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Original Articles

Fast Track Biopsy method: a rapid approach to preoperative diagnoses
V. Steri, A. Farnedi, E. Montinari, M.P. Foschini

Introduction. It is well known that the use of a microwave oven greatly reduces the time for histoprocessing. Objective: Apply the microwave-based histoprocessing method “Fast Track Biopsy” (FTB), previously described for breast needle-core biopsies, to samples of other organs.

Methods. From 01/09/2008 to 31/12/2008, 125 normal and neoplastic tissue samples (thickness from 0.2 and 1.2 cm) were collected, processed according to the FTB technique, stained with haematoxylin/eosin (H/E) and analysed with tissue-specific immunohistochemical (IHC) markers.

Results. The quality of both H/E and IHC stained sections was comparable to that obtained with standard methods. Sample thickness less than 0.5 cm gave better results.

Discussion. The FTB method is suitable for most tissue types, and is thus useful for preoperative diagnoses.

Diagnostic value of micro-histology in endometrial brushing
C. Manini, P.L. Montironi, A. Magistris, D. Stramignoni

Introduction. The efficacy of direct endometrial sampling by brushing in the collection of adequate and representative material is evaluated.

Methods. From January 1 2008 to October 31 2009, 195 women (age 29-82, mean 56, 113 postmenopausal), underwent endometrial brushing with Endoflower®. All samplings were performed in an outpatient setting. 137 patients had abnormal uterine bleeding (70 postmenopausal), 25 had asymptomatic endometrial thickening (≥ 4 mm), 6 had previous endometrial biopsies. The samples were fixed in a solution containing alcohol, water, EDTA and KOH, and centrifuged. The supernatant was filtered and the pellet embedded in paraffin.

Results. All patients reported that the technique was painless. Three cases suffered from shock. In 29 cases (15%), the sampling procedure was difficult due to cervical stenosis. A cellular sample large enough to prepare a cell-block was obtained in all cases. In 27 cases (14%), the sample was non-diagnostic. Cases were categorized as non-pathologic (negative) or pathologic (atypical and carcinoma). The correlation between cyto-histology on samples obtained with brushing and histology on biopsy or surgical specimen was possible in 46 cases (24%), with a diagnostic concordance of 93%. The rate of inadequate biopsies was 27% (8/30). 13 of 15 malignant neoplasias (2 carcinomas, 13 endometrioid adenocarcinomas) were correctly diagnosed in samples collected with Endoflower®. The sensitivity was 87% and specificity was 96%, with a positive predictive value of 92% and a negative predictive value of 90%.

Conclusions. Endometrial direct sampling with the Endoflower® device in an outpatient setting is well tolerated and well accepted by the gynaecologist. This sampling procedure allows preparation of cell-blocks. Endometrial cyto-histology is less expensive and invasive than other procedures and it could therefore be used in association with transvaginal sonography, even in institutions where liquid-based cytology is not in use.

The high expression of p53 in sporadic colorectal carcinoma is associated with metastasis and decreased survival
M. Pancione, N. Forte, S. Campione, A. Napolitano, D. Parente, L. Sabatino, A. Febraro, V. Colantuoni

Introduction. Alteration in the p53 tumour suppressor gene is an event that occurs frequently in human cancer, although its role as predictive and/or prognostic marker is still unclear. The aim of this study was to compare the expression profiles of p53 in colorectal carcinoma with clinicopathological features and survival rate at 5 years from diagnosis.

Methods. One hundred and twenty cases of primary sporadic colorectal cancers (CRCs) and 80 matched normal mucosas were analyzed by immunohistochemistry on paraffin-embedded specimens. The correlation between protein expression profiles, clinicopathological parameters and survival was investigated.

Results. In tumour tissues, the expression of p53 was high in 41 cases, low in 38 and negative in 41. A significant correlation was observed between increased p53 expression presence of lymph node (p = 0.002) or liver metastasis (p = 0.008). Moreover, higher levels of p53 were related with advanced tumour stage (III-IV; p = 0.007), poor survival and disease recurrence (< 0.01). Interestingly, in multivariate analysis p53 expression and distant metastasis were independent prognostic markers.

Discussion. Our results suggest that nuclear p53 accumulation in sporadic CRC may have prognostic significance and contribute to identification of patients at high risk of mortality. The current findings may be relevant for management of patients with CRC.

Lymphoepithelioma-like carcinoma of the endometrium

Only three cases of lymphoepithelioma-like carcinoma of the endometrium have been reported to date. We present a new case in a 67-year-old woman involving an exophytic mass that caused postmenopausal bleeding. Histologically, undifferentiated carcinomatous areas were intermingled with abundant lymphoid tissue. Epstein-Barr virus was not detected in either neoplastic or lymphoid cells.

Case reports

Extranodal Rosai-Dorfman disease of bone and nose: a case report and review of literature
S. Sellari- Franceschini, R. Lenzi, A. Tognetti, V. Seccia

Rosai Dorfman disease (RDD) is a rare benign condition of unknown origin, which was first described in 1969. By histopathology, the disease is composed of sinusoidal lymph node hyperplasia and abundant histiocytes with haemophagocytosis, particularly lymphocytes. It commonly affects lymph nodes, and rarely has an exclusively extra-nodal clinical presentation. Among the so-called “extranodal” sites, the head and neck region, and in particular the nose and paranasal sinuses, are frequently affected. RDD shows a highly variable clinical course that can be partly modified by medical therapy. We present a case of extra-nodal RDD, with nasal and osseous involvement, which has been followed-up for 19 years. We also discuss its presentation, the most relevant radiographic findings, treatment options and histological findings.

Sclerosing angiomatoid nodular transformation of the spleen associated with thrombocytopenia
J.L. Quirós, L. Manes, E. Bonandini, P. Vivaldi, P. Dalla Palma, M. Barbarechi

Sclerosing angiomatoid nodular transformation of the spleen, a recently described lesion of unknown pathogenesis, with a highly variable clinical course that is very often asymptomatic. Sclerosing angiomatoid nodular transformation may be a novo lesion or the final common pathway of various benign splenic conditions such as hamartoma, inflammatory pseudotumor and hemangiomata. We report the case of a 68 year-old woman with thrombocytopenia and a splenic mass, diagnosed as sclerosing angiomatoid nodular transformation.

Fibrous hamartoma of infancy of the labium majus: a typical lesion in an unusual site
G.M. Vecchio, A.E. Miano, G. Belfiore, E. Giurato, P. Amico, G. Magro

Fibrous hamartoma of infancy is a soft tissue subdermal fibromatos tumour that characteristically occurs in the first years of life. It is histologically composed of three different components that are
intimately admixed: well-defined bundles of fibro-myofibroblastic spindle-shaped cells, nodular proliferations of immature-looking mesenchymal cells set in a myxoid stroma, and mature adipose tissue. A wide intralesional and interlesional cellular composition is commonly observed. Fibrous hamartoma of infancy usually arises from subcutaneous tissue of the trunk, axilla, upper extremities and inguinal region. Only rarely has fibrous hamartoma of infancy been reported in genital organs, with only one case described in the labium majus. We report a rare case of fibrous hamartoma of infancy in the labium majus of a 1-year old female child. Ultrasonography revealed the presence of a mass-like lesion involving subcutaneous tissue, with ill-defined margins. We emphasize that fibrous hamartoma of infancy should be included in the differential diagnosis of soft tissue tumour-like and tumour lesions of the vulva in children. Awareness that fibrous hamartoma of infancy occurs at this site with irregular margins is important to avoid confusion with other lesions exhibiting a more aggressive behaviour.

**Critical issues in Pathology**

*When histologic diagnosis of pulmonary adenocarcinoma becomes difficult*


The differential diagnosis between pulmonary adenocarcinoma and several benign mimics can be a formidable challenge for the surgical pathologist, particularly in frozen sections and in small biopsies but sometimes in surgical specimens as well. In this review we will provide a practical guide to help the pathologist facing these problematic cases.
Fast Track Biopsy method: a rapid approach to preoperative diagnoses

V. STERI, A. FARNEDI, E. MONTINARI, M.P. FOSCHINI
Section of Anatomic Pathology, Department of Haematology and Oncology "L. e A. Seragnoli", University of Bologna, Bellaria Hospital, Bologna, Italy

Key words
Microwave • Histoprocessing • Preoperative diagnosis

Summary

Introduction. It is well known that the use of a microwave oven greatly reduces the time for histoprocessing. Objective: Apply the microwave-based histoprocessing method “Fast Track Biopsy” (FTB), previously described for breast needle-core biopsies, to samples of other organs.

Methods. From 01/09/2008 to 31/12/2008, 125 normal and neoplastic tissue samples (thickness from 0.2 and 1.2 cm) were collected, processed according to the FTB technique, stained with haematoxylin/eosin (H/E) and analysed with tissue-specific immunohistochemical (IHC) markers.

Results. The quality of both H/E and IHC stained sections was comparable to that obtained with standard methods. Sample thickness less than 0.5 cm gave better results.

Discussion. the FTB method is suitable for most tissue types, and is thus useful for preoperative diagnoses.

Introduction

Time is an important factor in terms of prognosis, therapy and general patients management. Furthermore, it is also important in limiting psychological distress of patients. For these reasons, processing techniques employing microwaves have been utilised to reduce the time of tissue fixation, dehydration and paraffin impregnation embedding of tissue.

In 2005, a method for rapid processing of breast needle biopsies based on microwaves was developed, called “Fast Track Biopsy” (FTB). In the same study, a rapid method for immunohistochemical staining was also introduced. The FTB method allowed histological analysis of breast needle biopsies with immunohistochemical characterization in a total time of 240 minutes instead of more than 24 hours using normal diagnostic procedures (NDP).

Rapid and accurate histological diagnosis is also desirable in other neoplastic diseases in order to improve diagnostic-therapeutic pathways.

The aim of this study was to validate the FTB technique in tissues of different organs to assess the possibility of adopting it as a diagnostic preoperative procedure.

As immunohistochemical stainings are sometimes employed to achieve a more accurate preoperative diagnosis, a rapid immunohistochemical method has also been tested on the same tissues.

Materials and methods

In the period between 01/09/2008 and 31/12/2008, 125 tissue samples were collected at the Section of Anatomic Pathology of the Department of Haematology and Oncology at the University of Bologna at Bellaria Hospital.

Tissue samples were obtained from surgical specimens, when dimensions allowed additional collection beyond that needed for normal diagnostic procedures (NDP), and fixed in 10%-buffered formalin for maximum of 2 hours.

To determine the maximum thickness of tissue samples for the analysis, samples for FTB processing were taken of increasing size from 0.2 to 1.2 cm thick.

These samples were processed with the automated microwave processor RHS-1 (Milestone Srl, Sorisole-Bergamo, Italy) following the protocol of FTB technique previously described by Ragazzini et al.

Acknowledgements

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samples were immersed in 10%-buffered formalin for 20 min at 50°C (instead of 12 hours at room temperature by NDP), dehydrated with absolute ethanol for 15 min at 65°C and, finally, soaked in paraffin for 25 min at 65°C under vacuum at 100 mB. All steps occurred in the presence of 600 W microwaves. The time required for microwave processing is 80 (± 5) minutes (instead of 14 hours by NDP) (all details listed in Table I).

Following this, samples were embedded in paraffin blocks, cut into 3 μm sections with a microtome and stained with haematoxylin/eosin (H/E) using an automatic stainer (Leica ST5020, Multistainer Workstation, Leica Microsystems, Milano, Italy). Embedding, cutting and staining with H/E lasted 40 (± 5) min.

