PATHOLOGICA

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

REVIEW
315 PDX-1 (Pancreatic/Duodenal Homeobox-1 Protein 1)
F. Pedica, S. Beccari, S. Pedron, L. Montagna, P. Piccoli, C. Doglioni, M. Chilosi

ORIGINAL ARTICLES
322 Lipomatous angiomyofibroblastoma of the vulva: diagnostic and histogenetic considerations
G. Magro, L. Salvatorelli, G. Angelico, G.M. Vecchio, R. Caltabiano
327 A peculiar fibroma-like lesion of superficial soft tissue: morphologic and immunophenotypic evaluation
M. Filotico, A. Damuri, R. Filotico

CASE REPORTS
330 Mixed stromal and smooth muscle tumours of the uterus: a report of two cases
335 Primary mediastinal angiosarcoma: a rare observation in a patient with 8-year-survival
M. Mlika, A. Berraies, M.S. Boudaya, A. Hamzaoui, F. El Mezni
338 Dermatofibrosarcoma protuberans of the vulva: a mesenchymal tumour with a broad differential diagnosis and review of literature
S. Gilani, B. Al-Khafaji

LEGAL ISSUE
348 La responsabilità del medico pubblico innanzi alla Magistratura Contabile dopo un innovativo indirizzo di coordinamento impartito dal Procuratore regionale del Lazio
G. Santeusanio, A.R. de Dominicis
351 Prospettive generali di rischio in Anatomia Patologica
E. de Dominicis, G. Santeusanio

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Società Italiana di Anatomia Patologica e Citopatologia Diagnostica, Divisione Italiana della International Academy of Pathology
CONTENTS

REVIEW

PDX-1 (Pancreatic/Duodenal Homeobox-1 Protein 1)
F. Pedica, S. Beccari, S. Pedron, L. Montagna, P. Piccoli, C. Dogliotti, M. Chilosi

The homeodomain-containing transcription factor pancreatic duodenal homeobox 1 (PDX-1) plays a key role in pancreatic development and β-cell function. It is a major regulator of transcription in pancreatic cells, and transactivates the insulin gene by binding to a specific DNA motif in its promoter region. Glucose also regulates insulin gene transcription through PDX-1. It has been shown that PDX-1 is required for maintaining pancreatic islet functions by activating gene expression and has a dual role in pancreatic development. It initially contributes to pancreatic formation during embryogenesis and subsequently regulates the pancreatic islet cell physiology in mature islets. Because of this key role in the embryologic development of the pancreas, PDX-1 expression has been investigated in pancreatic cancer cell lines and human tumors. Moreover, a few reports have described expression of PDX-1 in other human neoplasms and have investigated its potential role in differential diagnosis, but data on normal human tissues are lacking. Understanding the molecular mechanisms of pancreas formation, and especially the function of PDX-1, may contribute to the improved treatment and prevention of debilitating diseases such as diabetes, insulinomas and pancreatic carcinomas. Nevertheless, further studies are needed concerning its possible application in routine practice.

ORIGINAL ARTICLES

Lipomatous angiomysarcoma of the vulva: diagnostic and histogenetic considerations
G. Magro, L. Salvatorelli, G. Angelico, G.M. Vecchio, R. Calabubano

We report a rare case of angiomysarcoma (AMFB) of the vulva, composed predominantly of a mature fatty component, representing approximately 60% of the entire tumour. The tumour, designated as “lipomatous AMFB” should be interpreted as the morphological result of an unbalanced bidirectional differentiation of the presumptive precursor stromal cell resident in the hormonally-responsive stroma of the lower genital tract, with the adipocytic component overwhelming the fibroblastic/myofibroblastic one. The close admixture of adipocytes with spindled/epithelioid cells of the conventional AMFB resulted, focally, in a pseudo-infiltrative growth pattern and pseudo-lipoblast-like appearance, raising problems in differential diagnosis, especially with well-differentiated lipoma-like liposarcoma and spindle cell liposarcoma. Awareness of the possibility that vulvo-vaginal AMFB may contain large amount of lipomatous component is crucial to avoid confusion with other bland-looking spindle cell tumours containing infiltrating fat.

A peculiar fibroma-like lesion of superficial soft tissue: morphologic and immunophenotypic evaluation
M. Filotico, A. Damari, R. Filotico

Apical lesion of superficial soft tissue characterised by fibroma-like morphologic and immunohistochemical profile consisting of CD34+, Vim+, CD4+/-, CD31+/-, FLI1+ and INI-1 retained is described. The lesion entered into differential diagnosis with the so-called fibroma-like variant of epithelioid sarcoma, with the entities defined as ES-like/pseudomyogenic haemangiendothelioma and the recently identified entity defined as superficial CD34+ fibroblastic tumour. All of these entities share a common morphological structure, but differ in their immunophenotypic profile.

CASE REPORTS

Mixed stromal and smooth muscle tumours of the uterus: a report of two cases
N. Abid, R. Kallel, M. Mellodhi, H. Moif, L. Ayedi, A. Khabir, T. Boudawara

Mixed stromal and smooth muscle uterine tumours, defined as those containing at least 30% of each component as seen by routine light microscopy, are rare. This report describes the morphologic features of two such tumours diagnosed in 44-year-old and 50-year-old females complaining from recurrent uterine bleeding that was unresponsive to medical treatment. Morphological and immunohistochemical evaluations were performed, and a final diagnosis of mixed endometrial stromal nodule and smooth muscle tumour of the uterus was rendered in both cases.

Primary mediastinal angiosarcoma: a rare observation in a patient with 8-year-survival
M. Miika, A. Berreiai, M.S. Boudaya, A. Hammouti, F. El Mezni

Background. Vascular tumours of the mediastinum are rare, accounting for 1-2% of all mediastinal tumours in this location. Angiosarcoma are most often encountered as sporadic lesions, typically in the scalp or face of elderly patients. However, they can occur in any anatomic site. Mediastinal angiosarcomas (MA) are very rare with less than 50 cases reported.

Case report. The authors describe the case of a 38-year-old woman whose past medical history was consistent for a MA that was diagnosed in 2003. This tumour was treated by complete surgical resection followed by radiation therapy and chemotherapy. Diagnosis was based on histologic examination. In 2011, the patient presented a pleural localisation of the angiosarcoma and died one month after admission, 8 years after diagnosis of the MA.

Conclusion. MA is a very rare tumour causing a diagnostic dilemma. Clinical and radiologic findings are non-specific, and final diagnosis is based on histologic examination. The case described is unusual considering the long period of survival, which may be explained by the treatment modalities associating complete surgical resection, chemotherapy and radiation therapy.

Dermatofibrosarcoma protuberans of the vulva: a mesenchymal tumour with a broad differential diagnosis and review of literature
S. Giliani, B. Al-Khafaji

Dermatofibrosarcoma protuberans (DFSP) is a malignant cutaneous soft tissue tumour, which rarely presents in the vulva. We report an unusual case of this tumour involving the vulva. A 61-year-old female presented with a mass in the left mons pubis. Subsequent excisional biopsy of the mass was performed. Histologic evaluation of the specimen showed a spindle cell lesion consisting of fibroblast-like cells arranged in a storiform pattern. On average, there were 2 to 3 mitotic figures per 10 high power field (hpf). The neoplastic cells showed extension into the surrounding fibrofascial tissue. A panel of immunohistochemical stains including CD34, S-100, melan-A, HMB-45, vimentin and smooth muscle actin (SMA) were tested. The neoplastic cells showed diffuse staining with CD34 and vimentin, while the rest were negative. Based on the morphologic and immunohistochemical staining pattern, a diagnosis of DFSP was rendered. The patient underwent two subsequent resections before she had clear resection margins. The postoperative course was unremarkable. The patient is disease free without recurrence after a follow-up of 12 months. DFSP infrequently involves the vulva and should be considered in the differential diagnosis of other spindle cell lesions presenting in this unusual site. The role of immunohistochemical staining with CD34 is imperative in establishing the diagnosis. The rate of local recurrence is high, but it rarely shows metastasis. Treatment of choice is wide local surgical excision with close follow-up to detect reoccurrence.

Unusual presentation of metastatic adenoid cystic carcinoma: a challenge in aspiration cytology of the thyroid
B.J. Rocca, A. Barone, A. Gini, M.R. Ambroso, A. Disanto

Introduction. Adenoid cystic carcinoma is a malignant neoplasm most commonly originating in the salivary glands. Its occurrence elsewhere is rare and its metastasis to the thyroid gland has been described only once.

Case report. We describe the case of a 66-year-old man who presented for a swelling in the midline neck of six months duration. A solitary palpable nodule was identified in the isthmic region of the thyroid. Fine needle aspiration of the nodule revealed high cellularity, a partial microfollicle-like pattern and the presence of small hyaline globules. The neoplastic population was composed of monomorphic cells with basolateral appearance. Thyroid primitivity was excluded on the basis of the negativity for TTF1 and thyroglobulin. As the patient referred an ulcerative lesion of the inferior lip, fine needle aspiration cytology of the lesion was performed, yielding a diagnosis of adenoid cystic carcinoma.

Conclusion. The present case highlights the need to be aware of possible metastatic thyroid localisation of adenoid cystic carcinoma also originating in minor salivary glands of the oral cavity. This is a very rare event, but it should be taken into consideration and clinical and cytological findings must be carefully examined.

Gastrointestinal stromal tumour of the stomach with ossseous differentiation: a case report
A. Giorlandino, R. Calabubano, A. Carrerra, S. Lanzafame

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal neoplasm of the gastrointestinal tract, while osseous metaplasia of the tumour is an unexpected event. To date, no cases have been reported in the literature. Herein, we report a case of a 60-year-old man affected by a GIST with benign osseous metaplasia and mature bone formation. We also discuss the pathogenesis of intratumoural ossification and review the relevant literature. The prognostic significance of ossification in GIST remains unclear because of the limited cases reported.
PDX-1 (Pancreatic/Duodenal Homeobox-1 Protein 1)

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Key words

PDX-1 • Immunoistochemistry • Pancreatic development • Normal human tissues

Summary

The homeodomain-containing transcription factor pancreatic duodenal homeobox 1 (PDX-1) plays a key role in pancreatic development and β-cell function. It is a major regulator of transcription in pancreatic cells, and transactivates the insulin gene by binding to a specific DNA motif in its promoter region. Glucose also regulates insulin gene transcription through PDX-1.

It has been shown that PDX-1 is required for maintaining pancreatic islet functions by activating gene expression and has a dual role in pancreatic development. It initially contributes to pancreatic formation during embryogenesis and subsequently regulates the pancreatic islet cell physiology in mature islet cells.

Introduction

Pancreatic compartments have been demonstrated to derive from progenitor cells that express the pancreatic and duodenal homeobox gene (PDX-1) during pancreas development 1. The human pancreatic and duodenal homeobox-1 gene (PDX-1) is located on chromosome 13q12.1 near the CDX2 gene 2. In mouse and rat, the PDX-1 genes are localized on chromosomes 5 3, and 12 4, respectively.

PDX-1 (also known as IDX-1/STF-1/IPF1) 5–6 is a marker of all pancreatic and midgut progenitors, is expressed in precursors of the endocrine and exocrine (acinar and duct) compartments of the pancreas and is essential for development of the pancreas 7. PDX-1 is also expressed in the adjacent presumptive stomach and duodenum 7–7. This gene belongs to a Para-Hox gene cluster out of the major Hox cluster of homeodomain proteins 1 and its coding region comprises two exons 5: the first encodes for the NH2-terminal region of PDX-1 and the second for the homeodomain and COOH-terminal domain. The activation domain is located in the NH2-terminal region, while the homeodomain is responsible for DNA binding 9–10.

