size, either cystic or not cystic 2. It is classified in five main categories on the basis of the prevalent component or, in other words, on the basis of the apparent site of maldevelopment of the airway lesion 3. This classification system was not widely applied and it has been recently revised1. However, CPAM type 2 is characterized by the presence of bronchiolar-like structures forming cysts with a diameter comprised between 0.5 and 2 cm. In 95 % of CPAM type 2 cases only one lobe is affected, with a minimal preference for the inferior ones (55 %). It has a typical paediatric onset and manifests mainly in the newborns as distress respiratory syndrome 3. PS are localized lesions comprising lung parenchyma receiving their blood supply via aberrant systemic arteries and lacking continuity with the upper respiratory tract 4-5. PS can be distinguished in extra-lobar (EPS) and intra-lobar (IPS). The former is a pulmonary segment distinct from the normal lung, coated by its own pleura. In the second one, the accessory lung is annexed to the normal parenchyma and shares the same pleura with the normal one 6. IPS affects the basal lobes in 98 % of cases and, in particular, the posterior basal lobe (81 %) 2. In addition IPS, differently from EPS, is rare in early childhood. More than one CTM are frequently found in the same patient 6 and this suggests their common pathogenesis. However, the co-occurrence of both CPAM and IPS in the same individual is rare and the pathogenic mechanisms leading to their combination are unclear 7. In this work we describe a case of a congenital malformation in which CPAM type 2 and IPS are combined. Furthermore, we discuss the pathogenic mechanisms and make a critical review of the classification criteria of CTMs.
Case report

A baby girl was born by a full term delivery at the Obstetric Unit of Siena University Hospital. At birth she presented an acute respiratory distress syndrome. A chest MDCT (multirow detector computed tomography) showed a multicystic mass localized in the lower lobe of the right lung, with the largest cyst of diameter of 23 mm and presenting an air-fluid level within it. Moreover, the mass showed a systemic blood supply from a large arterious vessel originating from the abdominal aorta. Further, the pulmonary arterial vessels of the right lower lobe were interrupted after the origin of the apical branch. A right lower lobectomy was performed at the age of four months. At gross examination, the resected right lower lobe of the lung measured 7 x 5 x 6 cm. A systemic artery (maximum diameter 5 mm) was identified at the congested, red wine colored basal posterior segment located at the transition between thoracic and diaphragmatic surface. This artery branched off in the posterior basal segment of the basal pyramid (Fig. 1a-1b). The cut surface of lung parenchyma showed multiple cysts with a diameter varying between a few millimeters and 2 cm, occupying the entire basal pyramid. Abnormal vessels walls were as thick as those of the systemic arteries. There was no clear separation between the territory perfused by these vessels and the remaining parenchyma (Fig. 1c-1d). At microscopic examination, the walls of the cysts were formed by a central portion rich in blood and lymphatic vessels that were separated from the epithelial component by bundles of smooth muscle. Most of the cysts were lined by a ciliated epithelium (Fig. 2a-2b). The walls of the abnormal arterial branches were thick and contained continuous circular elastic fibers, similar to those seen in systemic elastic arteries. Their branches were directed in a disorganized way towards the posterior basal segment and formed a conglomeration of large vessels. These did not show any ordered connection with the adjacent cystic parenchyma that appeared congested and with areas of hemorrhagic infarction. Notably, the arterial vessels of all basal segments apart from the posterior one were hypoplastic. Lymphatic endothelial cells (positive for D2-40) were widely present in the lung parenchyma and dilated lymphatic vessels were present not only in their typical location but also in the inter-alveolar septa. The cells lining...
the cysts often showed the immunophenotype typical of bronchiolar epithelial cells, expressing for instance basal p63 and CK5 and apical TTF1 and CK7. Interestingly epithelial cells were also positive for D2-40 (Fig. 3).