In selected cases, immunohistochemical analysis for specific organ or tissue markers was performed, as listed in Table I. Dewaxing and antigens unmasking occurred simultaneously with the solution W-CAP TEC buffer pH 6 or W-CAP TEC buffer pH 8 (Bio-Optica, Milan, Italy) for 25 min at 98°C or, separately, by graded alcohol followed by enzymatic digestion with protease (LabVision, Fremont, CA, USA) for 30 min at room temperature, depending on the antibody protocol. Inhibition of endogenous peroxidases was performed by a 10 min incubation with H₂O₂ (3% in H₂O), followed by 5 min with Blocking Solution (LabVision, Fremont, CA, USA) to induce saturation of non-specific binding sites; both steps were carried out at room temperature.

Tab. I. Process timing.

<table>
<thead>
<tr>
<th>FTB procedure</th>
<th>Timing</th>
<th>NDP Procedure</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td></td>
<td>H&amp;E</td>
<td></td>
</tr>
<tr>
<td>Formalin fixation</td>
<td>20 min</td>
<td>Formalin fixation</td>
<td>From 12 to 48 hours depending on dimensions</td>
</tr>
<tr>
<td>Dehydration + Paraffin soaking</td>
<td>15′ + 16′ + 25′ = 56′</td>
<td>Dehydration + Paraffin soaking</td>
<td>14 hours</td>
</tr>
<tr>
<td>Inclusion, cutting and staining</td>
<td>40 min</td>
<td>Inclusion, cutting and staining</td>
<td>40 min</td>
</tr>
<tr>
<td>FTB procedure total timing</td>
<td>-/+ 2h</td>
<td>NDP procedure total timing</td>
<td>From 26h 40′ to 62h 40′</td>
</tr>
</tbody>
</table>

Tab. II. Panel of antibodies used for IHC analysis.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone/manufacturer</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle actin</td>
<td></td>
<td>1:100</td>
<td>None</td>
</tr>
<tr>
<td>CD 3</td>
<td>PS1, Neomarkers</td>
<td>1:20</td>
<td>W-CAP pH 8</td>
</tr>
<tr>
<td>CD 20</td>
<td>L26, Dako</td>
<td>1:100</td>
<td>W-CAP pH 6</td>
</tr>
<tr>
<td>CK-7</td>
<td>OvTL12/30, Dako</td>
<td>1:100</td>
<td>Enzymatic Digestion</td>
</tr>
<tr>
<td>CK-14</td>
<td>LL002, Neomarkers</td>
<td>1:400</td>
<td>W-CAP pH 6</td>
</tr>
<tr>
<td>CK-20</td>
<td>KS 20.8, Dako</td>
<td>1:40</td>
<td>W-CAP pH 6</td>
</tr>
<tr>
<td>CK-MNF116</td>
<td>MNF116, Dako</td>
<td>1:200</td>
<td>Enzymatic Digestion</td>
</tr>
<tr>
<td>MART</td>
<td>A103, Ventana</td>
<td>Prediluted</td>
<td>W-CAP pH 8</td>
</tr>
<tr>
<td>MUC-2</td>
<td>M53, Neomarkers</td>
<td>1:100</td>
<td>W-CAP pH 6</td>
</tr>
<tr>
<td>MUC-5</td>
<td>45M1, Neomarkers</td>
<td>1:200</td>
<td>None</td>
</tr>
<tr>
<td>SURFACTANT</td>
<td>PE-10, Dako</td>
<td>1:150</td>
<td>W-CAP pH 8</td>
</tr>
<tr>
<td>TTF-1</td>
<td>8C7G3/1, Neomarkers</td>
<td>1:200</td>
<td>W-CAP pH 6</td>
</tr>
</tbody>
</table>
Primary monoclonal antibodies, listed in Table II, were applied on sections for 60 min at room temperature. Following this, chromogenic detection was performed using the UltraVision Detection System (LabVision, Fremont, CA, USA), which entailed incubation with Antibody Enhancer for 20 min followed by HRP-Polymer for 30 min. Finally, DAB chromogen (Dako, Carpenteria, CA, USA) was applied for 3-5 min and sections were counterstained with haematoxylin after washing with water. Appropriate positive controls were added to each batch of slides. Negative controls consisted in omission of the primary antibody.

Immunohistochemical analysis required a total time of 210-240 minutes depending on unmasking protocol.

Results

In order to find the sample maximum thickness that could be processed by FTB, we compared the histologic sections obtained from 30 tissue samples with a thickness from 0.2 to 1.2 cm. The tissues analysed were: skin, adipose tissue, retroareolar tissue, breast carcinoma, lymph node and submandibulary gland.

When the thickness was ≤ 0.5 cm (17/30), the processing was comparable to that obtained with the conventional procedure and there were no artefacts caused by delayed fixation or incomplete paraffin embedding.

When the thickness was > 0.5 cm, sections of skin and adipose tissue (4/13) presented artefacts caused by delayed or incomplete fixation and insufficient paraffin embedding.

In the remaining samples of neoplastic and non-neoplastic breast, lymphoid and glandular tissues (9/13), the processing was comparable to the standard procedure even when the thickness was > 0.5 cm.

95 tissue samples were processed by FTB and divided as follows: tongue (2), submandibulary gland (7), parotid (1), thyroid (6), lung (8), stomach (2), colon (8), spleen (2), gallbladder (3), appendix (1), adipose tissue (5), breast (13), retroareolar tissue (2), nipple (1), lymph node (7), oral cavity squamous cell carcinoma (4), lung adenocarcinoma (2), colon adenocarcinoma (4), stomach adenocarcinoma (2) and breast carcinoma (15).

The H/E sections were ready in 120 minutes after arrival in the laboratory, and all were suitable for histological analysis (95/95). Specifically, there were no artefacts...
due either to delayed fixation or to incomplete paraffin embedding (Fig. 1).

To assess the antigenicity of FTB-processed tissues, 48 samples were selected and analyzed by immunohistochemistry. They were subdivided as follows: skin (5), submandibular gland (5), thyroid (5), colon (5), lung (8), spleen (1), stomach (2), gallbladder (1), adipose tissue (5), lymph node (5), oral cavity squamous cell carcinoma (1), lung adenocarcinoma (2), colon adenocarcinoma (2) and stomach adenocarcinoma (1).

The immunohistochemical investigations required between 210 and 240 min, and all the sections were positive for major tissue-specific antigens (48/48) (Fig. 2).

Discussion

In the present study, tissue samples from different organs with or without neoplastic alterations were analyzed by the FTB method. The quality of microwave histoprocessing of samples up to 0.5 cm thick was comparable to that obtained with the standard processing method. In larger samples (up to 1.2 cm), differences depending on the type of tissue were observed. Specifically, skin and adipose tissue showed artefacts due to incomplete fixation, while in lung, glandular and lymphoid tissues processing was optimal. The quality of the sections stained with H/E was comparable to sections of the same histological specimens processed according to standard procedures, and there were no differences in both cytoplasmic and nuclear staining. The immunogenicity of different tissues was not affected by microwave processing: the quality and intensity of immunohistochemical staining of tissue-specific markers was comparable to that obtained with the automated immunostaining procedure currently applied.

Our data show that the histological and immunohistochemical sections obtained by rapid microwave processing are indistinguishable from those conventionally processed for small tissue samples (up to 0.5 cm thick). Therefore, the method here studied is optimal for small samples such as preoperative biopsies. For larger samples, the quality of processing was closely related to the characteristics of the tissue examined. In some cases, it will be necessary to adjust specific parameters such as microwave power, temperature, pressure and time duration of single steps in order to optimize histological processing.

We conclude that the FTB rapid method can be extended to different types of tissues, in addition to breast, as it provides rapid histological and immunohistochemical results (H/E in 120 minutes + IHC in 240 minutes) and ensures a reliable and accurate diagnosis. This could mean a significant reduction of waiting time and stress for patients since the pathologist could communicate a histological diagnosis to the clinician in just a few hours. FTB rapid method could represent an improvement in the management of individual cases, and a significant decrease in hospitalization costs.
References


Diagnostic value of micro-histology in endometrial brushing

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Key words
Endometrial cytology • Direct intrauterine sampling • Endometrial brushing • Abnormal uterine bleeding

Introduction. The efficacy of direct endometrial sampling by brushing in the collection of adequate and representative material is evaluated.

Methods. From January 1 2008 to October 31 2009, 195 women (age 29-82, mean 56, 113 postmenopausal), underwent endometrial brushing with Endoflower®. All samplings were performed in an outpatient setting. 137 patients had abnormal uterine bleeding (70 postmenopausal), 25 had asymptomatic endometrial thickening (> 4 mm), 6 had atypical endometrial cells on pap-smear, 9 patients needed preoperative controls for uterine prolapse, 11 were treated with tamoxifen and 7 had other problems. The samples were fixed in a solution containing alcohol, water, EDTA and KCO₃, and centrifuged. The supernatant was filtered and the pellet embedded in paraffin.

Results. All patients reported that the technique was painless. Three cases suffered from shock. In 29 cases (15%), the sampling procedure was difficult due to cervical stenosis. A cellular sample large enough to prepare a cell-block was obtained in all cases. In 27 cases (14%), the sample was non-diagnostic. Cases were categorized as non-pathologic (negative) or pathologic (atypical and carcinoma). The correlation between cyto-histology on samples obtained with brushing and histology on biopsy or surgical specimen was possible in 46 cases (24%), with a diagnostic concordance of 93%. The rate of inadequate biopsies was 27% (8/30). 13 of 15 malignant neoplasias (2 carcinosarcomas, 13 endometrioid adenocarcinomas) were correctly diagnosed in samples collected with Endoflower®. The sensitivity was 87% and specificity was 96%, with a positive predictive value of 92% and a negative predictive value of 90%.

Conclusions. Endometrial direct sampling with the Endoflower® device in an outpatient setting is well tolerated and well accepted by the gynaecologist. This sampling procedure allows preparation of cell-blocks. Endometrial cyto-histology is less expensive and invasive than other procedures and it could therefore be used in association with transvaginal sonography, even in institutions where liquid-based cytology is not in use.

Introduction
Endometrial carcinoma is most frequent in developed countries¹,², and may be an incidental finding in a biopsy specimen. Most often it is found in patients investigated for abnormal uterine bleeding³. The endometrium can be studied by a variety of methods that are often used simultaneously. Endometrial cytology on specimens obtained with direct sampling in an outpatient setting, in spite of its high sensitivity in detecting carcinoma, is not widely used in Western countries, probably because of the high percentage of inadequate samples. However, the commercial availability of a flexible sampling device led to an increase in the number of satisfactory tests. This kind of device allows the sampling of the entire uterine cavity including the fundus and the tubal corners with limited cervical contamination. In addition liquid-based cytology reduces technical artefacts and makes it possible to obtain small histology samples with residual tissues⁴. The aim of this study was to evaluate if the endometrium can be successfully investigated on samples obtained with a brushing device (Endoflower®RI-MOS), followed by filtering, instead of liquid-phase treatment, for cytology before paraffin embedding for “micro-histology”.

Patients and methods
From January 1, 2008 to October 31, 2009 195 consecutive unselected patients were enrolled in the study. The median age was 56 years (range 29-82); 82 were in the pre- or peri-menopausal period, and 113 were in menopause (Tab. I). 137 women (70 in menopause) were referred for abnormal uterine bleeding, 25 for thickened…

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endometrium, 6 for abnormal endometrial cells in pap smear, 9 for routine check-up before surgery for uterine prolapse, 11 for follow-up in tamoxifen treatment and 7 for unspecified reasons. An additional 12 patients (7 in menopause) could not be enrolled because of stenosis of the cervical canal. After providing informed consent, patients underwent endometrial brushing performed by 15 different gynaecologists in an outpatient setting using the Endoflower® device (Fig. 1). This device measures 3 mm in diameter; it has umbrella-shaped tips that are rotated inside the uterine cavity and then withdrawn inside the introducer to prevent cervical contamination during removal. After removal, the device was cleaned with gauze to remove cervical cells and the umbrella-shaped tips were exposed and immersed in a fixative solution made out of ethanol, water, EDTA and KCO3. Together with samples, all clinical information was provided to the pathologist (including the reason of the test, endometrial thickness, therapies, physical examination, pregnancies, last menses, sampling conditions). Samples were centrifuged 3000 rpm 10’, the supernatant was filtered through a 5 micron pore membrane, and cells were transferred to slides for Papanicolaou staining (Fig. 2). The pellet was processed as if it were a biopsy and embedded in paraffin. From this block, 4 micron sections were stained with H&E (Fig. 3), PAS and Papanicolaou. Cytological diagnosis was made according to the following four categories: negative, presence of atypical cells, presence of carcinoma cells, inadequate for diagnosis.