PDX-1 is a pancreas-specific homeoprotein, β- and δ-cell-specific and responsible for transcription and expression of insulin and somatostatin. PDX-1 activity is central to the regulation of a number of glucoregulatory genes within the β-cells, including insulin 11, islet amyloid polypeptide (IAPP) 12, glucose transporter type 2 (GLUT2) 13 and glucokinase 14. It regulates the balance between the exocrine (acinar and ducts) and endocrine progenitors that differentiate within the pancreas 15, depending on glucose levels through phosphorylation 16 and nuclear translocation 17.

Anatomical observations of amniotic embryos demonstrated that pancreas progenitors develop from a segment of the dorsal endoderm and separately from paired lateral domains of endoderm.

When the gut-tube closes, the lateral pancreatic endoderm domains fuse at the ventral region of the gut to form the ventral pancreatic bud, while the dorsal pancreatic endoderm goes on to form the dorsal pancreatic bud. In the end the two buds fuse with the ventral descendants
populating part of the “head” of the pancreas and the dorsal descendants forming the rest of the gland 18. The ventral bud develops adjacent to the hepatic diverticulum, while the dorsal bud arises on the opposite side of the gut tube. When the stomach and duodenum rotate, the ventral bud and hepatopancreatic orifice rotate and fuse with the dorsal bud. The ventral bud forms the uncinate process, while the dorsal bud forms the other part of the pancreas. The ventral duct fuses with the distal part of the dorsal duct to become the main duct of Wirsung and the proximal part of the dorsal duct becomes the duct of Santorini 18.

Mice homozygous for a targeted mutation in PDX-1 selectively lack a pancreas, but the duodenum has a normal C-shaped form 7. In homozygous mutants there is no pancreatic duct, but the common bile duct is present 7. PDX-1 knockout mice exhibit pancreatic agenesis and abnormal formation of the pylorus and duodenum 7, 19, 20. Defects in this gene are a cause of pancreatic agenesis, which can lead to early-onset insulin-dependent diabetes mellitus, as well as maturity onset diabetes of the young type 4 (MODY4) 21.

PDX-1 is first detected at embryonic day 8.5 (E8.5) in the dorsal endoderm of the murine gut when it is still an open tube and expressed in the dorsal and ventral pancreatic buds and in the intervening endoderm of the presumptive duodenum at E9.5 21. Its high expression is maintained in all epithelial cells of the pancreatic bud until E10.5, after which it decreases and is present later in the differentiated β-cell 22.

When the pancreatic epithelium proliferates and invades the mesenchyme around, the mesenchyme itself sends signals to the pancreatic epithelium to promote cellular differentiation and morphogenesis. In fact, without mesenchymal signals epithelial cells fail to grow and acini do not form 23. In PDX-1-null mice the pancreatic mesenchyme forms a hollow bud-like structure without epithelium 24.

Beyond β- and δ-cells, a lower expression of PDX-1 is present in the pancreatic acinar cells, epithelium of the duodenum, Brunner’s glands of the duodenum and pyloric glands of the stomach 21, 25.

Miyatsuka T et al. demonstrated that persistent expression of PDX-1 induces acinar-to-ductal metaplasia in a cell-autonomous manner 26. This occurs because up-regulation of PDX-1 causes activation of signal transducer and activator of transcription (STAT-3), which has been described in mouse models of pancreatic metaplasia 27.

Fukuda et al. 13 demonstrated that PDX-1 inactivation leads to loss of the major duodenal papilla and formation of brown pigment stones in the common bile duct. They also showed that PDX-1 null mice do not develop peribiliary glands or mucin-producing cells in the common bile duct. On the other hand, the re-upregulation has been reported in human patients and several mouse models of pancreatic cancer and pancreatitis 28, 30.

Extrahepatic bile ducts derive from PDX-1 positive cells in the foregut endoderm, sharing the common origin with the ventral pancreas but not with the liver 31, while the intrahepatic biliary cells derive from hepatoblasts. The liver itself derives from the ventral foregut and it needs signals from the cardiac mesoderm (such as fibroblast growth factor, FGF) 32 to develop 33.

The endoderm contains the precursors that give rise to the epithelium of both the gut and associated organs, such as the liver, pancreas and respiratory tract. In fact, the respiratory system arises from the ventral foregut endoderm 34.

### PDX-1 and Diabetes

Thomas IH et al. 35 described the combination of severe exocrine pancreatic insufficiency and permanent neonatal diabetes which suggested the possibility of pancreatic agenesis and, by association, the presence of a PDX-1 mutation.

Regarding acquired diabetes, Macfarlane et al. 36 identified 3 mutations in the β-cell transcription factor PDX-1 associated with type 2 diabetes. All 3 mutations (C18R, D76N and R197H) resulted in reduced binding of the protein to the insulin gene promoter and decreased insulin gene transcription.

Considering possible new strategies in treatment of diabetes mellitus, Yuan et al. 37 recently generated mesenchymal stem cells that are able to secrete insulin with stable transfection of the PDX-1 gene. The authors demonstrated that overexpression of PDX-1 in mesenchymal stem cells alone is sufficient in induction of insulin gene expression and insulin secretion. Another group 38 developed a strategy to generate human insulin producing cells using a 3D culture system with peripheral blood cells.

There is promising progress in redirecting various cell types to behave like β-cells and to produce insulin. However, in-depth knowledge of post-translational modifications of the PDX-1 protein and its interaction with other regulatory proteins will be fundamental to develop new treatments for diabetes mellitus 39.

### PDX-1 as a Target for Anti-Cancer Therapy

Recently, Wu et al. 40 have described the possibility to utilise Pdx-1 as a target gene for pancreatic cancer. In fact, Pdx-1 has been described as a potential molecular target for pancreatic cancer 41, 42 and the authors 40 have recently described the possibility to use RNA interference (RNAi) as a powerful new tool for targeted gene therapy.

### PDX-1 Expression in Human Normal and Neoplastic Tissues

Only a few reports investigated the immunohistochemistry of PDX-1 in human tissues, most of them focusing on gastrointestinal tissue (Tab. I).

Buettner et al. 43 investigated normal human pancreatic tissue and found that PDX-1 is mainly expressed in the cytoplasm and nuclei of endocrine and ductular cells in normal adult pancreas 43. In fact, although PDX-1 acts as a transcription factor, it can also be found in an inactive form in the cytoplasmic compartment since activation and nuclear translocation in pancreatic cells is regulated by glucose 36.
These Authors found that in normal antrum mucosa, nuclear and cytoplasmic expression of PDX-1 is found in epithelial cells in the neck region of the glands, while in normal body mucosa PDX-1 was mostly negative. Moreover, the gastric parietal cells in biopsies with pancreatic metaplasia had moderate to strong immunoreactivity for PDX-1, which was also found in the cytoplasm and in the nuclei of metaplastic acinar cells as well as cells adjacent to metaplastic areas in about half of cases with pancreatic metaplasia. PDX-1 was also present in the cytoplasm and nucleus of hyperplastic endocrine nodules and in the adjacent gastric glands in cases with atrophic body gastritis. Thus, PDX-1 may represent an important pathogenic factor for the development of pancreatic metaplasia and endocrine cell hyperplasia.

Leys CM et al. showed that fundic mucosa was devoid of cells with true nuclear PDX-1 immunoreactivity, even if normal antral mucosa had nuclear staining for PDX-1, especially in cells at the base of the glands. In case of antralisation of the gastric fundus, PDX-1 expression was not present, but intestinal metaplasia stained strongly for nuclear PDX-1. Nuclear PDX-1 expression was observed in 50% of antral-derived cancers and 40% of fundic cancers. PDX-1 expression did not correlate with clinical outcome.

Sakai et al. studied PDX-1 expression in 30 of 39 corpus tumors and intestinal metaplasia. The authors hypothesised that intestinal metaplasia may develop due to formation of pseudopyloric glands in the corpus because PDX-1 together with MUC6 (marker of pseudopyloric metaplasia) is significantly higher in well differentiated carcinomas than undifferentiated type. Thus, they suggested that intestinal metaplasia and differentiated type carcinomas arise on the basis of pseudopyloric/pyloric glands.

Park et al. studied PDX-1 expression in pancreatic neoplasms. They observed that PDX-1 expression was present in more than 50% of cells in PanIN, IPMN, and well differentiated neuroendocrine tumours.

Tab. I. Literature review table on PDX-1.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Antibody</th>
<th>Subject</th>
<th>Results (PDX-1 staining)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buettner et al., 2004</td>
<td>Polyclonal rabbit anti-PDX-1 antibody (see reference for details, 1:1000)</td>
<td>Atrophic corpus in gastritis</td>
<td>4/10 areas of pancreatic metaplasia and parietal cells adjacent to these areas endocrine nodules in 10/10 cases</td>
</tr>
<tr>
<td>Sakai et al., 2004</td>
<td>Produced by the authors</td>
<td>Normal and neoplastic stomach</td>
<td>pseudopyloric glands and intestinal metaplasia. differentiated type carcinomas (39/43, 90.7%) T1 carcinomas (42/43, 97.7%) undifferentiated type (33/52, 63.5%) T2–4 (50/52, 57.7%) carcinomas.</td>
</tr>
<tr>
<td>Leys et al., 2006</td>
<td>The guinea pig anti-PDX-1 polyclonal antibody (Gastroenterology 2005;128:1292 - 305)</td>
<td>Gastric adenocarcinoma</td>
<td>antral glands 47/104 gastric fundic cancers 23/46 gastric antral cancers</td>
</tr>
<tr>
<td>Liu et al., 2007</td>
<td>Goat anti-PDX-1 monoclonal antibody (Santa Cruz, USA).</td>
<td>Pancreatic cancer</td>
<td>41.1% of pancreatic cancer samples (especially at the leading edge of tumori, correlating with grading)</td>
</tr>
<tr>
<td>Ballian et al., 2008</td>
<td>Rabbit polyclonal antibody against the N-terminal of PDX-1 peptide 4</td>
<td>Several human tissues</td>
<td>In this report, levels of PDX-1 expression were quantified in a primary colorectal tumour, a metastasis and in benign tissue from a single patient</td>
</tr>
<tr>
<td>Srivastava et al., 2009</td>
<td>PDX-1 Goat Polyclonal (1:100 Microwave; Santa Cruz Biotechnology, CA)</td>
<td>Well differentiated neuroendocrine tumours</td>
<td>60% in stomach 80% in duodenum 0% in ileum and lung 55% in appendix 17% in rectum 28% in pancreas</td>
</tr>
<tr>
<td>Park et al., 2011</td>
<td>PDX-1 mouse monoclonal antibody that recognizes the C-terminus of PDX-1 (amino acids 91 to 283) MAB2419, clone 267712; R&amp;D Systems, Minneapolis, MN</td>
<td>Pancreatic neoplasms</td>
<td>40.6% of Panin in more than 50% of cells 35.2% of IPMN in more than 50% of cells 2/3 mucinous cystic neoplasms 9/67 pancreatic adenocarcinomas 47.7% of well differentiated endocrine tumours</td>
</tr>
<tr>
<td>Chan et al., 2012</td>
<td>PDX-1 Santa Cruz (sc-14662, Polyclonal goat 1:50)</td>
<td>Neuroendocrine tumours</td>
<td>pancreas 72% bronchopulmonary 10% appendix 17% cecum, colon, ileum and rectum 0%</td>
</tr>
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cancer and precursor lesions. PDX-1 nuclear labeling was present in non-neoplastic islet cells, centroacinar cells and intralobular and interlobular ducts. PDX-1 was strongly expressed in precursor lesions of pancreatic adenocarcinoma such as intraductal papillary mucinous neoplasm (35.2%), pancreatic intraductal tumours (40.6%) and mucinous neoplasms (2 of 3 cases), but the degree of dysplasia was not correlated with the intensity of staining of PDX-1.

Pancreatic adenocarcinoma had variable positivity (13.4% with strong positivity) and well differentiated neuroendocrine tumors were positive in 38.6% of cases.