**Discussion**

Cystic lesions are very frequent features in many congenital pulmonary abnormalities. However, the mechanism of their formation is still not clear and the criteria used to classify cystic lesions such as CPAM are not well defined. Several studies report a high frequency of association (20%) between CPAM and other congenital lesions, both CTMs and not-CTMs. CPAM type 2 can be sometimes accompanied by bronchial atresia, EPS and, rarely, IPS. The persistence of primitive bronchial arteries reaching the pulmonary buds before the development of pulmonary arteries is observed in most cases of IPS. Langston suggests that the presence of malformations such as cystic lesions of CPAM type could be responsible for the lack of physiological involution of embryonal vessels. Lung buds are enveloped in a continuous mesenchymal surround throughout development. Blood vessels develop at the same time of airways within this mesenchymal component by capillary vasculogenesis that leads to the foregut-mesodermal pulmonary plexus. The latter needs a connection with the arterial system. At first, this plexus is connected to the primitive branchial arteries. However, after the thirteenth week of embryonal life, it is reached by branches of the pulmonary arteries that have been developing by angiogenesis from the aortic sac. This event leads to the regression of the previously established systemic connections. This complex process is under the direct control of molecular interactions between mesodermal and endodermal components regulating the lung development at different stages. We have described a case of CTM associated with both vascular and airways lesions (IPS and CPAM type 2 respectively). The morphology of this rare malformation suggests the presence of an abnormal regulation of the interaction between mesoderm and endoderm that ultimately leads to the altered development of both components. The morphological aspects of CPAM type 2 may be a consequence of a defective and interrupted endodermal differentiation in the pseudo glandular phase. In fact, at the end of this stage, which lasts from the sixth to the sixteenth week of embryonal life, airways are completely formed and their branches reach the level of the acinus. The impairment of the mesodermal component may have caused a defective development of the pulmonary plexus, the lack of connection between this and the pulmonary arterial system and the persistence of the one with the systemic circulation. This is supported by histological and radiological evidences of hypoplasia of the pulmonary arteries in the basal segments of the right inferior lobe. In addition, the abnormal expression of D2-40 (oncofetal protein M2A), documents the additional presence of an anomaly in the development of the lymphatic system. This is in agreement with the hypothesis of a pathological mesodermal differentiation. Moreover, the persistence of this oncofetal protein in the...
epithelial cells suggests the endodermal component is also altered \(^4\). In our case, the abnormal interaction between endodermal and mesodermal components of lung buds has happened at a specific time point, presumably around the thirteenth week of embryonal life, which corresponds to the pseudo glandular stage just before the establishment of connections between mesodermal plexus and pulmonary circulation. So, according also to the recently proposals for the classification of pulmonary malformations \(^1\), CPAM would not be an hamartomatous lesion and the classification proposed by Stocker \(^3\) based on the apparent “site of maldevelopment” might be incorrect. The right classification criteria may consider the time rather than the site of malformation. This new type of approach based on the temporal aspects of the endoderm/mesoderm interaction, suggested also by Clements et al \(^15\), could lead to a more modern and correct classification of all types of CTMs.

**References**

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Case report

Neuroglial heterotopia of the scalp

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Key words
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Summary
Heterotopic glial nodules of the scalp are non hereditary congenital malformations composed of mature brain tissue isolated from the cranial cavity. The majority of these lesions are found in the nasal region and occur rarely on the scalp. They are frequently diagnosed in newborn infants. However, they may rarely be found in adults. The pathogenesis of these lesions remains unknown.

We describe the case of a temporal scalp nodule in a 50 year-old man. At the time of the excision, the mass was not associated with intracranial connection. Histological examination revealed neural tissue staining with S100-protein and the glial fibrillary acidic protein (GFAP).

Background
Glial heterotopias are rare, benign congenital lesions, corresponding to the presence of extra cranial ectopic brain tissue without connection to the central nervous system. His pathogenesis is still unclear. They result from abnormal extra cranial sequestration of neural tissue during the embryogenesis. These malformative lesions mostly occur in childhood, before the first year and are often located at the nose. The location at the scalp is rare. Only 13 cases of scalp localized lesions are reported in the English medical literature. We report a rare case of glial heterotopia of the scalp, discovered incidentally in a 50 year-old man.