81 patients underwent subsequent hysteroscopy or hysterectomy and histology samples were collected. In 46 cases, both brushing samples and biopsies were adequate, and diagnoses were compared to evaluate the sensitivity and specificity of Endoflower® sampling, subdividing diagnoses in 2 major groups: pathological (including atypical hyperplasia, adenocarcinoma and carcinosarcoma) and non-pathological (proliferative, secretive and atrophic endometrium and polyps).

Results

All patients judged the procedure as tolerable. In 3 cases, lipothymia or syncope occurred not requiring intensive care. Complications such as bleeding or infections were not observed either immediately afterwards or later. In 29 cases (15%), the sampling was difficult because of stenosis of the cervical canal: therefore, in 11 cases cervical dilators were used. All samples provided the possibility to perform a micro-biopsy embedded in paraffin. When cytology was suitable for diagnosis, samples were considered as diagnostic even if histology was inadequate.

In 152 cases (78%) the diagnosis was “negative for atypical cells”, in 7 cases was “presence of atypical cells” and in 9 cases: “presence of carcinoma cells” (Tab. II).

In 27 cases (14%) the sample was inadequate. Samples were judged inadequate because of the absence or scarcity of endometrial cells in cytology slides and micro-biopsy histology slides. All inadequate samples were studied clinically and with transvaginal sonography, 8 patients underwent hysteroscopy and biopsy (2 endometrial polyps, 4 normal endometrium, 2 inadequate sample), 3 patients repeated cytology brushing (normal endometrium) and 5 patients had a cervical polyp.
After Endoflower\textsuperscript{®} brushing, 81 patients underwent hysteroscopy and/or hysterectomy and histology sampling. Eight of 30 (27\%) hysteroscopy biopsies were inadequate for diagnosis because of scarcity of endometrial tissues, while the Endoflower\textsuperscript{®} sample of the same patients was adequate and allowed diagnosis of atrophic endometrium, negative for malignancy. Of 6 inadequate brushing samples, 2 were reported in the histology diagnosis as endometrial polyps and 4 as normal endometrium.

When cytology Endoflower\textsuperscript{®} brushing samples and histology samples of hysteroscopy biopsies or hysterectomy were both adequate in the same patients, the diagnoses were compared. The concordance of diagnoses was 93\%; the sensitivity of the cytological procedure was 87\% and the specificity 96\%; the positive predictive value was 92\% and the negative predictive value was 90\%.

Thirteen of 15 cases of malignant endometrial disease were correctly detected in Endoflower\textsuperscript{®} specimens. The 2 false negative diagnoses were a small well-differentiated endometrioid adenocarcinoma in a polyp and a carcinosarcoma with inward growth. The only false positive for malignancy, reported as "presence of atypical cells", was a polyp with reactive/repair changes of the surface epithelium.

**Discussion**

Since abnormal uterine bleeding is seldom due to malignant neoplasias (8\% in postmenopausal patients), invasive procedures should be discouraged as first-line diagnostic tools. The endometrium should be sampled in an outpatient setting with minimally invasive methods, which are well accepted by patient and physicians and do not require anaesthesia\textsuperscript{5}. Transvaginal sonography is well tolerated and has high sensitivity. However, being based on endometrial thickness, it is not of help in type II endometrioid carcinomas that often grow in an atrophic endometrium. In addition, endometrial thickness may be overestimated, as it happens in tamoxifen induced oedema\textsuperscript{6}. Hysteroscopy with biopsy is the gold standard for diagnosis of endometrial diseases in patients with abnormal uterine bleeding or suspect disease after transvaginal sonography\textsuperscript{7}. However, hysteroscopy has some drawbacks. Firstly, it is an invasive test that is not always well tolerated by patients in spite of the use of mini or flexible devices\textsuperscript{8}\. Second, all histology sampling devices have a larger diameter as compared to cytology sampling devices. Moreover, hysteroscopy cannot always be performed in an outpatient setting, which can lead to increased costs. Lastly, in menopausal women the biopsy specimen may be inadequate due to uterine atrophy\textsuperscript{10}\. Endometrial cytology with direct sampling and smear slides is easily performed, well tolerated and relatively...

<table>
<thead>
<tr>
<th>Tab. II. Cytological diagnosis.</th>
</tr>
</thead>
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<td>Negative for atypical cells (normal)</td>
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<td>Presence of atypical cells</td>
</tr>
<tr>
<td>Presence of carcinoma cells</td>
</tr>
<tr>
<td>Inadequate</td>
</tr>
</tbody>
</table>

Fig. 3. Tissue fragments obtained from a cell-block showing normal proliferative endometrium (haematoxylin & eosin, 4X).
inexpensive, but is not commonly used because excess blood or overlapping cells frequently hamper correct diagnosis; in addition, rigid devices do not allow a complete sampling of the uterine cavity leading to many false negative diagnoses. Flexible brushing devices sliding inside an outer sheath allow adequate sampling of the whole uterine cavity including the fundus and tubal corners without contamination by endocervical tissues. In some instances, such as severe atrophy, direct brushing sampling provides adequate samples more often than biopsy. Up to now, flexible brushing devices have been used in conjunction with liquid phase cytology that reduces the number of inadequate samples due to excess of blood and overlapping cells in smears. However, liquid phase cytology is more expensive than traditional cytology, and requires both dedicated instruments and specific training of the cytopathologist.

In our study, 15 different gynaecologists were able to provide adequate samples in an outpatient setting with a well tolerated procedure. Just 12 (7%) patients observed in the same period could not be sampled because of stenosis of the cervical canal. There were relatively few complications, which did not require intensive care. Micro-biopsies embedded in paraffin were obtained in all cases allowing several slides for conventional histology stains and leaving additional sample for ancillary techniques. This procedure is as expensive as a standard biopsy and less expensive than liquid phase cytology. It requires longer time compared to simple cytology, but histology slides are available the day after the sampling. The interpretation of micro-biopsy slides does not require specific training since they are comparable to standard biopsies. When we are dealing with the diagnosis of adenocarcinoma precursor changes and the differential diagnosis of adenocarcinoma versus atypical hyperplasia, cytology diagnostic criteria are not sufficient and histologic criteria are a major diagnostic tool. Cytology slides obtained by membrane filtration were similar to liquid phase slides without overlapping cells as found in smear slides; they provided adequate samples in some cases of severe endometrial atrophy that did not allow adequate micro-inclusion. In fact, even a small number of malignant cells in a cytology slide allows a diagnosis of malignancy. The excess blood was eliminated by means of a haemolytic fixative. With our procedure, just samples with a few or no endometrial cells had to be discarded, and the percentage of inadequate samples (14%) was lower than the frequency of inadequate biopsies reported in the literature with different sampling techniques, ranging from 16% to 76%. A main reason for inadequacy was the presence of endometrial or cervical polyps (6/20 cases) that probably hamper correct brushing. Our data are in agreement with what is reported in the literature concerning the same sampling technique coupled with liquid phase cytology (13% of inadequate samples).

In our study, brushing samples were more often diagnostic compared to biopsies taken during hysteroscopy in the same patients (27% inadequate). The concordance with histology (93%) could be underestimated since in only 46 cases both samples were adequate and comparable. While 13 of 15 malignant cases were correctly diagnosed, in 2 cases the brushing technique failed to collect cells useful for correct diagnosis, since one lesion was inside an endometrial polyp and the other was located deep inside the uterine wall. The only false positive, reported as “presence of atypical cells”, demonstrates the difficulty of differential diagnosis of reactive/repair changes versus malignant changes.

Conclusions

Direct sampling of the uterine cavity using a flexible device with an outer sheath is easy to perform, well tolerated by patients and devoid of major complications. The sample obtained with this procedure allows obtaining cytology slides by means of membrane filtration and micro-biopsies embedded in paraffin. The advantages of cytology, such as adequate sample even in case of severe atrophy, are coupled with the advantages of histology, such as the availability of the structural pattern and some additional sample for further investigations. Membrane filtered cytology slides are comparable to liquid phase cytology slides.

The procedure presented in this study may be a useful diagnostic tool in selected clinical cases: abnormal uterine bleeding, increased endometrial thickness and tamoxifen-treated patients. In addition, it may play a role in tailoring surgery in patients suffering from uterine lesions different from endometrial diseases (e.g., prolapse). In conjunction with transvaginal sonography, this procedure improves diagnostic accuracy by reducing the number of invasive and expensive procedures and hysteroscopies.

References


The high expression of p53 in sporadic colorectal carcinoma is associated with metastasis and decreased survival

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Key words
p53 • Colorectal • Cancer • Carcinoma • Metastasis

Summary

Introduction. Alteration in the p53 tumour suppressor gene is an event that occurs frequently in human cancer, although its role as predictive and/or prognostic marker is still unclear. The aim of this study was to compare the expression profiles of p53 in colorectal carcinoma with clinicopathological features and survival rate at 5 years from diagnosis.

Methods. One hundred and twenty cases of primary sporadic colorectal cancers (CRCs) and 80 matched normal mucosas were analyzed by immunohistochemistry on paraffin-embedded specimens. The correlation between protein expression profiles, clinicopathological parameters and survival was investigated.

Results. In tumour tissues, the expression of p53 was high in 41 cases, low in 38 and negative in 41. A significant correlation was observed between increased p53 expression presence of lymph node (p = 0.002) or liver metastasis (p = 0.008). Moreover, higher levels of p53 were related with advanced tumour stage (III-IV; p = 0.007), poor survival and disease recurrence (p < 0.01). Interestingly, in multivariate analysis p53 expression and distant metastasis were independent prognostic markers.

Discussion. Our results suggest that nuclear p53 accumulation in sporadic CRC may have prognostic significance and contribute to identification of patients at high risk of mortality. The current findings may be relevant for management of patients with CRC.

Introduction

The human TP53 encodes a nuclear protein that plays a central role in cell-cycle arrest, apoptosis, cellular senescence and differentiation in response to endogenous and exogenous stress signals. TP53 mutations are very common and have been described in more than 50% of human cancers. For these reasons, it is recognized as one of the most important tumour suppressor genes. The prevalence of mutations makes p53 an attractive molecular target for cancer therapy. Under normal physiological conditions, p53 is rapidly degraded by ubiquitin-ligases such as MDM2. However, different types of stress such as DNA damage, hypoxia and heat shock can also lead to activation and stabilization of the protein. The activity of p53 can also be deregulated through alterations of upstream or downstream genes including c-myc, ras and some viral proteins. The biological responses activated by p53 are due mainly to its ability to modulate gene expression by binding directly to specific target sequences on DNA (p53-responsive elements). Recent studies have revealed that gain of function mutations of p53 can promote genetic instability and tumour progression, inhibiting important factors involved in DNA repair. Additionally, new genes have been described under the transcriptional control of p53 that reveal a more complex role of the protein in cancer progression. Several studies have shown that p53 overexpression in various malignancies is related to specific clinicopathological parameters and to unfavourable prognosis. However, the clinical significance of TP53 alterations in CRC progression remains controversial.