Ballian et al. 46 reported that PDX-1 expression in 10 colon cancer specimens was significantly elevated in both the nucleus and cytoplasm of malignant cells, with lower levels found in benign tissues. In the same report, the authors 46 found high PDX-1 protein levels in metastases. Two different reports about the diagnostic utility of PDX-1 in neuroendocrine tumors (NET) found somewhat discrepant results in gastrointestinal tract endocrine tumors.

Srivastava et al. 47 observed PDX-1 expression in both gastrointestinal and pancreatic NET, a subset of rectal NET and more than half from the appendix. PDX-1 was absent in ileal and pulmonary NET. They concluded that PDX-1 can be used together with other 3 markers (NE-2, SP-55, Cdx-2 and TTF-1) to distinguish the origin of NET and more than half from the appendix. PDX-1 was specific and moderately sensitive for gastrointestinal NET; for a similar pattern of NET, while CDX-2 was very specific and sensitive for pancreatic NET, which may help in defining the primary site of origin of NET. PDX-1 in combination with Cdx-2, TTF-1 and CK7, which may help in defining the primary site of origin of NET. PDX-1 was specific and moderately sensitive for pancreatic NET, while CDX-2 was very specific and sensitive for gastrointestinal NET; for a similar pattern was seen for TTF-1 in bronchopulmonary NET.

In another report, Chan et al. 48 found that PDX-1 was positive in 72% of pancreatic NET, 10% of bronchopulmonary and 4% of GI tract NET. They concluded that PDX-1 can be used together with other markers (NE-SP-55, Cdx-2 and TTF-1) to distinguish the origin of well-differentiated NET in the gastro-enteric-pancreatic axis.

Materials and methods

We collected routinely available normal human tissues from stomach (fundus, corpus, antrum), duodenum, colon, appendix, liver, gallbladder, pancreas, tonsil, spleen, thymus, lung, thyroid, breast, skin, prostate, seminal vescicles, bladder, lymph node, kidney, adrenals, pituitary gland, ovary, uterus, salivary glands and cardiac muscle.

We performed immunohistochemistry, immunofluorescence and Western blot analysis in human normal tissue sections using previously described reagents and protocols 49.

Immunohistochemical analysis

We used a rabbit monoclonal antibody specific to a synthetic peptide of 46 kDa, corresponding to residues on the C-terminus in human PDX-1 antibody (Epitomics, clone EPR3358(2)), which was diluted 1:3000 and antigen retrieval was performed by incubation in buffer ER2 pH 8−9 for 15 min at 95°C. Detection was performed by a polymer-based system (Bond Polymer Refine Detection, Leica Biosystems, Nussloch, Germany) with an automated stainer (Leica Bond-Max).

Moreover, we used chromogranin A antibody (mouse monoclonal, Dako: da 3, 1:2500) and cytokeratin 7 (OV-TL12/30, BioGenex, 1:400) to perform double immunohistochemical staining and double immunofluorescence analyses.

Immunofluorescence analysis

Sections (3 μm) were collected on polarised slides and let dry for 1 hour at 60°C, and then deparaffinized in xylene for 20 min. Next, sections were hydrated with 100%, 85% and 75% ethanol and rinsed in distilled water. Furthermore, sections were treated for antigen retrieval with citrate buffer at pH 6, previously heated at 100°C for 30 min. Afterwards, sections were washed in running water and distilled water, then incubated with protein block solution for 10 min. The protein blocking solution in excess was eliminated and incubated with primary antibody for 1 hour at room temperature in a wet room; they were then washed 3 times with PBS and incubated with secondary antibody conjugated with fluorochrome for 30 minutes in a wet dark room at room temperature.

Later, sections were washed with PBS and incubated with alcoholic solution with 0.5% Sudan black for 10 min and then washed again with PBS. Excess PBS was eliminated and 20 μl of DAPI were added directly before application of a cover slip. The procedure was repeated for the secondary antibody. Sections were then examined with fluorescence microscope.

First primary antibody staining sites were visible in green, second primary antibody staining sites in red and double staining sites in yellow. Counterstained nuclei were visible in blue light. We applied PDX-1 (1:500) and chromogranin (1:2000) antibodies.

Western blot analysis

For each sample, 20 serial 10 μm sections of fresh frozen tissue were collected in an Eppendorf tube; 150 μl of cell lysis buffer (Cell Signaling Technology) was added prior to heating at 100°C for 5 min. Samples were cooled for 5 min on ice, centrifuged at 14,000 × g for 15 min and supernatants were transferred to a fresh tube and stored at −20°C. Protein quantification was performed by using the BioRad protein assay kit according to manufacturer’s instructions. 25 μg of extracted lysates was resolved on a 10% polyacrylamide SDS-PAGE gel in a BioRad Mini Protean tetra cell system at 150 V for 1 h.
Electrophoresed proteins were transferred onto a nitrocellulose membrane at 250 mA for 90 min. Membranes were blocked in TBST plus 5% non-fat dry milk for 1 h at RT with constant shaking. They were incubated overnight at 4°C with the indicated antibodies, washed three times with TBST and incubated with the specific secondary anti-mouse or anti-rabbit peroxidase-conjugated anti IgG antibody. After three washes with TBST, immunoblots were visualized with ECLplus (Amersham/GE Healthcare Europe GmbH, Munich, Germany). Expression levels of PDX-1 were quantified by ImageJ densitometric analysis. An anti-β-actin antibody (ab6276, Abcam, Cambridge, UK) was used as a control for protein loading.

Results

We found PDX-1 to have well defined nuclear staining and to be heterogeneously expressed only in the digestive tract with some differences in the different organs. In particular, we confirmed previous results since in the pancreas PDX-1 stained normal endocrine islets (Fig. 1A), pancreatic ducts and ductules (Fig. 1 A, B), but not acini. Moreover, both the Wirsung duct and intrapancreatic bile duct were positive (Fig. 1 C, D) showing a strong staining nuclear pattern.

In the liver, PDX-1 stained the bile duct epithelium of the major and minor branches of the biliary tree and peribiliary glands (Fig. 1E), but was not expressed in normal hepatocytes. Moreover, PDX-1 was widely and strongly expressed in the gallbladder epithelium (Fig. 1F).

In the gut tube, PDX-1 was strongly expressed in the mucosa of antrum (Fig. 2A) and duodenum (Fig. 2B), but not in the rest of the stomach or in the oesophagus. The ileal (Fig. 2C), appendiceal (Fig. 2D) and colonic mucosa (Fig. 2E) expressed PDX-1 in only a few scattered cells. These PDX-1 positive cells tended to be located at the base of the crypts and were characterised by a fusiform small nucleus with scant cytoplasm. Some of these scattered PDX-1 positive cells co-expressed chromogranin (Figs. 2E and 3).

Moreover, we found weak staining for PDX-1 in the adrenal gland and granulosa cells of the ovary, although Western blot analyses showed these were false positive cases, confirming the results in colon (Fig. 4). All other tissues were negative for PDX-1.

Conclusions

PDX-1 is expressed in the human digestive tract, and in particular in the duodenal and duodenal-pancreatic district. In the liver, PDX-1 stains only the biliary tree, but hepatocytes are negative.

These results can be explained by the embryology of pancreas and liver since they both develop from the ventral pancreatic bud 18. Moreover, along the rest of the digestive tube, it stains scattered small cells in the small intestine (other than duodenum) and large bowel. The differences between duodenal staining and the rest of the intestine can also be explained by embryology.

The duodenum arises from two adjacent regions of the gut tube: the foregut and midgut. The junction between these two regions lies at the mid-point of the duodenum, at the level of the entry of the bile duct. These cells are worthy of further investigation because they some co-stain chromogranin and some do not. This peculiar char-
characteristic, together with their morphology and anatomical location, may suggest that they could represent the “stem cell” niche of the intestine. The endoderm also forms the lining of three accessory organs that develop immediately caudal to the stomach. The hepatic diverticulum is the tube of endoderm that extends out from the foregut into the surrounding mesenchyme. The mesenchyme induces this endoderm to proliferate, to branch and to form the glandular epithelium of the liver. A portion of the hepatic diverticulum (the region closest to the digestive tube) continues to function as the drainage duct of the liver, and a branch from this duct produces the gallbladder 19. PDX-1 expression marks a pluripotent population of cells that give rise to all cell types of the neonatal pancreas (endocrine, exocrine and duct) and epithelium of the duodenum and posterior stomach 1. These data can explain the different distribution of PDX-1 expression among the gut tube.

Since PDX-1 is restricted to a few precise districts, it can be useful in suggesting the origin of endocrine neoplasms when unknown, but more data are needed to demonstrate its sensitivity and specificity in routine practice. Until now, only 3 reports have investigated PDX-1 expression in well differentiated neuroendocrine tumours 45 47 50, but they applied 3 different PDX-1 antibodies.

Moreover, our antibody was different from those reported in the literature and demonstrated to have a true positivity when it detected strong and precise nuclear staining, while other reports 43 46 also considered cytoplasmic staining with different clones as true positivity.

The common result presented herein is that the PDX-1 gene expressed specifically in the duodenum and biliary pancreatic tree and is a valid marker for this district. More reports are needed comparing different PDX-1 antibodies to better define which is best in term of sensitivity and specificity.

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Lipomatous angiomyofibroblastoma of the vulva: diagnostic and histogenetic considerations

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Key words
Angiomyofibroblastoma • Vulva • Lipomatous variant • Differential diagnosis

Summary
We report a rare case of angiomyofibroblastoma (AMFB) of the vulva, composed predominantly of a mature fatty component, representing approximately 60% of the entire tumour. The tumour, designated as “lipomatous AMFB”, should be interpreted as the morphological result of an unbalanced bidirectional differentiation of the presumptive precursor stromal cell resident in the hormonally-responsive stroma of the lower genital tract, with the adipocytic component overwhelming the fibroblastic/myofibroblastic one. The close admixture of adipocytes with spindled/epithelioid cells of the conventional AMFB resulted, focally, in a pseudo-infiltrative growth pattern and pseudo-lipoblast-like appearance, raising problems in differential diagnosis, especially with well-differentiated liposarcoma and spindle cell liposarcoma. Awareness of the possibility that vulvo-vaginal AMFB may contain large amount of lipomatous component is crucial to avoid confusion with other bland-looking spindle cell tumours containing infiltrating fat.

Introduction
Bland-looking mesenchymal tumours of the lower female genital tract comprise lesions which arise specifically in the vulvo-vaginal region, and soft tissue tumours that can occur at other sites of the body. Among the former lesions, at least four distinct entities can be recognised: aggressive angiomyxoma, angiomyofibroblastoma, cellular angiofibroma and myofibroblastoma. Interestingly, overlapping morphological and immunohistochemical features have been noticed not only among these lesions, but also with spindle cell lipoma, and mammary and soft tissue myofibroblastoma. Apart from these similarities, there is increasing evidence that spindle cell lipoma, cellular angiofibroma, mammary, soft tissue and vulvo-vaginal myofibroblastoma share the same chromosomal aberration, namely 13q14 deletion, as indicated by FISH analyses showing monoallelic deletion of RB1 and FOXO1.