Case report
A 50 year-old man presented with a swelling of the occipital scalp which was noticed at birth and increased progressively in size. At physical examination the lesion was firm and alopecic (Fig. 1). Clinically, the diagnosis of benign adnexal tumor was made and a surgical resection of the lesion was performed. Preoperatively, the lesion did not adhere to the occipital bone and had no connection with the brain. Grossly, the tumor was nodular, white, ill-defined and measured 1.2 cm of diameter. Histological examination showed, showed a well-circumscribed lesion of the deep dermis composed of mature glial tissue (Fig. 2). It consisted of a dense network of columns and clusters of neural cells within a fibrillar, fibrous and hyaline tissue (Fig. 3). Sections of nerves were also seen. The immunohistochemical study showed a diffuse and intense staining of cells with GFAP (glial fibrillary acidic protein) and S100 protein.
Neuroglial heterotopia of the scalp (Fig. 4). The cytokeratin was negative. These features confirmed the diagnosis of a neuroglial heterotopia. At 3 years of follow-up, the patient was asymptomatic and there was no recurrence.

Discussion

Glial heterotopia is a rare, non-hereditary malformative lesion. It is defined by the presence of an ectopic glial or neuroglial tissue outside the brain. It must be distinguished from meningeal heterotopias that are more frequent and derived from the brain and the spinal cord meninges.

Several other names have been brought to describe this entity: brain heterotopias, astrocytoma, glioma, teratoma and choristoma.

The term cerebral heterotopia was first introduced by Lee and Mac Laurin in 1955 to describe the nasal glioma.

This congenital lesion is found mainly in the nose and less frequently on the palate, tongue, orbit, lung and chest wall. The location at the scalp is extremely rare. Only 13 cases were described in the English medical literature. Gray et al reported 2 cases of glial heterotopia of the scalp, in a series of 11 children with heterotopic neural tissue.

The exact pathogenesis of these lesions is unknown, although hypothetical etiopathogenesis mechanisms have been postulated. First, heterotopic brain may derive from an encephalocele that subsequently loses its communication with the brain. An alternative theory suggests that ectopic brain in remote locations may result from sequestration of extracranial embryonic neural tissue. The third hypothesis suggests that heterotopic brain derives from isolated rests of displaced pluripotent neuroectodermal cells.

This lesion is usually diagnosed in children, rarely in adults. It has a preferentially occipital, parietal or sometimes median location. It is a nodular lesion often discovered at birth and increasing proportionately with the child’s growth. It is solitary, circular, skin-colored, pink or bluish and mobile. It is most often bald scalp plaque sometimes surrounded by a crown of hair. It measures 2 to 4 cm in diameter.

The CT scan is a complementary study that is necessary in preoperative planning to determine the extent and location of a mass. On CT scan, a heterogeneous hyperattenuated mass with or without a cyst is a common feature.

Grossly, the lesion is firm, nodular and has a grey-white cut surface. Histological examination showed a glial
proliferation made of clusters of round or oval cells, composed mainly of astrocytes sitting in a neurofibrillary and richly vascularized tissue. These cells have central nuclei, without atypia and nucleoli. The cytoplasm is fibrillar and finely granular. Some astrocytes are giant and multinucleated like ganglion cells. An associated neural component or sometimes ependymal or choroid plexus structures can be found. However, purely glial appearance is most frequently described in the literature\textsuperscript{2,5}.

The diagnosis of glial heterotopia is essentially histological helped by immunohistochemistry witch shows positivity of glial component to GFAP (glial fibrillary acidic protein) and S100 protein\textsuperscript{1,2}.

The glial heterotopia and encephalocele have the same clinical and histological appearance but encephalocele is connected to the sub-arachnoid space by a cavity\textsuperscript{1}.

The treatment consists of total excision of the tumor. If the excision is incomplete, residual nerve tissue that persist can cause a recurrence. Furthermore, no malignant transformation has been reported in the literature. Our patient showed no recurrence or secondary lesion with a decline of 3 years.

References