Immunohistochemistry (IHC) is the most commonly used method to investigate p53 expression, based on the observation that TP53 mutants display increased protein stability and accumulate in the nuclei of tumour cells. In this study, in order to elucidate the role of p53 in sporadic CRC, we investigated its expression levels by IHC.
in a group of 120 patients, using matched normal mucosa as a control. Patients were followed for more than 5 years after initial diagnosis. The expression levels of p53 were correlated with clinical and pathological parameters and survival.

**Material and methods**

**Patients and Tumours**

One hundred and twenty patients who had undergone surgical resection for CRC between 1999-2004 at the Department of Surgery of Fatebenefratelli Hospital (Benevento, Italy) were investigated. The analysis was performed also on 80 samples of matched normal colon mucosa. Informed consent was obtained from all patients. None had a history of hereditary CRC or had received chemotherapy/radiotherapy prior to resection or had taken non-steroidal anti-inflammatory drugs (NSAID) on a regular basis. At the time of diagnosis, samples were graded by a pathologist according to the criteria of the International Union against Cancer (UICC) and the Tumour-Node-Metastasis (TNM) classification system. The mean age of patients was 71.5 ± 14.6 years, and there were 77 males and 43 females. Clinical and pathological characteristics are summarized in (Tab. I). Follow-up was available for all patients, with a median post-operative duration of 56.3 months. Overall length of survival was calculated starting from the date of first surgery. The average survival of patients was 69.05 months (range 11-78 months).

**Immunohistochemistry**

Specimens of tumour tissues and adjacent normal mucosa and were taken during surgery, fixed in 10% formalin and embedded in paraffin. Four μm thick-sections were cut from paraffin-embedded tissue blocks, mounted on poly-lysine coated slides, deparaffinized, rehydrated and treated at 95 °C for 45 min in 10 mM citrate buffer (pH 6.0) to unmask epitopes. Sections were subsequently rinsed with 3% hydrogen peroxide at room temperature to quench endogenous peroxidase activity. The antigen was visualized using the ABC method with an automated instrument from Ventana Medical Systems. The antibody used against human p53 protein was the pre-diluted mouse monoclonal antibody [Ventana Medical Systems (Bp53-11)], which recognizes both mutated and wild-type forms of p53. The antibody was incubated at 37°C for 40 min, and after the reaction with secondary antibody and peroxidase, tissues were stained for 5 min with DAB chromogen and counterstained with haematoxylin, air dried and cover-slipped. In some cases, experiments were performed in duplicate, and a positive control that over-expressed p53 was also used.

**Evaluation of staining**

The evaluation of the score was done using a semiquantitative method based on the intensity of nuclear staining as: (1 = low, 2 = moderate, 3 = intense), and taking into account the percentage of positive cells as: (Score 0: ≤ 1%; Score 1: {1% - 25%}; Score 2: {25% - 50%}; Score 3: {50% - 75%}; Score 4: {75% - 100%}). The final score, classified as negative (0-2), low (3-5), or high (6-7), was obtained by adding the two scores. The assessment was made by two independent pathologists who were blinded to previous results and clinicopathological features. Discruptant cases were re-evaluated and scored by consensus. Microscopic investigation and photographic acquisitions was performed with a Nikon Eclipse E600 light microscope.

**Statistical analysis**

Association between p53 expression levels and clinicopathological parameters was assessed using the

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<tr>
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<tr>
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<td>&gt; 55</td>
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<tr>
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<td>&gt; 3-7 cm</td>
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<td>&gt; 7 cm</td>
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</tr>
<tr>
<td>G1/G2</td>
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<tr>
<td>G3</td>
<td>19</td>
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<tr>
<td>T stage</td>
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<td>T4</td>
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<td>Liver Metastasis</td>
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<td>M1</td>
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</tr>
<tr>
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Proximal: caecum, ascending and transverse colon; Distal: descending and sigmoid colon, rectum.
χ2 test. The direction and the strength of association between the variables were evaluated and compared with the spearman’s rho correlation coefficient, a non-parametric version of Pearson’s correlation coefficient. The Kaplan-Meier method was used to estimate survival; differences between subgroups were analyzed and compared with the log-rank test. Overall length of survival was measured starting from the day of the first surgery. Prognostic factors (clinical and molecular) were examined by univariate and multivariate analyses (Cox proportional hazards model). Data were reported as mean ± SD, and mean values were compared using the Mann-Whitney test. Results were considered statistically significant when a p < 0.05 was obtained. All statistical analyses were carried out with the SPSS (version 15.0) for Windows (SPSS Inc., Chicago) software package.

**Results**

**IMMUNOHISTOCHEMICAL ANALYSIS OF p53**

In adjacent non-neoplastic colonic mucosa, focal nuclear p53 positivity was observed only in 12 cases (15%) (Fig. 1A). In 41 (34.1%) of 120 primary tumours, p53 immunoreactivity was classified as negative (Fig. 1B); 38 cases (31.8%) were considered to have a low expression profile (Fig. 1C), while 41 cases were classified as high (34.1%) (Fig. 1D).

**RELATION BETWEEN p53 EXPRESSION LEVELS AND CLINICOPATHOLOGICAL PARAMETERS**

p53 expression profiles were correlated with clinicopathological characteristics. Interestingly, a significant correlation was found with the presence of both lymph node (p = 0.002) (Fig. 2A) and distant...
correlation between p53 status and colorectal cancer metastasis. Comparison between p53 expression levels and presence/absence of lymph node metastasis (panel A) or liver metastasis (panel B). The p value for each graph is reported. N0: absence of lymph node metastasis; N1-2: presence of lymph node metastasis; M0: absence of distant metastasis; M1: presence of distant metastasis.

**Tab. II.** Correlation between p53 expression levels and relevant clinicopathological parameters.

<table>
<thead>
<tr>
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<th>p</th>
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<td>31</td>
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Proximal: caecum, ascending and transverse colon; Distal: descending and sigmoid colon, rectum; AD: adenocarcinoma; AD-MUC: adenocarcinoma with a mucinous component below 50%; MUC: adenocarcinoma with a mucinous component above 50%; * p < 0.01

metastases (p = 0.008) (Fig. 2B). No significant associations were found with patient age, tumour location, depth of invasion and degree of differentiation (Tab. II). In addition, cases with nuclear p53 accumulation were found to have significant relation with tumour histology and advanced tumour stage (III-IV; p < 0.01). The relationships between relevant clinicopathological features and p53 expression are shown in Table II.

**CORRELATION WITH SURVIVAL**

Finally, the p53 expression levels were correlated with the 5-year survival rate. The median follow-up of all patients was 56.3 ± 20.4 months, whereas the 5-year survival rate, from initial diagnosis, was 75.4% in stage I-II, 69.7% in stage III and 42.6% in stage IV. Overall survival of the cohort was 69.05 months, and cancer-related death occurred in 38 cases (31.6%). When p53 status was correlated with the overall survival (OS) and disease free survival (DFS), we observed a sig-
significant relationship between increased p53 levels and shorter survival compared to groups with negative or low expression (p = 0.004) (Fig. 3A, 3B). Moreover, mean survival was significantly increased for patients with negative or low p53 levels compared to the group with high expression (Tab. III). To evaluate whether the p53 expression levels have prognostic significance, we used a Cox proportional hazards model. The analyses were performed in all patients taking into account both clinical and molecular factors. Univariate analysis for (OS) and (DFS) showed that three prognostic factors (p53 expression, distant metastases and tumour stage) were strongly related to the prognosis (Tab. IV). Interestingly, multivariate analysis revealed that p53 status and distant metastases were independent prognostic factors when adjusted for tumour stage (p < 0.05, Tab. IV).

Tab. III. p53 expression levels and overall survival.

<table>
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<th>Status</th>
<th>n</th>
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</table>

ES: standard error of the mean.

Tab. IV. Univariate and multivariate (Cox proportional hazard analysis) in all CRC-patients.

### Univariate OS

<table>
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### Univariate DFS

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### Adjusted HR OS

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### Adjusted HR DFS

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Ad: adenocarcinoma, Ad/Muc: mucinous adenocarcinoma or adenocarcinoma with a mucinous component below 50%, OS: overall survival, DFS: disease free survival, Diff.: Degree of differentiation, Dist. met: Distant metastasis, dx: proximal location; sx: distal location, HR: Hazard Ratio, 95% CI: 95% Confidence Interval; * p < 0.05; ** p < 0.01.
Discussion

The aim of this study was to investigate p53 expression levels in order to shed light on the role of this marker in sporadic colorectal cancer progression. Despite extensive studies, the clinical significance of p53 expression in CRC is still controversial. TP53 is one of the most frequently mutated tumour suppressor genes in CRC, and many p53 gene mutations result in a more stable protein that accumulates within the nuclei of tumour cells. However, the biological significance of abnormal p53 accumulation in tumour cells is still unclear, as well as the relation between p53 expression in tumours and response to chemotherapy. IHC is one of the most sensitive techniques to investigate the status of p53, based on the concept that genetic changes of TP53 result in accumulation of the corresponding protein in tumours.

In this study, we report that p53 is over-expressed in about 35% of primary tumours analyzed, while it was low or undetectable in the remaining cases. When the expression of p53 was related to clinicopathological characteristics, we found an interesting association. Tumours showing elevated accumulation of p53 in the primary tumours were significantly associated with regional lymph node involvement and the presence of distant metastases. A similar relationship was also observed for tumour stages III and IV.

Two common mutants of p53 frequently observed in human tumours have recently been shown to trigger tumorigenesis, promoting the genetic instability and metastasis formation in a mouse model. These data might help to explain our current findings and support the conclusion that increased levels of p53 contribute to metastatic progression of CRC. Another striking finding of our study was the association between p53 expression and survival. Overall survival was significantly lower for patients with high p53 expression. In contrast, better outcome was observed for patients with low or negative expression. Interestingly, tumours with elevated accumulation of p53 also showed more recurrences and poorer prognosis compared to other groups. Multivariate analysis showed that p53 is an independent prognostic factor.

These results strongly indicate that p53 expression may be a novel marker to predict prognosis and aggressiveness of CRC. Expression of p53 may also provide useful information when selecting the patients for adjuvant radiotherapy. In conclusion, we demonstrate that in sporadic CRCs the expression levels of p53 are closely related to prognosis. The relationship between increased p53 and metastatic progression of CRC merits further investigation.

References

Lymphoepithelioma-like carcinoma of the endometrium

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Key words
Lymphoepithelioma-like • Carcinoma • Endometrium

Summary
Only three cases of lymphoepithelioma-like carcinoma of the endometrium have been reported to date. We present a new case in a 67-year-old woman involving an exophytic mass that caused postmenopausal bleeding. Histologically, undifferentiated carcinomatous areas were intermingled with abundant lymphoid tissue. Epstein-Barr virus was not detected in either neoplastic or lymphoid cells.

Introduction
Lymphoepithelioma (LE) is a poorly differentiated carcinoma of the nasopharynx composed of sheets of large atypical epithelial cells intermingled with a benign inflammatory infiltrate that is rich in lymphocytes and plasma cells. A consistent feature is the association of this type of carcinoma with Epstein-Barr virus (EBV), which is detectable in all tumour cells. Lymphoepithelioma-like carcinomas (LELC) can also originate outside the nasopharynx, but resemble a lymphoepithelioma histologically. Lymphoepithelioma-like carcinomas may be found in almost any epithelial organ, including the lung, thymus, stomach, tonsils, larynx, salivary glands, renal pelvis and urinary bladder, breast, and, in the female genital tract, uterine cervix, vulva, vagina and ovary. LELCs are prevalent in males between 50 and 70 years of age. To the best of our knowledge, only three cases of this unusual cancer occurring in the endometrium have been described to date. Herein, we present the fourth case of endometrial LELC that was not associated with EBV infection.