Angiomyofibroblastoma (AMFB) is an uncommon, benign mesenchymal tumour that usually involves the vulva and vagina, but it can also occur at other sites such as the urethra, perineum, inguinal area, fallopian tube, vagina, scrotum, spermatic cord or pararectal region in males. Clinically, most AMFBs present as slowly-growing, subcutaneous painless masses which are often misdiagnosed as Bartholin’s gland cyst, hydrocele of the canal of Nuck, or aggressive angiomyxoma. Only rarely have tumours with features similar, but not identical, to AMFB been reported in unusual sites, such as the oral cavity. Although mesenchymal lesions labelled as angiomyofibroblastoma-like tumours have been reported in the male genital tract, most represent cellular angiofibroma, and not “true” AMFBs as originally described in the vulvo-vaginal region. According to the original description, the term AMFB is referred to the two main components of the tumour: blood vessels and stromal cells. AMFB contains numerous, sometimes ectatic, small- to medium-sized blood vessels which are, at least focally, surrounded by clusters of spindled to epithelioid cells. These cells are usually arranged in cords, trabeculae, or single cell files and set in a ma...
Lipomatous angiomyofibroblastoma of the vulva

matrix that varies from myxoid to hyaline, AMFB only rarely undergoes sarcomatous transformation with local recurrence. Immunohistochemical expression, albeit variable, of desmin and less frequently α-smooth muscle actin, seems to confirm that neoplastic cells are myofibroblastic in nature, but the occurrence of a prominent fatty component as an integral part of the tumour is extremely rare; the term “lipomatous AMFB” has been proposed for such tumours. We herein report a rare case of lipomatous AMFB of the vulva, emphasizing pathological features, and providing histogenetic and differential diagnostic considerations.

Clinical history

A 56-year-old woman presented with a painless, solitary, 4.5 cm mass in the vulva that appeared to be well-circumscribed and soft in consistency on physical examination. Preoperative ultrasonography confirmed a well-circumscribed mass in the vulva. Complete surgical excision of the mass, including a rim of adjacent, grossly normal tissue, was performed. No local recurrence has been experienced 2 years after surgery.

Materials and methods

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

Results

Grossly, the tumour consisted of a well-circumscribed, incompletely encapsulated nodular mass measuring 4.5 cm in greatest diameter. The cut surface showed a lipomatous tumour with interspersed fibrous areas. Calcifications, haemorrhage, and necrosis were absent. Histologically, at low magnification, a well-circumscribed lesion, composed predominantly (60% of the entire tumour) of mature adipose tissue, was seen (Fig. 1). The overall appearance was that of a lipomatous tumour containing dispersed, irregularly-shaped cellular areas and thick fibrous septa (Fig. 1). The fatty component was represented by mature adipocytes lacking nuclear pleomorphism. The non-adipocytic component was represented by conventional AMFB, namely proliferation of bland-looking spindled to epithelioid cells haphazardly set in a fibrous stroma and frequently arranged around small-sized blood vessels (Figs. 2, 3). Mono- or bi-nucleated epithelioid cells, at least focally, were closely packed to form small clusters. Tumour cells had an appreciable pale to eosinophilic cytoplasm and were variably set in a loose oedematous to fibrous stroma containing thin to thick wavy collagen fibres (Fig. 3). Mitotic activity was very low (< 1 mitosis x 50 HPF). Atypical mitoses, nuclear atypia and necrosis were not observed. Mast cells...
were readily identified in the fibrous stroma. The adipocytic and the spindled/epithelioid components were variably admixed: in some areas, the former component was represented by small islands of conventional AMFB completely surrounded by mature adipose tissue (Fig. 1), while in other areas the spindled to epithelioid cells were closely intermingling with adipocytes, resulting in a pseudo-infiltrative growth pattern of the former cells towards the latter cells (Fig. 4). In the areas that contained the juxtaposition of the two components, adipocytes focally varied in size and shape, exhibiting, at least focally, a univacuolar lipoblast-like appearance (Fig. 5). However, true lipoblasts, namely adipocytes showing hyperchromatic indented or sharply scalloped nucleus, were lacking. Neoplastic cells showing hybrid features between the two components, namely spindled/epithelioid cells with varying degrees of intracytoplasmic accumulation of lipids in the form of single large non-membrane-bound droplet or multiple small droplets, could not be identified, even after meticulous examination of the entire tumour. Immunohistochemically, the spindled/epithelioid cells were diffusely positive for vimentin, bcl2-protein (Fig. 6) and CD99 (Fig. 7), and focally for desmin. No immunostaining was obtained with any other antibodies tested. Mature adipocytes were S-100 positive. Based on morphological and immunohistochemical findings, a diagnosis of “lipomatous AMFB” was rendered.
Discussion

Vulvar AMFB is currently included in the category of the “specific stromal tumours of the lower female genital tract”, together with aggressive angiomyxoma, cellular angiofibroma and myofibroblastoma. Although diagnosis of AMFB is usually straightforward if typical morphology is encountered, diagnostic problems may arise with unusual morphological variants, such as the “lipomatous variant”.

Herein, we report on a rare case of benign spindle cell stromal tumour of the vulva, with prominent (60% of the entire tumour) mature fatty component. Due to this morphology, the tumour was closely reminiscent of a lipomatous tumour, especially spindle cell lipoma, well-differentiated lipoma-like liposarcoma or spindle cell liposarcoma. However, morphological and immunohistochemical findings were consistent with a fibroblastic/myofibroblastic tumour that fits within the spectrum of AMFB, representing the uncommon lipo-matous morphological variant, and thus the descriptive term “lipomatous AMFB of the vulva” seems to be most appropriate. The following morphological and immunohistochemical features, typically described in most cases of AMFB of the vulvo-vaginal region, support this diagnosis: i) intrasessional fat was an integral component of the tumour and not the result of entrapping, as it was identified either at the periphery or in the centre of the tumour; ii) the non-lipomatous component exhibited typical morphological and immunohistochemical features of AMFB. Interestingly, we found that, apart from focal immunostaining of desmin, both bcl-2 protein and CD99 were strongly and diffusely expressed in our case. Although these molecules may be potentially exploitable for differential diagnostic purposes, we underline that these markers are not specific, and are also reported in most cases of vulvo-vaginal myofibroblastomas.

The origin of a large amount of adipose tissue in vulvo-vaginal AMFB is still unclear. Some authors have speculated that lipomatous AMFB may arise from a perivascular or pericytic stem cell, which may differentiate into a myofibroblastic and fatty lesion under unknown stimuli. We were not able to identify cells with intermediate morphological and immunohistochemical features of fibroblasts/myofibroblasts and mature adipocytes. This argues against the hypothesis that the fatty component is the result of a metaplastic process from a fully mature cell type (fibroblast/myofibroblast) into another (adipocyte). Therefore, mature adipose tissue in lipomatous AMFB seems to arise “ex novo” from precursor stromal cells. As previously postulated for “benign stromal tumours of the breast”, a category of lesions which share several morphological, immunohistochemical and cytogenetic findings with the benign stromal tumours of the lower female genital tract, it can be speculated that AMFB, like vulvo-vaginal myofibroblastoma, may arise from a presumptive precursor cell of hormonally responsive stroma, and capable of multidirectional mesenchymal differentiation, including fibroblastic, myofibroblastic and lipomatous differentiation. In this regard, lipomatous AMFB should be interpreted as a bimorphic tumour that reflects the plasticity of precursor cells to undergo a dual fibroblastic/myofibroblastic and lipomatous differentiation, with the former component overwhelming the latter. As most cases of AMFB may contain a small component of adipose tissue, we speculate that there is a continuous spectrum of lipomatous differentiation in this tumour, ranging from a few islands to a large amount of adipose tissue.

As lipomatous AMFB contains a prominent fatty component, the main differential diagnosis includes spindle cell lipoma, lipoma-like well-differentiated liposarcoma and spindle cell liposarcoma. Unlike lipomatous AMFB, spindle cell lipoma contains neither epithelioid cells nor abundant capillary-like blood vessels. In addition, spindle cell lipoma lacks the tendency of neoplastic cells to aggregate around blood vessels and is usually a CD34-positive and desmin-negative tumour. Lipoma-like well-differentiated liposarcoma contains adipocytes with hyperchromatic and atypical nuclei, as well as atypical stromal cells in the fibrous septa intersecting the adipocytic component. All these features are lacking in lipomatous AMFB. In addition, the detection of lipoblasts, which however are not always present, argues against a diagnosis of AMFB. Spindle cell liposarcoma is a distinct, relatively rare, clinicopathological entity usually occurring in the deep and superficial soft tissues of shoulder girdle, upper limbs, groin, buttock and thigh. Notably, the spindle cell component of spindle cell liposarcoma is fibroblastic/myofibroblastic in nature, being variably stained with desmin and CD34. However, it is distinguishable from lipomatous AMFB for the presence, even if only focally, of lipoblasts with cytological features that closely resemble the differentiation of human embryonic fat. Although lipoblast-like cells can be encountered in the areas of lipomatous AMFB in which spindle epithelioid cells closely intermingle with adipocytes, however, correct interpretation of the context, namely identification of areas with the features of conventional AMFB, is crucial for pathologists to avoid misdiagnosis of malignancy. Fibromatosis is a locally-recurring lesion that rarely occurs in the vulva. Unlike lipomatous AMFB, fibromatosis exhibits infiltrating borders entrapping fat, and is composed of long sweeping cellular fascicles embedded in a variable fibrous stroma. The three different morphological phases, namely proliferative, involutional and residual, typically coexisting concurrently in the same case of fibromatosis, are lacking in lipomatous AMFB. Immunohistochemically, fibromatosis expresses β-catenin, α-smooth muscle actin, while desmin is usually absent or only focally expressed.

In conclusion, the present case is unusual in that it was difficult to recognize as AMFB, owing to the large amount of its lipomatous component. Awareness by pathologist of the possibility that vulvo-vaginal AMFB may exhibit a dominant fatty component is crucial to avoid confusion with other benign or malignant bland-looking spindle cell tumours containing or infiltrating fat.
References


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A peculiar lesion of superficial soft tissue characterised by fibroma-like morphology and an immunohistochemical profile consisting of CK+, VIM+, CD34+, CD31+/−, FLI1+ and INI-1 retained is described. The lesion entered into differential diagnosis with the so-called fibroma-like variant of epithelioid sarcoma, with the entities defined as ES-like/pseudomyogenic haemangioendothelioma and the recently identified entity defined as superficial CD34+ fibroblastic tumour. All of these entities share a common morphological structure, but differ in their immunophenotypic profile.

A 26-year-old man presented for a subcutaneous slow-growing, painless swelling on the left wrist lasting about two years. The patient was subjected to the surgical excision of the mass located in subcutaneous tissues, close to the tendons of the extensor muscles.

Materials and methods

At macroscopic examination, the surgical specimen was oval shaped, measured 1-8 x 1 cm, and the sectional area showed a pearly, homogeneous appearance. The outer surface was smooth. The formalin-fixed, paraffin-embedded sample was stained with haematoxylin-eosin and investigated with the following immunohistochemical antibodies: VIM (monoclonal 1:50 Dako), CK AE1-AE3 (monoclonal 1:50 Dako), EMA (monoclonal 1:50 Dako), SMACT (monoclonal 1:50 Dako), CALP (monoclonal 1:50 Dako), DESM (monoclonal 1:50 Dako), MYOGL (polyclonal 1:500 Sigma Aldrich), S100 (polyclonal 1:400 Dako), Ki-67 (monoclonal 1:75 Dako), CD34 (monoclonal 1:20 Dako), CD31 (monoclonal 1:20 Dako), INI-1 (polyclonal 1:500 Ventana Riche), FLI-1 (polyclonal 1:50 Santacruz Biotech).

Results

Histologic findings: a solid proliferation with a spindled cellular fibroblast-like component (Fig. 1a) was seen that was sometimes myoid-like (Fig. 1b). Among these elements pleomorphic, atypical cells with voluminous, hyperchromatic, irregular nucleus, sometimes multiple (Fig. 1c, d) were found with faintly acidophilic cytoplasm. Mitotic activity was virtually absent. The stroma was very loose and faintly fibrillar. The elements were assembled into variously oriented fascicles. The results of immunohistochemistry are summarized in Table I and show the following immunophenotypic profile: CK+ (Fig. 2a), VIM+ (Fig. 2b), CD34+ (Fig. 2c), CD31 (Fig. 2d), INI-1-retained (Fig. 2e), and FLI-1+ (Fig. 2f).

Discussion

In the interpretation of this case numerous diagnostic hypotheses were considered that were progressively discarded. The age of the patient, localisation of the lesion, its slow and indolent growth and the immunophenotypic profile,
characterized by the simultaneous expression of cytokeratin, vimentin and CD34, would support a diagnosis of epithelioid sarcoma (ES). The morphological structure, predominantly spindle-celled, is different from the classic description of this tumour, lacking the pseudogranulomatous aspects, scalloped necrosis and the epithelioid cellular component.

Since 1992 there have been five cases with the same morphologic characteristics and simultaneously expressing VIM and CK reported in the literature. These lesions were labeled as "fibroma-like variants of ES". In 1999, in a review of 112 cases of ES, seven were attributed to the aforementioned variant, 50% of which had documented expression of CD34. In 2003, seven cases with similar morphologic characteristics and expressing an immunophenotypic profile consisting of VIM+, CK+, CD34+, CD31+ and FLI-1+, were categorised as "ES-like haemangiendothelioma". In the fifth edition of Enzinger & Weiss’s Soft Tissue Tumors (2008), two separate entities were reported; one was described as a fibroma-like variant of ES, and the other as ES-like haemangiendothelioma. The former is CD34+ and CD31-, and the latter CD34- and CD31+. In the same year, a brief report of 29 cases with the same morphology and immunophenotypic pattern (CK+, VIM+, CD34-, INI-1 intact) were considered to be "pseudomyogenic (fibroma-like) variants of ES". In 2011, based on a study of 30 cases, the same authors, using a wider panel of antibodies, giving an immunophenotypic profile consisting of CK+ (100%), CD34+(0%), CD31+ (50%), FLI-1+ (100%), INI-1 intact (100%), redefined the lesion as “pseudomyogenic haemangiendothelioma”. In a comment on this report in the same journal, in a Letter to the Editor, the authors of an earlier report suggested that the morphologic and immunophenotypic identity of the lesion termed by them as ES-Like haemangiendothelioma was the same as the one called by the authors as pseudomyogenic haemangiendothelioma.

Very recently a study entitled “Superficial CD34-Positive Fibroblastic Tumor” was published in which, on the basis of 18 cases, a peculiar neoplasm of the superficial soft tissues was described, hitherto not reported. The neoplasm was found in adult subjects, mainly located in the extremities, and was morphologically characterised by a fascicular growth pattern of spindled cells, with striking, often bizarre cellular pleomorphism. All cases showed strong, diffuse CD34 positivity, focal cytokeratin expression (69%), SMARGCB1 (INI-1) retained, and Ki-67 < 1%. S100, SM-act, desmin and FLI-1 were all negative. Thirteen patients were available for follow-up; 12 were alive without evidence of disease. Only one patient developed locoregional lymph node metastases seven years after marginal excision of the tumour. The authors concluded that this was a distinctive low-grade mesenchymal neoplasm of intermediate (borderline) malignancy.

A literature review shows that over time, around the core basis of a lesion morphologically defined as fibroma-like and immunophenotypically characterised by the simultaneous expression of CK and VIM, a variety of antibody associations have been created. Different denominations have also been assigned to such associations. The differences occur mainly in the expression of CD34, CD31, FLI-1 and INI-1. Currently, according to the Stanford’s criteria, classic ES shows positivity for CD34 in 50% of cases, associated with loss of INI-1, and negativity for CD31 and FLI-1. In fact, in one of the aforementioned reports, 50% of the cases showed positivity for CD34 and negativity for CD31, consistent with classic ES. In other reports, constant negativity for CD34 is seen, associated with CD31+, FLI-1+ and INI-1 retained. From these data it is evident that it is justifiable in the first case the definition of fibroma-like ES and, conversely, in the second that of ES-Like/Pseudomyogenic Hemangiendothelioma. While on one hand the loss or retention of INI-1 is currently considered a discriminating element to establish or exclude an ES, the expression of CD31 and FLI-1 supports an endothelial histogenesis. The recently described entity with its distinctive immunophenotypic profile (CK+, VIM+, CD34+, Ki-67 < 1%, FLI-1- and INI-1-retained) adds a

Tab. I. Immunophenotypic profile.

<table>
<thead>
<tr>
<th>VIM</th>
<th>CK</th>
<th>EMA</th>
<th>SMA</th>
<th>CALP</th>
<th>DESM</th>
<th>MYOGL</th>
<th>S100</th>
<th>Ki67</th>
<th>C34</th>
<th>CD31</th>
<th>INI1</th>
<th>FLI1</th>
</tr>
</thead>
<tbody>
<tr>
<td>+diff</td>
<td>+diff</td>
<td>rare</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>&lt;1%</td>
<td>+diff</td>
<td>+f</td>
<td>ret.</td>
<td>+diff</td>
</tr>
</tbody>
</table>

f: focal; diff: diffuse; neg: negative; ret: retained.
new subgroup to this complex group of lesions. With regards to the prognosis of these lesions, the literature does not mention differences between the classical form of ES and its fibroma-like variant, while the ES-like/pseudomyogenic haemangioendothelioma and the superficial CD34+ fibroblastic tumour are considered low-risk lesions (borderline).

Conclusions

The lesion that we observed with its immunophenotypic profile (CD34+, CD31+, FLI-1+, INI-1-retained) cannot be compared to any of the above-described fibroma-like entities with which it shares a common morphological structure for the following reasons: - It cannot be considered as a fibroma-like variant of ES because it has intact INI-1; It cannot be considered as an ES-like/pseudomyogenic haemangioendothelioma because it has intense expression of CD34. - It cannot be considered as a CD34+ fibroblastic superficial tumour because it has intense expression of both CD31 and FLI-1. It is unclear if these various immunophenotypic expressions are indicative of different pathological entities or variants of a single entity, and further study will be needed to better to define this entity.

References

Mixed stromal and smooth muscle tumours of the uterus: a report of two cases

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Key words
Mixed • Stromal • Smooth muscle • Tumour • Uterus

Summary
Mixed stromal and smooth muscle uterine tumours, defined as those containing at least 30% of each component as seen by routine light microscopy, are rare. This report describes the morphological features of two such tumours diagnosed in 44-year-old and 50-year-old females complaining from recurrent uterine bleeding that was unresponsive to medical treatment. Morphological and immunohistochemical evaluations were performed, and a final diagnosis of mixed endometrial stromal nodule and smooth muscle tumour of the uterus was rendered in both cases.

Introduction
Small areas of smooth muscle differentiation are commonly seen in otherwise typical endometrial stromal neoplasms, and vice versa, while tumours exhibiting both prominent endometrial stromal and smooth muscle differentiation are relatively rare. In the most recent WHO publication, such tumours are called “mixed or combined stromal and smooth muscle tumours” and defined as tumours containing more than 30% of each component. Two cases of mixed stromal and smooth muscle tumours of the uterus with a review of the available literature are presented herein (Tab. I).

Case 1
A 44-year-old woman was evaluated for dysmenorrhoea. Examination revealed a uniformly enlarged uterus of 16 weeks’ size. Transvaginal ultrasound revealed a well-defined hyperechoic heterogeneous nodule located in the left lateral aspect of the uterus and measuring 9×8×7 cm with increased vascularity on colour Doppler. A myomectomy was performed. Grossly, the tumour was well circumscribed, but non-encapsulated; the cut surface was myxoid and showed an admixture of soft tan nodules and firm white whorled nodules (Fig. 1). Histologically, it consisted of aggregates of typical smooth muscle cells with bland nuclei and scant to moderate eosinophilic cytoplasm with indistinct cytoplasmic borders, intermixed with ‘small darkly staining cells’ whorled around small arterioles. Both components were cytologically bland, they were present in the tumour in similar proportions and surrounded by a hyalinised abundant stroma. The smooth muscle areas strongly immunostained with smooth muscle actin (SMA), desmin, calponin and h-caldesmon and lacked CD10. The stromal component was positive for CD10 and negative for all smooth muscle markers (Fi2). A diagnosis of mixed stromal and smooth muscle tumour was made even though characterisation of the stromal tumour by histology (endometrial stromal nodule or endometrial stromal sarcoma) was not possible because the interface tumour/surrounding myometrium was not available. A subsequent hysterectomy with bilateral salpingo-oophorectomy was performed, and extensive sampling revealed no microscopic residual tumour. The postoperative course was uneventful with no recurrence or symptoms after 1-year follow-up.

Case 2
A 50-year-old woman presented with a 5-month history of left flank pain and metrorrhagia that was unresponsive to medical treatment. Physical examination revealed a non-tender palpable suprapubic mass. Pelvic ultrasonography showed an enlarged uterus with 14×10 cm isoechoic mass increased vascularity on colour Dop-
Mixed stromal and smooth muscle tumors of the uterus

Ploter suggesting fibroid. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. Grossly, the uterus was bulky measuring 18x16x11 cm. Cut sections of the uterus showed a well-circumscribed, non-encapsulated, whitish homogenous mass measuring 15x11x10 cm present within the myometrium, distorting the endometrial cavity, but distinct from it. Histologically, the tumor consisted of two cell types. In some areas, the tumor showed smooth muscle features and consisted of spindle cells with moderate amounts of eosinophilic cytoplasm and cigar-shaped regular nuclei. In other areas (40% of the tumor), however, tumor cells showed typical features of endometrial stromal tumors and resembled stromal cells of proliferative endometrium. Mitoses were rare (<1/10HPF). The tumor had well circumscribed margins with no infiltration of the surrounding myometrium. The smooth muscle component was strongly positive for smooth muscle actin.

Tab. I. Summary of clinical and pathological features of previously reported cases of mixed stromal and smooth muscle tumors of the uterus.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Number of cases</th>
<th>Clinical data</th>
<th>Particular pathological findings</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olivia et al. (1998)</td>
<td>15</td>
<td>Patients age: 29-68 (mean 46 years)</td>
<td>SC: ESS in 6 cases and ESN in 9 cases; the SC predominated in 5 cases and was desmin (-) in all cases; SMC: predominated in 7 cases; showed moderate cytological atypia, mitotic figures 4/10 HPF and focal tumor cell necrosis in only one case; Areas of sex-cord differentiation in one case</td>
<td>One tumour with infiltrative margins recurred 4 years later as pure ESS; 6 patients were alive and free of disease; Data not available for 8 patients</td>
</tr>
<tr>
<td>Reena et al. (2005)</td>
<td>2 tumors in the same patient</td>
<td>40-year-old, uterine bleeding</td>
<td>SC: ESN; SMC: benign appearance</td>
<td>NA</td>
</tr>
<tr>
<td>Pamecnik et al. (2006)</td>
<td>1</td>
<td>52-year-old uterine bleeding</td>
<td>SC: high grade stromal endometrial sarcoma (CD10+ and MiB1=35%), with minor foci of low grade ESS that are CD10+ and MiB1=1%; SMC: benign appearance</td>
<td>No recurrence 8 month after the surgery</td>
</tr>
<tr>
<td>Pandey et al. (2010)</td>
<td>2</td>
<td>42-year-old, abdominal mass and uterine bleeding</td>
<td>SC: ESN; SMC: benign appearance</td>
<td>NA</td>
</tr>
<tr>
<td>Shintaku et al. (2013)</td>
<td>1</td>
<td>74-year-old uterine bleeding</td>
<td>SC: low grade ESS with foci of anaplasia (CD10+); SMC: benign appearance</td>
<td>Metastasis in the lung 9 months later; histological examination showed an anaplastic spindle cell sarcoma (CD10+ and Desmin-).</td>
</tr>
<tr>
<td>Dunder P et al. (2012)</td>
<td>1</td>
<td>73-year-old, uterine bleeding</td>
<td>SC: low grade ESS with myxoid changes; SMC: benign appearance, epitheloid cells</td>
<td>NA</td>
</tr>
<tr>
<td>Geetha et al. (2008)</td>
<td>1</td>
<td>49-year-old uterine bleeding</td>
<td>SC: ESN; No smooth muscle component identified on light microscopy; Desmin immunoreactivity in 50% of the tumor cells</td>
<td>NA</td>
</tr>
</tbody>
</table>

SC: stromal component; SMC: smooth muscle component; ESN: endometrial stromal nodule; EES: endometrial stromal sarcoma; NA: not available/ +: positive/ -: negative

Fig. 1. Gross appearance of the tumour showing an admixture of soft tan nodules (▲) and firm white whorled nodules (●).
(SMA) and h-caldesmon, and focally and weakly CD10-positive, contrasting with the endometrial component which was stained only with CD10 (fig. 3). The final diagnosis was mixed stromal and smooth muscle tumour. The postoperative course was uneventful with no recurrence or symptoms after 3-years follow-up.