Case report
A 62-year-old woman was admitted to Siena Hospital for postmenopausal bleeding. Her past medical history did not reveal any major disease, and physical examination was unremarkable. Laboratory data were within normal limits. Colposcopy was normal, and there was no abnormal cervical cytology. Hysteroscopy showed the presence of an exophytic mass and a leiomymatous lesion of the uterus. The patient underwent endometrial biopsy: the diagnosis was that of an “undifferentiated carcinoma”, but complete work-up was negative for distant metastases.

A total hysterectomy was performed with bilateral adnexitomy and iliac lymphadenectomy. Pathological evaluation of the specimen revealed a poorly differentiated carcinoma with a marked inflammatory response, and on the basis of morphological, immunohistochemical and molecular biology findings, a diagnosis was made of EBV-negative, undifferentiated LELC of the endometrium. The patient was staged as stage IB, according to International Federation of Gynaecology and Obstetrics (FIGO) classification. The patient did not receive adjuvant therapy. Eight months after surgery she was free of disease, as confirmed by nuclear magnetic resonance (NMR).

Correspondence
Maria Raffaella Ambrosio, Dipartimento di Patologia Umana e Oncologia, Sezione di Anatomia Patologica, Policlinico Universitario “Le Scotte”, via delle Scotte 6, 53100 Siena (SI), Italy - E-mail: maradot@libero.it
Materials and methods

Representative samples of the surgical specimen were fixed in 10% buffered formalin and embedded in paraffin, according to standard procedures. Tissue sections (4 mm thick) were cut and stained with haematoxylin and eosin. Immunohistochemical stains were performed on other sections of each block employing the Ultravision Detection System anti-Polyvalent HRP (LabVision, Fremont, CA, U.S.A.; Bio-Optica) and using diaminobenzidine (DAB; Dako) as chromogen. The following antibodies were studied: AE1/AE3, CK7, CK20, EMA, vimentin, oestrogen and progesterone receptors, caldesmon, desmin, α-inhibin, CD10, CD117, HMB-45, Melan A, p53, Ki67, D2-40, CD3, CD20, CD79a, CD30, CD45, and LMP (Tab. I). Negative controls were obtained by replacing the specific antibody with non-immune serum immunoglobulins at the same concentration as the primary antibody. Sections were then counterstained with Harris haematoxylin, dehydrated in alcohol, cleared in xylene and cover-slipped.

Cell proliferation was assessed by counting the number of Ki-67-positive tumour cell nuclei in at least 10 high power (x 400) fields.

In situ hybridization was performed on sections from formalin-fixed, paraffin-embedded samples using an oligonucleotide probe for EBV-encoded RNA (EBER-1; PNA Probe/Fluorescein; DakoCytomation Denmark A/S).

Results

Upon gross examination, the uterus measured 7 x 4 x 2.5 cm and weighed 50 g. The endometrial cavity was 6 cm in length and it was filled by a vegetant, exophytic, tan mass that macroscopically extended to less than half the myometrial wall. Furthermore, there was a subserosal leiomyomatous lesion of 1 cm. The cervix and both adnexa were unremarkable.

Wcap: Wax capture antigen retrieval solution; MW: microwave.

<table>
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<th>Antigen Retrieval</th>
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<td>RN7</td>
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<td>CS 1-4</td>
<td>1:25-1:50</td>
<td>Trypsin</td>
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Tab. I. Monoclonar Antibodies
lymphoepithelioma-like carcinoma of the endometrium

by fibrous tissue (Fig. 1b). The carcinomatous areas were characterized by cells with indistinct margins, round to oval vesicular nuclei, prominent nucleoli and scant cytoplasm (Fig. 1b, bottom right). Minute areas of coagulative necrosis were present. Nuclear atypia and several mitotic figures were observed.

In some areas, the lymphoid tissue formed follicular structures with germinal centres. The density of the lymphoid tissue varied from areas with a few lymphocytes and plasma cells to areas where abundant lymphoid cells broke the tumour islands into small groups of cells.

At immunohistochemistry, the carcinomatous cells stained for cytokeratin AE1/AE3 and cytokeratin 7 (Fig. 1c), vimentin, EMA and p53, while they were negative for CD10, α-inhibin, desmin, caldesmon, HMB-45, CD117, Melan-A, CD45, and LMP. The tumour was positive for both oestrogen (Fig. 1d) and progesterone receptor. The proliferation index (Mib-1) was about 30%. The lymphoid tissue contained a mixture of T cells (CD3 positive) and B cells (CD20 positive), with numerous CD30 positive blasts.

The cervix was not involved by the tumour; both adnexa were atrophic without microscopic evidence of neoplasia. There was no lymph node metastasis.

In-situ hybridization for EBV was negative in both the carcinomatous and lymphoid cells.

Discussion

The term lymphoepithelioma was introduced in 1921 to refer to an undifferentiated carcinoma of the nasopharynx with a dense lymphocytic component. It is characterized by nests of undifferentiated epithelial cells with indistinct cell borders, large, vesicular nuclei with evident nucleoli, moderate atypia and a synecytial growth pattern, and infiltrated by a prominent benign reactive lymphocytic infiltrate. Epstein-Barr virus (EBV)
is frequently present in the malignant epithelial cells of this carcinoma.

LELC is a subtype of poorly differentiated squamous cell carcinoma, identical to the LE described in the nasopharynx but originating in other anatomic sites, including thymus, tonsils, larynx, lung, stomach, salivary glands, breast, skin, and thyroid gland. In the female genital tract, LELC has been described in the uterine cervix, vulva, vagina, and ovary. To the best of our knowledge, only three cases of this unusual cancer have previously been described in the endometrium.

The relationship between EBV and LELC in general is controversial. Although EBV-positive and EBV-negative LELC have been observed in a range of anatomical locations, some LELC at specific anatomic sites have never been proven to be associated with EBV. There is no satisfactory explanation for EBV being commonly present in certain anatomic sites, but not in others. Due to the restricted anatomic distribution of EBV, it was believed in the past that only foregut-derived organs (salivary gland, stomach, thymus, and lung) were susceptible to EBV-associated carcinogenesis, perhaps because these organs are in close proximity to sites of natural viral replication. The portal of entry of the virus is thought to be the oropharyngeal mucosa, which also serves as the site of production of the virus, which is periodically shed in the saliva. The infection of lymphocytes permits systemic dissemination, and following primary infection EBV lies latent in a few lymphocytes for the duration of life. However, EBV is associated with LELC in a wide range of locations that do not fit with any anatomic or embryologic derivation. According to other authors, EBV-associated LELC might be related to racial or geographic influences rather than to the anatomic location of the tumour. In conclusion, the absence of the EBV genome in most LELC cases implies that EBV is not a necessary factor in the aetiology or pathogenesis of LELC, and that the degree of racial or geographic influence on the association of EBV with lymphoepithelioma-like carcinoma varies according to the anatomic location.

In the case described herein, the differential diagnosis was made with an endometrioid or serous type carcinoma of the uterus, as well as stromal sarcoma, carcinosarcoma, and metastatic tumours, including lymphoepithelioma, melanoma, and lymphoma. The absence of glands and papillae, the cytologic features and the rich lymphoid stroma excluded the diagnosis of primitive endometrial carcinoma. Negativity for CD10, α-inhibin, desmin, caldesmon, and CD117, ruled out pure mesenchymal and mixed Mullerian tumours.

A diagnosis of melanoma was ruled out due to Melan-A and HMB-45 negativity. AE1/AE3 and CK7 and CK20 stains, as well as the immunophenotype of the lymphoid cells, were helpful in supporting the epithelial nature of the lesion. Furthermore, positivity for vimentin and oestrogen and progesterone receptor confirmed the uterine origin of the neoplasia, excluding the possibility of a secondary lymphoepithelioma.

When stratified according to stage, LELC has a favourable prognosis with a low frequency of regional lymph node metastasis, in contrast to nasopharyngeal LE. This is probably due to the presence of prominent lymphoid tissue, which may represent a host response against the tumour, and to its biological characteristics, including responsiveness to chemotherapy. However, the limited number of endometrial LELC makes it very difficult to assess their prognosis. Vargas and Merino reported two of these tumours, both in postmenopausal women. One patient presented with stage IVb (involvement of endocervical canal, left adnexa, lymph nodes, mesocolon and sigmoid colon serosa), and was treated with chemotherapy. The patient was alive with no evidence of tumour 9 months later. The other patient presented with stage IIIc, received radiation and chemotherapy and died of disease 1 year after diagnosis. Rahimi reported a case of a patient with a stage Ib tumour who did not receive chemotherapy after surgery and was alive one year after diagnosis. The present case was FIGO stage IB, and the patient was well eight months after surgery.

We did not find any association with EBV infection: LMP was negative and PCR did not detect EBV sequences in either neoplastic or lymphoid cells. This is in agreement with the other cases of endometrial LELC described so far and with what is known about LELCs of non-foregut-derived organs and LELCs in non-Asian patients.

On the basis of these findings, further studies are necessary to understand why these tumours, which have the same morphology as LE, often have a different prognosis and show a variable presence of the virus. In fact, the biologic significance of the finding of EBV in LELC is unclear. The prognostic importance of EBV also remains unknown because of the relatively small numbers of cases and short follow-up in different reports. An analysis of additional cases, including long-term follow-up, would be necessary in order to gain insight into the biological behaviour of this rare tumour.
References


Extranodal Rosai-Dorfman disease of bone and nose: a case report and review of literature

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ENT Unit, Neuroscience Department of the University of Pisa, Italy; *U.O. Anatomia Patologica II, Azienda Ospedaliero Universitaria Pisa, Italy

Summary

Rosai-Dorfman disease (RDD) is a rare benign condition of unknown origin, which was first described in 1969. By histopathology, the disease is composed of sinusoidal lymph node hyperplasia and abundant histiocytes with haemophagocytosis, particularly lymphocytes. It commonly affects lymph nodes, and rarely has an exclusively extra-nodal clinical presentation. Among the so-called “extranodal” sites, the head and neck region, and in particular the nose and paranasal sinuses, are frequently affected. RDD shows a highly variable clinical course that can be partly modified by medical therapy. We present of a case of extra-nodal RDD, with nasal and osseous involvement, which has been followed-up for 19 years. We also discuss its presentation, the most relevant radiographic findings, treatment options and histological findings.

Introduction

Sinus histiocytosis with massive lymphadenopathy (SHML) was initially described by Rosai and Dorfman in 1969. It is a rare histiocytic proliferative disorder classified as an idiopathic histiocytosis. Although the most frequent clinical manifestation of Rosai-Dorfman disease (RDD) is bilateral cervical lymphadenopathy, the disease may occur in the absence of detectable nodal involvement in 25-43% of cases. Low-grade fever, leukocytosis, elevated erythrocyte sedimentation rate (ESR) and polyclonal hypergammaglobulinemia are often present. Despite infective and/or immunological theories, the cause and pathogenesis of this disease remain unclear.

Foucar et al. analyzed 423 cases collected from world literature, 48 of which presented nasal cavity and/or paranasal sinus (rhinosinusal) involvement, and 42 presented two different sites of extranodal involvement. After this report, an additional cases of RDD involving the rhinosinusal area without cervical adenopathy have been published.

Here the authors present an additional case of RDD with a 19-year-follow up that had extranodal disease primarily involving a fibula and secondarily the nasal cavities, without cervical or other nodal lymphadenopathy, and discuss its evolution and therapy.

Clinical history

In February 1990, a 26-year-old woman presented with a painful swelling of the left medial malleus. A radiograph showed a well defined lytic lesion of distal left tibia, and technetium scan revealed an increased uptake in the same area. This lesion was curetted in April 1990 with an initial pathological diagnosis of “eosinophilic granuloma”. After intervention, she continued to feel occasional pain in the same site. In 1991, she began to complain of nasal obstruction with occasional bleeding. An otolaryngologist attempted to remove a small polypoid lesion from the right nasal fossa, but had to interrupt the operation due to serious bleeding. Later on, anosmia and bilateral nasal obstruction progressively appeared, and she was referred to our centre in 1992. ENT showed bilateral nasal obstruction resulting from diffuse submucosal thickening mainly involving the right nasal cavity; nasal mucosa appeared thin and bled easily. Neither lymphadenopathy nor other lesions were present in the head and neck area.
A CT scan revealed a solitary soft tissue-like nasal mass involving the nasal septum and the anterior portion of superior and middle turbinates bilaterally. The paranasal cavities appeared normal. Preoperative routine blood tests were normal.