Discussion

Mixed endometrial stromal and smooth muscle tumours of the uterus have been the focus of limited number of studies, partly because of under-recognition of these tumours and their uncommon occurrence\(^1\)\(^2\). The histogenesis of these tumours is uncertain. It has been suggested that multipotential cells are present in the uterus that can differentiate into both endometrial stroma and smooth muscle\(^4\). In their original report, Oliva et al.\(^5\) found a high frequency of t(7;17)(p15;q21) translocation, resulting in the fusion of the JAZF1 and JJAZ1 genes, in endometrial stromal tumours with smooth muscle differentiation. As this translocation represents the most common cytogenetic alteration observed in low-grade endometrial stromal tumours, the authors suggested that the endometrial stromal and smooth muscle components of these tumours have the same origin, either from a common precursor cell with pluripotential differentiation or from endometrial stromal cells that have undergone smooth muscle metaplasia. They also proposed use of the detection of this chromosomal abnormality in the diagnostic of stromal tumours with smooth muscle differentiation when the smooth muscle component is predominant\(^5\).

The clinical presentation does not differ from that of uterine tumours of pure endometrial stromal or smooth muscle origin, with uterine bleeding representing the most common symptom as seen in Table 1. On radioimaging, the softer areas of endometrial stroma may be misinterpreted as either cystic degeneration or uterine leiomyosarcoma. Specific diagnosis therefore requires evaluation of the entire tumour\(^6\).

In almost all reviewed cases, mixed stromal and smooth muscle tumours of the uterus present as a solitary intramural nodule, but polypoid appearance and multiple tumours have been reported as well (Tab. 1). On gross examination, these tumours often show an admixture of soft tan to yellow nodules and firm white whorled nodules.
ules, or alternatively the areas of firm tissue may be seen at the periphery of softer nodules, which is not characteristic of smooth muscle tumours in general. To establish a diagnosis of mixed stromal and smooth muscle tumour, both components should occupy at least 30% of the neoplasm by haematoxylin and eosin staining. The components tend to be sharply demarcated, although they occasionally merge onto each other. The stromal elements are typical of endometrial stromal neoplasms, either a stromal nodule or a low-grade stromal sarcoma, and consist of uniform small cells with round nuclei and scanty cytoplasm with small thin walled blood vessels scattered uniformly throughout the stromal component. Zámečník et al. [9] reported areas of differentiation of the stromal component that appeared mainly as a high grade sarcoma with few reminiscent areas of low grade endometrial stromal sarcoma. In fact, the stromal component of the tumour described in that case represents 90% of the tumour’s surface; thus, from a quantitative point of view, the tumour would not meet the requirements for 30% smooth muscle component as defined by the WHO, and should not be considered as a mixed stromal and muscle tumour. The immunophenotype of stromal cells is identical to that observed in other endometrial stromal tumours. In most tumours, these cells do not stain for desmin or only scattered cells stain. Zámečník el al. described a case of mixed stromal and smooth muscle tumour of the uterus where the stromal component showed surprising immunoreactivity with HMB45 and negativity of other melanocytic markers such as melan A, tyrosinase and microphthalmia transcription factor; they thus suggested that mixed stromal and smooth muscle tumours of the uterus could represent partial differentiation towards perivascular epithelioid cell tumours (PEComa). Histological features of smooth muscle differentiation includes typical smooth muscle morphology reminiscent of that seen in leiomyomata or nodules with prominent central hyalinisation (the so-called starburst pattern) that merge with characteristic areas of stromal differentiation. Smooth muscle cells vary in appearance from typical spindle-shaped cells with abundant eosinophilic cytoplasm in the fascicles and aggregates to rounded epithelioid cells with clear or amphophilic cytoplasm in nodules. Despite their variable appearance, smooth muscle cells have a typical immunophenotype and show uniform strong positive staining for smooth muscle actin and desmin. Large thick-walled blood vessels are typically present in these areas. The smooth muscle component is usually benign; Olivia et al., however, reported a case with moderate cytologic atypia, focal tumour cell necrosis and 4 mitotic figures/10 high-power fields. Areas of sex cord-like differentiation and glandular elements have been described in mixed endometrial stromal-smooth muscle neoplasms; endometrial stromal nodule with both smooth and skeletal muscle components has also been described. Intravascular extension is a rare event in mixed stromal and smooth muscle tumours of the uterus; McCluggage et al. described an intravascular component located within the dilated vessels of the myometrium and the fallopian tube, consisting of a mixture of endometrial glands, endometrial stroma and smooth muscle, and exhibiting a zoning phenomenon. Mikami et al. reported a case of endometrial stromal sarcoma with extensive smooth muscle differentiation resembling intravenous leiomyomatosis with extension to the inferior vena cava and cardiac chambers; for that reason, the authors considered the neoplasm as a mixed stromal and smooth muscle tumour, although the smooth muscle component represented only 5% of the entire primary uterine tumour. Immunohistochemical staining should be interpreted with caution in these tumours; in fact, the smooth-muscle component is often positive for CD10 and smooth-muscle markers, as observed in our first case, and this profile may lead to diagnosis of pure smooth muscle neoplasia. Hence, immunohistochemical staining should be correlated with the different morphological components of the tumour. Conventional areas of endometrial stromal neoplasia should be positive for CD10, but not positive for more than one smooth muscle marker, being more positive for actin and desmin. The main differential diagnosis of combined stromal and smooth muscle tumours is with highly cellular leiomyomas, in which the densely cellular areas may resemble the endometrial stromal component. Cellular leiomyomas, however, show the presence of large blood vessels with thick muscular walls throughout the tumour and lack a starburst pattern. These tumours should also be distinguished from infiltration of the surrounding myometrium by a typical low-grade stromal sarcoma, and delineation requires close correlation between the gross and microscopic features with knowledge as to where the tissue blocks have been taken from. Notably, it is the endometrial stromal pattern that dictates the biological behaviour of the tumour. Those dominated by endometrial stroma with an infiltrating margin may recur or metastasise; therefore, similar to a conventional endometrial stromal nodule, thorough assessment of tumour borders is essential. In summary, we report two cases of mixed stromal and smooth muscle tumour of the uterus. Through this report, we emphasise that the immunohistochemical results in these tumours should be interpreted in correlation with the appearance of the different areas identified on histological examination, and that distinction from cellular leiomyoma is of primary importance because of different clinical behaviour and management.

References


Primary mediastinal angiosarcoma: A rare observation in a patient with 8-year-survival

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Key words
Mediastinum • Angiosarcoma • Hemangioendothelioma • Surgery

Summary
Background. Vascular tumours of the mediastinum are rare, accounting for 1-2% of all mediastinal tumours in this location. Angiosarcomas are most often encountered as sporadic lesions, typically in the scalp or face of elderly patients. However, they can occur in any anatomic site. Mediastinal angiosarcomas (MA) are very rare with less than 50 cases reported.

Case report. The authors describe the case of a 38-year-old woman whose past medical history was consistent for a MA that was diagnosed in 2003. This tumour was treated by complete surgical resection followed by radiation therapy and chemotherapy. Diagnosis was based on histologic examination. In 2011, the patient presented a pleural localisation of the angiosarcoma and died one month after admission, 8 years after diagnosis of the MA.

Conclusion. MA is a very rare tumour causing a diagnostic dilemma. Clinical and radiologic findings are non-specific, and final diagnosis is based on histologic examination. The case described is unusual considering the long period of survival, which may be explained by the treatment modalities associating complete surgical resection, chemotherapy and radiation therapy.

Background
Primary mediastinal sarcomas are very rare, representing 2-8% of the malignant mediastinal tumours and 1.4% of soft tissue sarcomas 1 2. These sarcomas are characterized by their poor behaviour that is essentially related to their development next to vital organs. Angiosarcomas are very rare, accounting for 1-2% of all mediastinal tumours. These tumours cause a diagnostic dilemma and necessitate multi-modal treatment procedures. We describe a new case of mediastinal angiosarcoma that was unusual due to the long-term survival of the patient. This case was documented by radiologic and histologic features.

Case presentation
The authors describe the case of a 38-year-old woman whose past medical history consistent of cholecystectomy performed in 2009 and a mediastinal angiosarcoma diagnosed in 2003. This tumour was treated by a complete surgical resection followed by radiation therapy and chemotherapy. Diagnosis was based on histologic examination. Microscopic features consisted in a tumour composed of multiple epithelioid cells with atypical and focally mitotic nuclei. Focal areas of necrosis were present (Fig. 1a). In June 2011, the patient presented with acute respiratory distress. Laboratory tests and tumoural markers (ACE, CA 27-29, CA 125) were within normal values. Physical examination was normal. A CT scan showed a right pleural effusion with pneumothorax. Many lesions of fibrosis were also noticed in the right lung (Fig. 2). Fine needle aspiration of the liquid was performed, and microscopic study concluded a mesothelial localisation of a sarcoma. Because of the increase of the dyspnoea, a talcage associated with surgical biopsy of the parietal pleural nodules through thoracoscopy was performed. Microscopic examination showed tumour proliferation consisting of sheets of epithelioid...
tumour cells with atypical nuclei (Fig. 1b). Immunohistochemical study showed expression of vimentin, CD31 and CD34 by tumour cells, revealing their vascular nature (Fig. 1c). A diagnosis of pleural localisation of angiosarcoma was retained. The evolution of the patient was marked by the increase of the dyspnoea, and exacerbation of thoracic chest pain followed by death one month after admission and 8 years after diagnosis of the mediastinal angiosarcoma.