In April 1992, the patient underwent a transnasal resection of the tumour that appeared to involve the nasal cartilage and the anterior edge of the perpendicular plate of the ethmoid bone. The post-operative course was uneventful. Histopathological analysis revealed a histiocytosis with a diffuse cytoplasmic positivity for S-100 protein. These findings were consistent with the diagnosis of Rosai-Dorfman disease (RDD) (Fig. 1).

Histopathological samples of the tibial lesion were re-reviewed and found compatible with a second localisation of an extranodal RDD.

The presence of an associated immune-mediated disease was then evaluated. General post-operative laboratory examinations did not reveal anaemia. The peripheral lymphocyte cell count was lowered (11.10%) and the neutrophil count elevated (81.70%). Abnormalities in serum proteins were detected: albumin and gamma-globulin were normal (57.3%), while alpha2-globulin (12.3%) and beta-globulin (12.2%) were elevated. Peripheral lymphocytes and serum protein levels were normal in following controls. Immunoelectrophoresis showed normal levels of IgM, IgG and IgA. ESR was normal. Anti-nuclear antibodies and ENA were absent. A RA-test was negative and CH-50 was normal. CD4 and CD8 counts were normal as was the CD4/CD8 ratio. A gallium scan showed a light uptake at the level of the floor of the nasal cavities and a wide area of uptake at the level of the medial aspect of the left distal tibia. (Fig. 2) A technetium scan confirmed this result and the scintigram appeared similar to the one performed before intervention on the tibia, two years earlier.

During the following two years, the patient complained of irregular pain and slight swelling of the operated leg, with worsening of nasal function as in case of rhinitis. Corticosteroid aerosol or spray was sufficient to restore normal functions and stop symptoms. These two different phenomena did not necessarily appear simultaneously. Physical examination revealed a progressively thickening of the residual ethmoidal perpendicular lamina and of the nasal spine. Nasal fossa patency was not significantly decreased by these two phenomena together, but only in the case of rhinitis. The patient discontinuously assumed oral steroids (methylprednisolone 16 mg per day, tapered on a monthly-base) until December 1995, when she decided to stop therapy definitively. Nasal and leg symptoms did not appear again. Physical examination did not reveal either nasal lesion progression or tibial swelling or pain.

The patient was regularly followed-up on an out-patient basis; she refused further imaging exams (e.g. scintigraphy) as she expressed desire to have children. Later,
she accepted to undergo a PET during her last control in March 2009 (Figs. 3, 4). Nineteen years after the first clinical observation, the disease is stabilized and the patient is clinically free-of-disease.

Discussion

Sinus histiocytosis with massive lymphadenopathy (SHML), also known as Rosai-Dorfman Disease (RDD), is a rare pathological disorder characterized morphologically by sinusoidal lymph node hyperplasia and abundant histiocytes with haemophagocytosis, particularly lymphocytes.

Characteristically, the most common clinical manifestation of the disease is a painless, massive, bilateral cervical lymphadenopathy, and it can be frequently associated with leukocytosis, elevated ESR, weight loss, hyperhidrosis, reversal of the T4/T8 ratio of circulating lymphocytes and polyclonal hypergammaglobulinemia. Sometimes the disease affects other organs, and it is seldom exclusively limited to the so-called extranodal sites.

The cause of the disease remains unknown, but an association with immune disorders and infectious agents has been documented; other infectious aetiologies such as the Epstein-Barr virus, cytomegalovirus and human herpes virus have been described in association with SHML, but were not demonstrated in our patient.

RDD is a relatively rare disease with 423 cases described in literature until 1990, when Foucar, Rosai and Dorfman made an extensive review of the literature; it mainly involves young people in their first decades of life, and males are slightly more often affected than females.

In the Foucar series, RDD involving bone without lymphadenopathy was found to be exceptionally rare, accounting for approximately 2% of cases. RDD without nodal involvement that affect the skeleton and other sites, as our case, is as uncommon as the exclusively skeletal form. In addition to the cases cited in the Foucar registry, other reports of solitary as well as multiple osseous lesions exist. The skeletal lesions involve long bones, skull, vertebrae, ribs, pelvis, phalanges and metacarpals, are typically osteolytic, and...
only rarely osteoblastic. The lesions are usually ill-defined with non-sclerotic borders; periosteal reaction and central calcification are not seen. Imaging techniques must be used to follow the progression of disease, and may reveal a gradual decrease in size and disappearance of the lesions. In the case of osseous RDD, bone metabolic imaging studies (such as scintigraphy with hydroxymethylene diphosphonate (HMDP), gallium CT scan, technetium CT scan) are mandatory in order to exclude additional localizations.

As far as the head and neck region is concerned, Foucar found that it was involved by disease in 22% of cases, with a strong predilection for the nasal cavity and paranasal sinuses. In 1993, Wenig et al. analyzed 14 cases of RDD of the head and neck region; 5 were located in the nasal cavity and 3 in the paranasal sinuses, where the disease was responsible for local symptoms, like nasal respiratory obstruction, anosmia and rhinorrhea, as in our case. All patients in the Wenig series underwent radical surgical excision, with control of disease in 3 cases. Gregor and Ninin reported a case of extranodal Rosai-Dorfman disease involving both the ethmoid sinuses infiltrating the cribiform plates and the floor of the anterior cranial fossa treated by craniofacial resection with no recurrence after 2 years. Goodnight et al. reported a case of Rosai-Dorfman disease involving the nasal cavity treated by endoscopic resection with no recurrence up to one year after intervention. Ku reported 2 cases of endoscopically-treated nasal RDD, with partial removal of the lesions and improvement of patient-reported symptoms.

Essentially, Rosai Dorfman disease is a histological diagnosis rather than a clinical one and, therefore, is seldom included in the clinical differential diagnosis. Microscopically, there is a polymorphous population of mature lymphocytes, plasma cells and numerous histiocytes with abundant pale to vacuolated cytoplasm and a large nucleus with prominent nucleoli. Many of these cells showed intact lymphocytes within their cytoplasm, a feature designated as lymphohagocytosis or emperipolesis (Fig. 1). There were small lymphoid aggregates and some plasma cells showing Russell bodies. There were no granulomas and no evidence of micro-organisms. Immunohistochemically, histiocytes were shown to be strongly reactive for S-100 protein. Immunophenotyping revealed a polyclonal population of plasma and lymphocytes. The histopathologic features of Rosai-Dorfman in extranodal sites are similar to the nodal disease except for the fact that fibrosis is more pronounced. The most striking histological feature of this condition is the presence of large aggregates of reactive plasma cells and lymphocytes alternating with pale areas composed of histiocytes. Immunohistochemical stains reveal histiocytic positivity for S100 protein, as in our case (Fig. 1).

From a histological point of view, differential diagnosis includes other infectious granulomatous diseases, sarcoidosis, Wegener’s granulomatosis, midline malignant granuloma, Langerhans cell histiocytosis, Hodgkin’s disease and fibro-inflammatory lesions. Radiographically, the disease must be differentiated from histiocytosis X, metastatic malignancy (including neuroblastoma), sarcoidosis, lipid storage disorders (Gaucher) and neurofibromatosis.

From a therapeutic point of view, there is no ideal protocol for treating Rosai-Dorfman disease as it is uncommon, the disease is self-limiting and seldom life threatening, rendering therapy unnecessary in most cases: in fact, the clinical course is quite variable, with alternating episodes of worsening or relief of symptoms. However, the outcome is usually good, with spontaneous resolution of the disease in about 50% of cases. Therapy becomes necessary in symptomatic or life-threatening lesions, even though a specific protocol does not exist. Surgery, radiotherapy and chemotherapy have all been advocated in RDD, but none are reported to consistently achieve sustained remission. Chemotherapy for Rosai-Dorfman disease includes corticosteroids, cytotoxic agents or a combination of both. Some responsiveness to cycles of chemotherapy similar to that commonly employed for low-grade lymphomas (cyclophosphamide, vincristine, methylprednisolone) has been reported. On the other hand, aggressive systemic therapy would appear to have no place in the treatment of localized disease. Radiotherapy has limited effects on the course of the disease, and should be used only in cases of orbital or laryngeal location. Radical surgery is not necessary for the disease, which invariably runs a benign course.

Conclusions

RDD is a rare disease that is challenging to treat, with an unpredictable and variable course. Diagnosis is not straightforward and is mainly histological; on occasion, as in our case, revision of histological specimens and adequate immunohistochemistry are mandatory to confirm suspicion of RDD, especially in the presence of granulomatous lesions. After diagnosis, total body scintigrams with gallium and technetium and/or PET must be performed to exclude other localizations. Therapy must be balanced knowing that RDD is a benign condition that can spontaneously regress. Medical therapy with corticosteroids is considered the gold-standard therapy for RDD, while surgery has a palliative role; steroid therapy, however, is not yet standardized.

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Sclerosing angiomatoid nodular transformation of the spleen associated with thrombocytopenia

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Key words
Spleen • Sclerosing angiomatoid nodular transformation of the spleen • Hamartoma • Hemangioma • Angiomatoid nodule • Thrombocytopenia

Summary
Sclerosing angiomatoid nodular transformation of the spleen, a recently described lesion of unknown pathogenesis, with a benign clinical course that is very often asymptomatic. Sclerosing angiomatoid nodular transformation may be a novo lesion or the final common pathway of various benign splenic conditions such as hamartoma, inflammatory pseudotumor and hemangioma. We report the case of a 68 year-old woman with thrombocytopenia and a splenic mass, diagnosed as sclerosing angiomatoid nodular transformation.

Introduction
The spleen is formed histologically of white pulp and red pulp. Tumours of the red pulp have a vascular origin, and reflect the variability of vascular structures of the normal spleen. Haemangiomas and lymphangiomomas are the most common benign tumours, whilst haemangiosarcoma is the most common malignant tumour. Sclerosing angiomatoid nodular transformation (SANT) is a rarely encountered benign lesion of the spleen, which must be distinguished from other angiomatoid tumours and tumour-like lesions.

Case report
A 68 year-old woman with Alzheimer's disease in treatment with rivastigmin and citalopram, was admitted to the emergency room of the “S. Chiara” Hospital of Trento, Italy for non-traumatic haematoma and epistaxis. At physical examination, she presented good general conditions, with petechiae in the oral mucosa and tongue, purpura in the arms and legs and bruises on her back. The spleen was not palpable. Laboratory studies revealed the following values: white blood cell 9.620/mm³, haemoglobin 12.9 g/dL, haematocrit 41% and platelet count 25,000/ mm³. Antibodies against Helicobacter pyloris 221u/ml and core virus hepatitis B were present; erythrocyte sedimentation rate was 78 mm/h.

Computed tomography (CT) of the abdomen revealed a mass in the spleen measuring 6.5 cm in its greatest dimension (Fig. 1) and an intra-abdominal lymphadenopathy of approximately 7 cm (Fig. 2). A bone marrow aspirate and biopsy were normal. The patient was treated with prednisone and infusion of immunoglobulin in high doses, showing rapid normalization of thrombocytopenia.

The clinical diagnosis was thrombocytopenic autoimmune disease and possible lymphoproliferative disease. Two months later a splenectomy was performed. On gross examination of the surgical specimen, the spleen was fragmented, had a total weight of 50 gm and the size of the fragments was 10 x 8 x 5.5 cm. The largest fragment had a nodular whitish tumour measuring 6.5 x 6 x 5.5 cm with multiple brownish-yellowish nodules (Fig. 3).