Discussion

Vascular tumours of the mediastinum are rare, accounting for 1-2% of all tumours in this location. Mediastinal sarcomas are very rare. In our department, 16 mediastinal sarcomas have been diagnosed over a 16-year-period, and this is the first case of angiosarcoma diagnosed during the same period. Angiosarcomas are most often encountered as sporadic lesions, typically in the scalp or face of elderly patients. However, they can occur in any anatomic site, including the deep soft tissue, breast, visceral organs and bone. Important predisposing conditions have been reported including radiation exposure, chronic lymphoedema, exposure to toxins such as vinyl chloride or thorotrast, or foreign bodies. Primary angiosarcomas of the mediastinum are rare with less than 50 cases reported in the English literature. Most tumours were located in the anterior mediastinum. Chest pain is the main presenting symptom. The patient age ranged from 25 to 62 years. Radiologically, tumours presented as a non-specific anterior mediastinal mass. Diagnosis is based on histologic examination, which may show typical features ranging from low-grade neoplasms with definitive vasoformation to high grade lesions with a more solid growth pattern and vast areas of necrosis. Immunohistochemical study demonstrates a vascular phenotype with immunoreactivity for factor VIII-related antigen, CD31 and CD34. Many differential diagnoses must be ruled out such as haemangioma or carcinoma, but the distinction is quite easy when based on histologic and immunohistochemical features. The most relevant mimickers of MA is epithelioid haemangoendothelioma, which is a vascular neoplasm of low to intermediate malignant potential that follows a nonaggressive clinical course. The distinction between both neoplasms is based only on microscopic findings without immunohistochemical study, which is unable to make any distinction. Another important consideration is the possibility that angiosarcoma may occur in association with a mediastinal germ cell tumour, which is considered as a sarcomatous transformation of these tumours and with very poor prognosis. This distinction is dependent on thorough sampling of the specimen. There is no consensus regarding management of MA due to its rarity, but surgical resection represents a mainstay. Thoracotomy has been used in almost all cases, but some authors report that, in patients with limited disease, endoscopic transthoracic approaches can reduce approach-related soft-tissue morbidity and facilitate recovery by preserving normal tissues of the chest wall. In our opinion, since MA is a malignant disease, it is rarely localised, resulting in the necessity of thoracotomic approaches. Other authors have recommended radical excision followed by post-operative radiotherapy, especially in cases where the tumour has been partially excised as the treatment of choice. It is extremely difficult to draw any conclusions about the value of adjuvant radiotherapy and/or chemotherapy, mainly because the numbers of patients are small. In our case, we can only suppose that the adjuvant therapy is implicated in the long survival of the patient. Many authors have reported that angiosarcomas of the
mediastinal angiosarcoma is a very rare tumour causing a diagnostic dilemma, especially with haemangioendothelioma and carcinoma. Clinical and radiological findings are non-specific, and final diagnosis is based on histologic examination. There is little consensus regarding treatment modalities because of its rarity of the cases, although radical excision followed by post-operative radiotherapy appears to be the treatment of choice. The case described herein is unusual due to the long period of survival, which may be explained by the treatment modalities consisting in complete surgical resection, chemotherapy and radiation therapy.

**Conclusion**

Mediastinal angiosarcoma is a very rare tumour causing a diagnostic dilemma, especially with haemangioendothelioma and carcinoma. Clinical and radiological findings are non-specific, and final diagnosis is based on histologic examination. There is little consensus regarding treatment modalities because of its rarity of the cases, although radical excision followed by post-operative radiotherapy appears to be the treatment of choice. The case described herein is unusual due to the long period of survival, which may be explained by the treatment modalities consisting in complete surgical resection, chemotherapy and radiation therapy.

**References**

Dermatofibrosarcoma protuberans (DFSP) is a malignant cutaneous soft tissue tumour, which rarely presents in the vulva. We report an unusual case of this tumour involving the vulva. A 61-year-old female presented with a mass in the left mons pubis. Subsequent excisional biopsy of the mass was performed. Histologic evaluation of the specimen showed a spindle cell lesion consisting of fibroblast-like cells arranged in a storiform pattern. On average, there were 2 to 3 mitotic figures per 10 high power field (hpf). The neoplastic cells showed extension into the surrounding fibroadipose tissue. A panel of immunohistochemical stains including CD34, S-100, melan-A, HMB-45, vimentin and smooth muscle actin (SMA) were tested. The neoplastic cells showed diffuse staining with CD34 and vimentin, while the rest were negative. Based on the morphologic and immunohistochemical staining pattern, a diagnosis of DFSP was rendered. The patient underwent two subsequent resections before she had clear resection margins. The postoperative course was unremarkable. The patient is disease free without recurrence after a follow-up of 12 months. DFSP infrequently involves the vulva and should be considered in the differential diagnosis of other spindle cell lesions presenting in this unusual site. The role of immunohistochemical staining with CD34 is imperative in establishing the diagnosis. The rate of local recurrence is high, but it rarely shows metastasis. Treatment of choice is wide local surgical excision with close follow-up to detect recurrence.

**Key words**

Dermatofibrosarcoma • Vulva • Local recurrence • Treatment

**Summary**

Dermatofibrosarcoma protuberans (DFSP) is a malignant cutaneous soft tissue tumour, which rarely presents in the vulva. We report an unusual case of this tumour involving the vulva. A 61-year-old female presented with a mass in the left mons pubis. Subsequent excisional biopsy of the mass was performed. Histologic evaluation of the specimen showed a spindle cell lesion consisting of fibroblast-like cells arranged in a storiform pattern. On average, there were 2 to 3 mitotic figures per 10 high power field (hpf). The neoplastic cells showed extension into the surrounding fibroadipose tissue. A panel of immunohistochemical stains including CD34, S-100, melan-A, HMB-45, vimentin and smooth muscle actin (SMA) were tested. The neoplastic cells showed diffuse staining with CD34 and vimentin, while the rest were negative. Based on the morphologic and immunohistochemical staining pattern, a diagnosis of DFSP was rendered. The patient underwent two subsequent resections before she had clear resection margins. The postoperative course was unremarkable. The patient is disease free without recurrence after a follow-up of 12 months. DFSP infrequently involves the vulva and should be considered in the differential diagnosis of other spindle cell lesions presenting in this unusual site. The role of immunohistochemical staining with CD34 is imperative in establishing the diagnosis. The rate of local recurrence is high, but it rarely shows metastasis. Treatment of choice is wide local surgical excision with close follow-up to detect recurrence.

**Introduction**

Mesenchymal tumours of vulva are rare. Dermatofibrosarcoma protuberans (DFSP) is a well differentiated sarcoma of dermal origin. It infrequently arises from vulva. Only a few cases have been reported in the literature.

**Case description**

A 61-year-old female presented with a left labial painless mass. She underwent excision of the lesion. Microscopic evaluation of the mass showed a spindle cell proliferation which consisted of fibroblast-like cells in a storiform arrangement (Fig. 1A and 1B). These neoplastic cells have an infiltrative pattern with extension into adjacent fibroadipose tissue (Fig. 2). On average, the mitotic rate was estimated at two to three per 10 hpf (Fig. 3). Overall, the morphologic pattern was uniform and most of the cells displayed bland cytologic features with no pleomorphism or necrosis (Fig. 4). To evaluate the nature of neoplastic cells, a panel of immunohistochemical stains including CD34, S-100, melan-A, HMB-45, SMA and vimentin was tested. The neoplastic cells showed a diffuse staining pattern with CD34 (Fig. 5A) and vimentin (Fig. 5B), while they were negative for S-100 (Fig. 5C), SMA (Fig. 5D), melan-A and HMB45. Based on morphologic features and the immunohistochemical staining pattern, a diagnosis of DFSP was rendered. The neoplastic cells were present at the resection margins. Later, the patient underwent two subsequent resections before she had clear margins of resection. The post-operative course was uneventful. The patient was discharged in a stable condition and is disease free after a follow-up of 12 months.

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Discussion

DSFP is a malignant cutaneous soft tissue tumour with frequent local recurrence. It is dermal in origin with low to intermediate potential for malignancy. It usually presents in the extremities and trunk, although it can present in unusual locations including vulva, breast and parotid.\(^6\) The mean age for presentation is 46 years, while cases of DFSP are reported in the vulva in different age groups. Imaging modalities including magnetic resonance imaging (MRI) are helpful to identify the extent of the disease. It can present as a slowly growing subcutaneous nodular mass. The overlying skin is usually attenuated. Microscopically, the epidermis may show atrophic changes with a cellular dermal spindle cell proliferation in a storiform pattern. The neoplastic cells show infiltrative configuration into adjacent adipose tissue at the base of tumour. Cytologically, neoplastic cells exhibit uniform bland looking fibroblast-like spindle cells with minimal atypia and absence of necrosis. The mitotic rate is variable and up to 5 per 10 hpf. Several morphologic variants of DFSP including myxoid, fibrosarcoma-like, pigmented variant or Bednar tumour have been described. Myxoid DFSP has predominantly myxoid background with spindle cell proliferation and intermixed capillary meshwork. Fibrosarcomatous variant can show similar morphologic features to conventional DFSP, but they usually have high mitotic activity and diminished staining pattern with CD34. Pigmented variants show pigmented dendritic cells admixed with neoplastic spindle cells. Immunohistochemical staining is helpful to confirm the diagnosis of DFSP and exclude other close differential diagnoses. The neoplastic cells in DFSP usually demonstrate a diffuse staining pattern with CD34. Intraoperative frozen sections are valuable in evaluating the margins of the lesion. This approach can guide the surgeon for appropriate management and can assist in a tissue preservation approach, specifically in the unusual sites such as the vulva.

The differential diagnosis is wide-ranging, encompassing...
Dermatofibromas, schwannomas, leiomyomas, spindle cell variant of melanoma, myxoid liposarcoma, and undifferentiated pleomorphic sarcoma (Tab. I). Dermatofibromas (DF) are benign fibrous histiocytic proliferations of plump spindle cells, usually involving the upper dermis with intervening collagen bands and fascicles. They usually show pseudoepitheliomatous hyperplasia, floret type multinucleated giant cells, dispersed foamy histiocytes and a variable number of mitoses. Histologically, neoplastic spindle cells show analogous morphologic features to those of DFSP, but they lack an infiltrative growth pattern into adjacent adipose tissue and stain positive with factor XIIIa. In contrast, DFSP display an infiltrative growth pattern and stains positive with CD34, while they usually lack giant cells and abundant collagen bands, which helps to differentiate it from DF.

Schwannomas are nerve sheath tumours. They can involve the vulva and present as clitoral hypertrophy. Microscopically, neoplastic cells have spindle cell morphology with areas of hypocellularity and hypercellularity, called Antoni A and Antoni B areas, respectively. Antoni A areas show nuclear palisading, whorling appearance and Verocay bodies. The neoplastic cells exhibit positive staining pattern with S-100 protein. While DFSP usually does not show hypocellular and hypercellular growth pattern and is negative for S-100 protein. Neurofibromas are benign tumours. They are usually spindle cell proliferations with distinct cell borders. The cells usually have tapered ends with a background mast cells and intervening collagen fibres. They usually lack mitotic figures. They stain positive with S-100 protein, while in comparison the neoplastic cells in DFSP show positive staining with CD34. Leiomyomas and its cellular variant consist of spindle-shaped smooth muscle proliferation which stain positive with SMA, unlike DFSP which exhibits positive staining with CD34 and negative for SMA. Melanomas can present at any location of body in different morphologic forms. The spindle cell variant of melanoma should always be considered in the differential diagnosis of DFSP. The neoplastic cells in melanoma stain positive with melan-A and HMB45 while they are negative for CD34, which aids in differentiating it from DFSP. Furthermore, myxoid liposarcoma can be confused with the myxoid variant of DFSP. The presence of CD34 staining and absence of lipoblasts supports the diagnosis of DFSP. Undifferentiated pleomorphic sarcoma rarely presents in the vulva, and very few cases have been reported in the literature. The neoplastic cells show marked pleomorphism, cyto-

<table>
<thead>
<tr>
<th>Series no.</th>
<th>Neoplastic entities</th>
<th>Morphologic and immunohistochemical staining features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dermatofibroma</td>
<td>Storiform arrangement of spindle cells, usually do not have infiltrative pattern and stains positive with Xllia.</td>
</tr>
<tr>
<td>2</td>
<td>Schwannoma</td>
<td>Hypocellular and hypercellular areas of neoplastic spindle cells. They usually stain positive with S-100 protein.</td>
</tr>
<tr>
<td>3</td>
<td>Neurofibroma</td>
<td>Benign neural tumour with spindle cell proliferation. They usually stain positive with S-100 protein.</td>
</tr>
<tr>
<td>4</td>
<td>Spindle cell melanoma</td>
<td>Cellular spindle cell neoplastic cells stain positive with melanoma makers including melan-A and HMB45.</td>
</tr>
<tr>
<td>5</td>
<td>Myxoid Liposarcoma</td>
<td>Characterized with stellate or fusiform cells, presence of lipoblast, plexiform capillary network, translocation (12;16) and absence of CD34 staining pattern support myxoid liposarcoma.</td>
</tr>
<tr>
<td>6</td>
<td>Undifferentiated pleomorphic sarcoma</td>
<td>Undifferentiated sarcoma with pleomorphic cells, having abundant mitotic figures. Areas of necrosis and giant cell formation can be seen.</td>
</tr>
</tbody>
</table>

Fig. 5. Spindle shaped neoplastic cells show diffuse staining with (a) CD34 and (b) vimentin (high power). They stain negative with (c) S-100 and (d) SMA (high power).
logic atypia, giant cells, abundant mitotic figures and foci of necrosis, a morphologic pattern, which is typically not observed in DFSP. Ultrastructurally, it shows areas of fibroblastic differentiation. Molecular and cytogenetic abnormalities in DFSP can be recognized by florescence in-situ hybridization (FISH). DFSP is associated with t (17; 22) translocation and COL1A1-PDGFB fusion. There is intensification of chromosome 17 and 22 or the presence of a supernumerary ring chromosome. These molecular markers aid in confirming diagnosis especially in difficult cases, with overlapping histologic features with other mimickers, and a non-conclusive CD34 staining pattern.