Microscopically, the tumour consisted of multiple angiomatoid nodules of various sizes in an extensive fibrosclerotic stroma with occasional dystrophic...
calcifications (Fig. 4). The nodules were round and sometimes convoluted. They were composed of irregular-shaped or slightly dilated vascular spaces lined by plump endothelial cells and extravasated erythrocytes and haemosiderin. The angiomatoid nodules were surrounded by onion-shaped fibrosis with the absence of many elongated fibroblast-like cells. Immunohistochemically, the vascular areas showed a complex mixture of endothelial cells with a phenotype resembling splenic sinusoids (CD34-/CD31+/CD8+) (Figs. 5, 6), capillaries (CD34+/CD31+/CD8-) (Fig. 7), and small veins (CD34-/CD31+/CD8-). The lining cells were focally positive for CD 68 (Fig. 8). The lesion was diagnosed as SANT.

Discussion

SANT is a rare, benign lesion of the spleen that has distinctive histopathological and tomographic characterics, but its actual pathogenesis is still unknown. The first case was described by Rosai in his textbook, under the name of multinodular haemangioma, reporting its histological description and the benign clinical course of the disease. In 2004, Martel et al, reported a series of 25 similar cases as a new entity, under the descriptive term of sclerosing angiomatoid nodular transformation of the spleen, whose histological aspect is different from other splenic lesions such as hemangiomas, hematomas and angiomatos. Subsequently, other cases have been reported, recognizing SANT as a specific entity.

There are several hypotheses regarding its origin. SANT could represent a curious nodular transformation in
response to an exaggerated non-neoplastic stromal proliferation. Alternatively it could be a form of splenic hamartoma or a variant of an inflammatory pseudotumor.

Most cases of SANT are asymptomatic, but a minority are associated with different diseases. Martel et al. mentioned a case with pancytopenia and raised erythrocyte sedimentation rate (ESR), and another case with anaemia. The first case reported in Italy showed a raised ESR, as in the present case.

There are rare reported cases of hamartomas associated with thrombocytopenia. One possible explanation may be that the lesional tissue derived from the red pulp acquires augmented phagocytic function (hypersplenisism). Angiomatoids nodules of SANT have immunohistochemical features similar to splenic red pulp, and it is tempting to speculate that they could acquire an increased phagocytic role and produce thrombocytopenia.

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Fibrous hamartoma of infancy of the labium majus: a typical lesion in an unusual site

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Key words
Fibrous hamartoma • Infancy • Labium majus • Ultrasonography • Differential diagnosis

Summary
Fibrous hamartoma of infancy is a soft tissue subdermal fibromatous tumour that characteristically occurs in the first years of life. It is histologically composed of three different components that are intimately admixed: well-defined bundles of fibro-myofibroblastic spindle-shaped cells, nodular proliferations of immature-looking mesenchymal cells set in a myxoid stroma, and mature adipose tissue. A wide intralesional and interlesional cellular composition is commonly observed. Fibrous hamartoma of infancy usually arises from subcutaneous tissue of the trunk, axilla, upper extremities and inguinal region. Only rarely has fibrous hamartoma of infancy been reported in genital organs, with only one case described in the labium majus. We report a rare case of fibrous hamartoma of infancy in the labium majus of a 1-year old female child. Ultrasonography revealed the presence of a mass-like lesion involving subcutaneous tissue, with ill-defined margins. We emphasize that fibrous hamartoma of infancy should be included in the differential diagnosis of soft tissue tumour-like and tumour lesions of the vulva in children. Awareness that fibrous hamartoma of infancy occurs at this site with irregular margins is important to avoid confusion with other lesions exhibiting a more aggressive behaviour.

Introduction
Fibrous hamartoma of infancy (FHI) is a rare benign soft tissue tumour, first described by Reye et al. in 1956 as a 'subdermal fibromatous tumour of infancy' 1. In 1965, Enziger 2 reviewed 30 cases of similar lesions for which the term “fibrous hamartoma of infancy” was proposed. This lesion usually develops in the first 2 years of life, with about 20% of cases detected at birth. There is a slight male predominance (ratio 2:1), without familial or syndromal association 1-3. Although FHI is nearly always solitary, multiple lesions can be encountered 4. FHI is a benign lesion that is cured by surgical excision, even if it may recur locally (15% of cases), especially following an incomplete resection 4. The recurrent cases are generally of non-destructive type and can be surgically re-excised accordingly 3. The main morphological criterion for diagnosis of FHI 3 is a variable mixture of the following components: i) intersecting fascicles of well oriented spindle-shaped cells with the features of fibro-myofibroblasts embedded in a variably fibrous stroma; ii) round to ovoid nests of undifferentiated/immature round to spindle to stellate cells set in myxoid stroma; iii) variable amounts of interspersed islands of mature adipose tissue. Notably, there is a considerable intralesional and interlesional cellular composition, and reported cases have a predominant fatty or fibrous component that can be reminiscent of lipoma or neurofibroma, respectively 3. Although there is agreement among various authors on the above-mentioned diagnostic criteria, the nature (reactive versus tumoural) of FHI is still matter of debate 5. However, the detection of chromosomal abnormalities, in two cases of FHI, strongly suggests the neoplastic nature of the lesion 5,7. In fact, two different translocations, a complex translocation involving chromosomes 6, 8, and 12, namely, t(6;12;8)(q25;q24.3;q13), and a reciprocal translocation, t(2;3)(q31;q21) reported in FHI have also been detected in other benign soft tissue tumours, such as tendon sheath fibroma and desmoplastic fibroblastoma 6,7. FHI characteristically arises from the subcutaneous tissue, especially in the trunk, axilla, upper extremities and inguinal region 5. Less frequently it may occur in the head and neck 8-9, hands and feet 10-11, ventricle and interventricular septum 12, and external genitalia 13-14.
To the best of our knowledge, only one case of FHI has been previously reported in the labium majus of a 6-year-old girl. We herein report a rare case of FHI occurring in the labium majus of a 1-year old female child. Awareness of this unusual clinical presentation for such a relatively common lesion in infancy is important to avoid preoperative misdiagnosis of a more aggressive lesion.

Case report

A 1-year old female child was presented to the Department of Paediatric Surgery because the parents noticed a slight asymmetry of the labia majora. Physical examination revealed a painless, ill-defined mass of the right labium majus. An ultrasound examination showed that subcutaneous tissue of the labium majus had an ill-defined mass-like appearance (measuring 3 cm in its greatest dimension) with a hyperechoic non-homogeneous pattern (Fig. 1). Due to the suspicion of a soft tissue neoplasm, the mass was surgically excised. Intraoperatively, the surgeon described a poorly circumscribed fibro-fatty mass and underlined the difficulties in defining its extension from the normally surrounding tissue.

Material and methods

The surgical sample was fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4 μm. Standard stains, including haematoxylin and eosin, as well as immunohistochemical analysis were performed. Immunohistochemical studies were performed with the labelled streptavidin–biotin peroxidase detection system using the Ventana automated immunostainer (Ventana Medical Systems, Tucson, AZ). The following antibodies were used: alpha-smooth muscle actin (Dako), desmin (Dako), vimentin (Dako), CD34 (Dako) and S100 protein (Dako).

Pathological findings

Grossly, the lesion measured 3 x 2.5 x 1 cm. The cut section revealed an ill-defined fibro-adipose tissue (Fig. 2). Histological examination revealed the typical features of FHI, namely, an infiltrating tumour-like lesion composed of a variable admixture of: i) interlacing, long fascicles of spindle-shaped cells set in a moderate fibrous stroma (Fig. 3); ii) scattered myxoid nodular areas of immature round, stellate and short spindle cells (Figs. 3-5); iii) mature adipose tissue variably intermixed with the above mentioned fascicles (Figs. 3, 4). A lymphocytic inflammatory infiltrate was focally seen. Immunohistochemically, the spindle-shaped cells of the interlacing fascicles were stained with vimentin and
α-smooth muscle actin, whereas the undifferentiated-looking cells of the nodular myxoid component were reactive exclusively to vimentin. Based on morphological and immunohistochemical features, a diagnosis of FHI was rendered.

Discussion

Only rarely FHI has been reported in external genital organs, with only one case reported to date in the labium majus of a 6-year-old girl. We herein present a case of FHI in a 1-year-old child who presented with an ill-defined subcutaneous mass in the right labium majus. Ultrasonography confirmed the presence of a tumour-like mass with irregular borders. Preoperatively differential diagnosis mainly included soft tissue tumours (lipomatous, vascular, fibro-myofibroblastic tumours), reactive pseudosarcomatous lesions (nodular fasciitis), and prepuberal vulvar fibroma, which is likely the same entity that Vargas et al. called “childhood asymmetric labium majus enlargement (CALME)”, a physiological expansion of normal indigenous vulvar soft tissues in response to hormonal surges of pre- and early puberty.

Unfortunately, clinical and imaging features are not reliable in distinguishing the above mentioned entities so that histological examination is needed. In our case, the diagnosis of FHI was histologically based. Differential diagnosis mainly revolved around lipofibromatosis and infantile fibrosarcoma. The former, considered by some authors as a variant of FHI, lacks, however, the primitive-mesenchymal cells that are always detectable in FHI. Although infantile fibrosarcoma may contain immature mesenchymal cells infiltrating fat, the fascicles of fibrous tissue with well oriented mature spindle cells are absent. Lastly, mitoses are easily seen in infantile fibrosarcoma, while they are usually absent in FHI.

In conclusion, the present case suggests that FHI should be included in the differential diagnosis of lesions involving the labia majora in children. Notably, it should be kept in mind that FHI has ill-defined margins, in order to avoid preoperative confusion with more aggressive lesions.

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Critical issues in Pathology

When histologic diagnosis of pulmonary adenocarcinoma becomes difficult

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Key words
Adenocarcinoma • Lung • Histology • Frozen section • Biopsy

Summary
The differential diagnosis between pulmonary adenocarcinoma and several benign mimics can be a formidable challenge for the surgical pathologist, particularly in frozen sections and in small biopsies but sometimes in surgical specimens as well. In this review we will provide a practical guide to help the pathologist facing these problematic cases.

Adenocarcinoma is the most frequent histotype of lung cancer. In the last few years, much effort has been devoted to its morphologic subtyping 1-13, to the histologic and immunohistochemical distinction with squamous cell and neuroendocrine carcinomas (particularly in poorly differentiated forms and in small biopsies) 14 15, and to its molecular characterization 16. These efforts are noteworthy as they can have a significant impact on patient care by providing a framework on which personalized treatment can be based. However, all these studies rely on the correct identification of malignant epithelial proliferation by the pathologist, a straightforward task in the majority of the cases but not in all: indeed, differential diagnosis between adenocarcinoma and some reactive/benign conditions can be one of the most challenging aspects of pulmonary pathology, particularly in frozen sections and in small biopsies but in surgical specimens as well. The main reasons for this can be attributed to the following:

• some adenocarcinomas are so bland that they are difficult to recognize as malignant;
• the number of neoplastic cells can be low;
• inflammation and fibrosis can obscure the tumour;
• some benign conditions can be so atypical as to closely mimic a carcinoma.

Quite surprisingly, only a relatively few number of articles 17-22 and textbooks 23-25 address this topic. Our goal is to provide a concise, practical guide to help the pathologist facing these problematic cases. While the following pages are focused on histology, it should be noted that on many occasions the contemporaneous evaluation of cytologic specimens can be very informative.

In difficult cases, differential diagnosis between pulmonary adenocarcinoma and its mimics should be based on a constellation of findings, and no single criteria is diagnostic in itself. It is useful for the pathologist to methodically balance the data in favour and against a diagnosis of adenocarcinoma: if any doubt persists, a firm diagnosis of malignancy should be avoided.

As a first step, it is important to consider the clinical and radiological scenario because it can favour either a reactive or a neoplastic process. If a carcinoma is clinically unlikely (for example, if the clinical presentation is acute and/or the lesion is radiologically diffuse and not a nodule), the pathologist should be very cautious and consider the possibility of a reactive simulator before making a diagnosis of malignancy. When available, the examination of the macroscopic specimen can also be helpful, particularly in frozen sections.

The histologic recognition of a difficult-to-diagnose adenocarcinoma begins at low magnification. Frequently, pulmonary adenocarcinoma stands out because of its architectural complexity: the neoplastic glands tend to be crowded and irregularly angulated/ramified, in contrast with most benign conditions in which the bronchioloalveolar spaces are generally more uniform and rounded. Cellular buds, papillae and cribriform structures are other architectural features...
that are very unusual in reactive conditions, and their presence favours adenocarcinoma.

When viewed at higher magnification, the cells of well differentiated adenocarcinoma generally display some degree of atypia, consisting of irregular nuclear contours with open or hyperchromatic chromatin and a high nuclear cytoplasmic ratio. Cytologic atypia can be mild and is better appreciated if the suspicious glands are compared with nearby benign bronchioles. The neoplastic cells are generally columnar and crowded, with overlapping nuclei and atypia that is quite uniform. In contrast, reactive pneumocytes are generally flat to cuboidal and less crowded, with fewer cells lining the alveolar spaces; their atypia can be striking but varies from cell to cell, with large bizarre pneumocytes arranged side-by-side with bland flattened cells. A monotonous proliferation of columnar crowded cells is the clue to differentiate adenocarcinoma from reactive pneumocytes.

In peribronchiolar metaplasia and honeycombing, the proliferating cells are bland and some are ciliated: in the lung, cilia are absent in adenocarcinoma and for practical purposes their presence in an epithelial proliferation is diagnostic of a benign lesion.

The degree of atypia is particularly minimal in mucinous bronchioloalveolar carcinoma, however the simple presence of a row of mucinous cells on normal alveolar walls, without intercalated ciliated cells, is diagnostic of malignancy, no matter how bland these cells are. Extracellular mucin pooling the alveoli can be a clue to search for neoplastic mucinous cells, which can be very limited in number.

In non-mucinous bronchioloalveolar carcinoma, cytologic atypia tends to fade toward the periphery, and in evaluating these lesions one has to concentrate on the most atypical regions, which are generally near the centre of the nodule. A sharp demarcation from surrounding benign pneumocytes is another criteria favouring adenocarcinoma, since in benign conditions the cells tend to blend into the adjacent epithelium. Intranuclear inclusions and intracytoplasmic Mallory-like material can be present both in reactive and neoplastic pneumocytes, although when intranuclear inclusions are numerous they favour adenocarcinoma.

The evaluation of the histologic background is equally important. In the presence of an acute lung injury with fibrin, hyaline membranes and organizing pneumonia, atypical pneumocytes are more likely to be reactive. One has to be very cautious before making a diagnosis of malignancy in these cases: the quantity of atypical cells and the degree of architectural and cytologic atypia must be disproportionate to the acute background before rendering an unequivocal diagnosis of adenocarcinoma. An acute background over-shadowing the atypical cells favours a reactive process, whereas the extension of atypical cells beyond the area of acute lung injury favours adenocarcinoma.

Occasionally, adenocarcinomas are associated with extensive inflammation and/or fibrosis, which can obscure neoplastic cells. In difficult cases, deeper high-quality haematoxylin-eosin staining and some immunohistochemical stains can be helpful. p63 or cytokeratin 5/6 can assist in distinguishing adenocarcinoma from peribronchiolar metaplasia/honeycombing because they highlight a rim of basal cells, present in the latter but absent in the former (and also absent in reactive pneumocytes). Some primary adenocarcinomas of the lung, namely...
When histologic diagnosis of pulmonary adenocarcinoma becomes difficult

Fig. 1. Four different examples of pulmonary adenocarcinomas composed of bland cells. The key to recognize these cases as malignant is the identification of a population of cells that is mildly but uniformly atypical, columnar and crowded (compare with reactive pneumocytes in Fig. 4). Two other features favouring adenocarcinoma are the abrupt transition with the surrounding parenchyma and the lack of cilia (the irregular cellular borders seen in B should not be confused with the long cilia shown in Fig. 5B). Note in particular the blandness of the mucinous bronchioloalveolar carcinoma illustrated in D: in the lung, a row of mucinous cells is diagnostic of adenocarcinoma, no matter how bland the cells.

Fig. 2. Three different examples of pulmonary adenocarcinomas with a paucity of neoplastic cells. A) Transthoracic fine-needle biopsy of a pulmonary mass in a 44-year-old man, smoker, with clinical evidence of brain metastases. Most of the biopsy is composed of fibro-elastotic tissue. B) At the edge of the biopsy, a group of detached cells is found. Despite their paucity, the uniform atypia with overlapping nuclei and the absence of cilia were considered enough, in this clinical situation, for a diagnosis of adenocarcinoma. In difficult cases, knowledge of the clinical data helps to put the microscopic findings into context. C) Transbronchial biopsy in a 61-year-old man with a pulmonary mass; at low magnification the parenchyma appears normal. D) At higher magnification, a few alveoli are lined by uniform mucinous cells. The subsequent surgical specimen confirmed a diagnosis of mucinous bronchioloalveolar carcinoma. E) Transbronchial biopsy in a 67-year-old woman with a pulmonary opacity (case courtesy of Dr. E. Nigroli, Cesena). Most of the biopsy consists of an inflammatory background. F) At higher magnification, a few neoplastic glands are present: here the criteria of malignancy was the uniformity of the cells and the complex cribriform/papillary configuration.

As a general rule, immunohistochemical stains should be used only to confirm a haematoxylin-eosin-based impression, and their results should always be evaluated within the overall context.

The main criteria to differentiate adenocarcinoma, reactive pneumocytes and peribronchiolar metaplasia are summarized in Table I, and some examples of adenocarcinomas and its mimics are shown in Figures 1 to 9.

mucinous (colloid) carcinomas, mucinous bronchioloalveolar carcinomas and adenocarcinomas with enteric differentiation, can variably express cytokeratin 20 and CDX-2: in the lung both antibodies are negative in reactive conditions, but also the majority of pulmonary adenocarcinomas are negative, and thus they are useful only when positive. p53 can be expressed in pulmonary adenocarcinomas but also in reactive pneumocytes, and its diagnostic utility in an individual case is limited.
Fig. 3. An adenocarcinoma obscured by a fibrotic background in a 54-year-old man with idiopathic pulmonary fibrosis (IPF, case courtesy of Prof. C. Capella, Varese). A) At low magnification the surgical biopsy shows a scarring process. An epithelial proliferation is present in the lower part of the picture, but it is partially obscured by the fibrosis and could be easily missed. B) Focally, the complexity of the epithelial proliferation is more evident: note the crowded, angulated glands with a disordered distribution. C) At higher magnification the cells are uniformly atypical and columnar, with overlapping nuclei and no cilia: these cytologic features are diagnostic of adenocarcinoma. D) An immunohistochemical stain for p63 further excluded an exuberant peribronchiolar metaplasia showing the absence of basal cells in the neoplastic glands: as an internal control, note the positive staining in the basal cells bordering the normal bronchiole in the left lower corner.

Fig. 4. Reactive pneumocytes mimicking adenocarcinoma in a transbronchial biopsy performed in a 62-year-old woman with diffuse alveolar damage (DAD) secondary to methotrexate. These markedly atypical but benign pneumocytes are more flattened, less crowded and less uniform than adenocarcinoma: large bizarre cells are present side-by-side with small bland cells (compare with the monotony of adenocarcinoma shown in Figures 1 to 3). The acute background with fibrin is a further clue of their reactive nature.

Fig. 5. Peribronchiolar metaplasia mimicking adenocarcinoma. A) Surgical lung biopsy in a 54-year-old man showing fibrosing nonspecific interstitial pneumonia (NSIP) with marked peribronchiolar metaplasia (case courtesy of Dr. A. Dubini and Dr. V. Poletti, Forlì). Although very exuberant, the epithelial proliferation seems to emanate from the bronchiole in an ordinate manner, and the alveolar spaces retain their rounded contours (compare with the disordered architecture of adenocarcinoma shown in Fig. 3B). B) At higher magnification the proliferating cells are bland and focally show long cilia. In the lung, long cilia are not present in adenocarcinoma and are a helpful clue of benignancy.
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Fig. 6. Benign entrapped alveoli mimicking adenocarcinoma in a surgical biopsy performed in a 50-year-old man with a 1 cm pulmonary nodule. A) Frozen section of the nodule showing an exuberant epithelial proliferation, more evident at the periphery. B) At higher magnification the epithelial cells are very bland and are associated with a stroma rich in equally bland spindle cells. The lack of atypia, the presence of a mesenchymal component and the age of the patient argue against a diagnosis of adenocarcinoma. C) Permanent section confirms a stellate nodule with an exuberant epithelial proliferation intermingled with a mesenchymal component. D) Pan-cytokeratin illustrates the exuberant nature of the epithelial component. E) The mesenchymal cells are reactive for smooth muscle actin. The final diagnosis was inflammatory myofibroblastic tumor with benign entrapped alveoli.

Fig. 7. Sclerosing haemangioma of the lung, a potential mimic of adenocarcinoma particularly in frozen sections 29-32. A) Frozen section of a pulmonary nodule in a 58-year-old woman. Macroscopically, the nodule was well-circumscribed and shelled out from the surrounding lung, an unusual finding for carcinoma. The low magnification shows the coexistence of solid, papillary and sclerotic areas; this variegated appearance is a second clue suggesting a sclerosing haemangioma. B) The third clue is the recognition of the characteristic bland, round to oval interstitial cells. C) Focal cytologic atypia is not unusual in sclerosing haemangioma and should not deter from the diagnosis if the other criteria are present. D) Transthoracic fine-needle biopsy of a well-circumscribed pulmonary nodule in a 54-year-old woman, showing the characteristic dual population of sclerosing haemangioma. Both superficial and interstitial cells were immunoreactive for TTF-1 and EMA whereas only superficial cells were positive for pan-cytokeratin: this typical immunoprofile 32 is helpful to confirm diagnosis in difficult cases.

Fig. 8. Other benign/low grade simulators of pulmonary adenocarcinoma 33. A) Papillary adenoma is a rare benign/low grade neoplasm, generally well circumscribed but sometimes with infiltrative features 34, composed of papillae lined by bland pneumocytes. It differs from adenocarcinoma for the lack of cytological and architectural characteristics of malignancy, and from sclerosing haemangioma for the lack of TTF-1-positive interstitial cells. To complicate diagnosis, rare bronchial-type papillomas can present as peripheral parenchymal nodules 35. B) A typical carcinoid mostly composed of papillary fronds. The characteristic bland cytology and the presence of more conventional areas are key to consider the possibility of a carcinoid and to perform the appropriate immunostains. C) Atypical adenomatous hyperplasia 1 23 36 37 is a precursor of peripheral adenocarcinoma of the lung, and consists of a small, generally peribronchial proliferation of mildly atypical pneumocytes. It differs from nonmucinous bronchioloalveolar carcinoma since it is smaller (generally less than 6 mm) and less atypical, but the limits are blurred. D) Micronodular pneumocyte hyperplasia (case courtesy of Prof. T.V. Colby, Scottsdale, U.S.A.) is a rare benign proliferation of plump pneumocytes lining thickened alveolar septa, occurring mostly (but not exclusively) in patients with tuberous sclerosis 38 39. It differs from atypical adenomatous hyperplasia because the cells are less atypical, the margins are more circumscribed and the interstitium is more prominent, but occasionally differential diagnosis can be difficult.
Reference
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