Wide surgical excision and post-excision follow-up is the standard approach in the management of DFSP. Due to the high local recurrence rate (20-49%), the role of Mohs micrographic surgery (MMS) is substantial in the accurate microdissection of margins. It is challenging to obtain negative margins in deep-seated lesions, so the use of MMS is limited. However, the Tubingen variant of Mohs surgery is useful to achieve negative margins with preservation of uninvolved tissue. Use of imatinib and radiotherapy is supportive predominantly in cases with advanced disease. Fibrosarcomatous differentiation in DFSP is associated with an increased risk for recurrence. Metastasis is rare with only a few reported cases. Clinical and radiologic follow up is recommended to identify early recurrent diseases.

DFSP is a malignant soft tissue mesenchymal tumour of dermal origin. It is less frequently encountered in the vulva. The differential diagnosis consists of a wide spectrum of diseases ranging from benign neoplasms to undifferentiated sarcomas. The characteristic morphologic pattern and immunohistochemical staining with CD34 is extremely helpful in confirming a diagnosis of DFSP.

References

Unusual presentation of metastatic adenoid cystic carcinoma: a challenge in aspiration cytology of the thyroid

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Key words
Fine needle aspiration cytology • Adenoid cystic carcinoma • Metastasis to thyroid • Minor salivary glands

Summary

Introduction. Adenoid cystic carcinoma (ACC) accounts for 3-5% of all salivary gland tumours and occurs in both minor and major salivary glands 1, the former representing the most common malignancy 2. It can also arise from submucosal seromucinous glands of the larynx and trachea as well as the lung 3. When it presents outside these locations, diagnosis is more challenging. Cytologically, the tumour is characterised by clusters of cohesive monomorphic small to round epithelial cells, with a high nuclear/cytoplasmic ratio, round nuclei, coarsely granular chromatin, prominent nucleoli and scant cytoplasm. The clusters surround globules of homogenous, acellular hyaline magenta-stained material. Dispersed naked nuclei are common as well as spherical aggregates, rosette-like groups, papillary structures and solid nests of cancer cells 3. The tumour often has a relentless clinical course with local recurrences and high incidence of distant metastases, owing to its infiltrative growth 3. The lungs and central nervous system are sites of predilection of distant metastases 4-6. Thyroid metastases from non-salivary gland ACC has been described in rare cases 6,9,10. In our extensive review of the literature, we found only one case of salivary gland ACC metastatic to thyroid in a patient with ACC of parotid gland 6,11. Herein, we describe the first case of ACC of a minor salivary gland metastatic to the thyroid.

Case report
A 66-year-old man presented for the onset of a swelling in the midline neck lasting six months. The swelling was slowly progressive and was not associated with pain or compressive symptoms. The patient was clinically euthyroid. On physical examination, a solitary palpable nodule was identified in the isthmic region of the thyroid. Fine needle aspiration of the nodule revealed high cellularity, a partial microfollicle-like pattern and the presence of small hyaline globules. The neoplastic population was composed of monomorphic cells with basaloid appearance. Thyroid primitivity was excluded on the basis of the negativity for TTF1 and thyroglobulin. As the patient referred an ulcerative lesion of the inferior lip, fine needle aspiration cytology of the lesion was performed, yielding a diagnosis of adenoid cystic carcinoma.

Conclusion. The present case highlights the need to be aware of possible metastatic thyroid localisation of adenoid cystic carcinoma also originating in minor salivary glands of the oral cavity. This is a very rare event, but it should be taken into consideration and clinical and cytological findings must be carefully examined.

Acknowledgements
The authors would like to thank Prof. Piero Tosi (University of Siena) for his expert review of the manuscript.

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Unusual presentation of metastatic adenoid cystic carcinoma: a challenge in aspiration cytology of the thyroid

Thyroid. Ultrasonography revealed a 16 x 14 mm mixed echoic lesion in the thyroid parenchyma, involving trachea and soft tissues, without lymphoadenopathies. In the suspicion of subacute thyroiditis, fine needle aspiration cytology (FNAC) of the nodule was performed; wet fixed and air dried smears were made and stained with May-Grunwald Giemsa (MGG) and Papanicolaou. FNAC revealed a highly cellular smear with a monomorphic population of basaloid cells in tight clusters (Figure 1A). There was a partial microfollicle-like pattern (Figure 1B). The individual cells were round, oval or slightly angulated with fine, granular chromatin, indistinct nucleoli, scant cytoplasm and indistinct borders. D) Small roughly spherical hyaline globules of basement membrane material were found, staining magenta with MGG, and showing marked variability in size.

"Ropy colloid" was observed. Since an adenoid cystic growth pattern with typical hyaline globules has been reported in tumours other than ACC, differential diagnosis considered numerous entities both primitive (follicular, papillary, anaplastic and medullary carcinoma) and metastatic (mainly from salivary glands and from the laryngotraechal complex). Thyroid primitivity was excluded on the basis of the negativity for TTF1 and thyroglobulin. In addition, computed tomography of neck and thorax did not reveal additional lesions in either major salivary glands or the laryngotraechal complex or lungs. The patient referred an ulcerative lesion of the internal surface of the inferior lip, and thus a FNA was carried out which found the same cytologic features as the thyroid nodule (Fig. 2A-B). The final diagnosis was ACC of minor salivary gland metastatic to thyroid. Diagnosis was then confirmed by histological examination of the thyroidectomy specimen, which showed an ACC with cribriform and solid pattern (Fig. 2C-D).

Discussion

Thyroid metastases are uncommon, and most originate from the upper and lower respiratory tract, kidney and breast. Primary ACC of the thyroid gland has never been described, and metastases of ACC to the thyroid are rare and mostly originate from the laryngotraechal complex and breast. Only one previous case of ACC of major salivary glands (the parotid) metastatic to thyroid has been described, while no metastases of ACC to the minor salivary glands have been observed to date. In this report, we describe the first case of minor salivary gland ACC metastatic to the thyroid. ACC represents one of the most malignant tumours of the minor salivary glands. It displays unique features, such as slow but aggressive growth, early invasion of peripheral nerves and/or blood vessels and a high incidence of recurrence and distant metastases, mostly in the lungs. The presence of a significant percentage of solid growth, as in our case, implies poor prognosis. It has to be remembered that an ACC-like pattern may also be found in primary malignant tumours of the thyroid, mainly in papillary carcinoma. However, in these cases, the hyaline globules are early psammoma bodies or thick colloid instead of basement membrane substance of myoepithelial origin. In addition, several cytologic parameters may be helpful in differential diagnosis between thyroid metastases of ACC and primary ACC-like malignant tumours of the thyroid (Tab. 1). The FNA smears from the thyroid nodule in our case showed all the cytologic features that are diagnostic of
ACC described in the literature. Moreover, computed tomography of the neck and thorax was negative, and the negativity for TTF1 and thyroglobulin ruled out primary thyroid carcinoma. The present case highlights the need to be aware of possible metastatic thyroid localization of ACC also originating in minor salivary glands of the oral cavity. This is a very rare event, but it should be taken into consideration, and clinical and cytological findings must be carefully examined. In our case, correct diagnosis was made only after an accurate physical examination of the patient which identified an ulcerative lesion of the oral cavity that resulted to be ACC.

**References**


**Tab. I. Differential diagnosis between metastatic adenoid cystic carcinoma and primary malignant tumours of the thyroid gland with adenoid cystic pattern.**

<table>
<thead>
<tr>
<th>ACC</th>
<th>PTC</th>
<th>FTC</th>
<th>MTC</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pattern</strong></td>
<td>cribiform</td>
<td>papillae</td>
<td>microfollicular</td>
<td>discohesive</td>
</tr>
<tr>
<td><strong>Cytoplasm</strong></td>
<td>basaloid</td>
<td>squamoid</td>
<td>minimal</td>
<td>moderate, with NE granules</td>
</tr>
<tr>
<td><strong>Nucleus</strong></td>
<td>oval with coarse chromatin</td>
<td>round with grooves, distinct nuclei and inclusions</td>
<td>round with fine chromatin</td>
<td>round to spindle, with distinct nuclei and</td>
</tr>
<tr>
<td><strong>Other features</strong></td>
<td>hyaline globules</td>
<td>ropy colloid, psammoma bodies</td>
<td>no colloid</td>
<td>amyloid</td>
</tr>
</tbody>
</table>

AC: anaplastic carcinoma; ACC: adenoid cystic carcinoma; FTC: follicular thyroid carcinoma; MTC: medullary thyroid carcinoma; NE: neuroendocrine; PTC: papillary thyroid carcinoma.
Gastrointestinal stromal tumour of the stomach with osseous differentiation: a case report

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Key words

GIST • Osseous differentiation • C-KIT

Summary

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal neoplasm of the gastrointestinal tract, while osseous metaplasia of this tumour is an unexpected event. To date, no cases have been reported in the literature. Herein, we report a case of a 60-year-old man affected by a GIST with benign osseous metaplasia and mature bone formation. We also discuss the pathogenesis of intratumoural ossification and review the relevant literature. The prognostic significance of ossification in GIST remains unclear because of the limited cases reported.

Introduction

Gastrointestinal stromal tumours (GISTs), accounting for 0.1%-3.0% of all gastrointestinal malignancies, are the most common mesenchymal neoplasm of the gastrointestinal tract and should be differentiated from other mesenchymal tumours. GISTs occur primarily in older patients of either sex, with annual incidences between 11 and 19.6 per 10\textsuperscript{6} population worldwide. GISTs show a wide spectrum of histological appearances and clinical features. Histologically similar tumours span a clinical spectrum from benign to aggressively malignant, and this has lead to a number of attempts to better identify pathological features (both morphological and molecular) that may predict subsequent behaviour, or suggest a specific clinical syndrome. More than half of all GISTs occur in the stomach and about one-third in the small intestine. Most GISTs arise sporadically, but may also occur in association with a number of clinical syndromes, including Carney triad and Carney-Stratakis syndrome. Some cases are reported during pregnancy. The most common clinical presentations include gastrointestinal bleeding, vague abdominal complaints and incidental findings. Small intestinal tumours may cause obstruction with or without intussusceptions. Typically, malignant GISTs disseminate by coelomic spread within the abdomen and/or metastasise to the liver and other organs such as the ovary. Extra-abdominal metastases are extremely unusual. GISTs may also occur in association with other malignancies and may be discovered incidentally during management of other tumours. GIST may range from an innocuous mural or subserosal nodule to a large complex mass that may be transmural in the gastric or intestinal wall, or present as multiple peritoneal nodules. Histologically, about 25% of gastric GISTs have spindle or epithelioid morphology, and a number of cases have mixed features. Most typical spindle cell GISTs are moderately or highly cellular and are relatively monomorphic, with the cells arranged in sheets, ill-defined fascicles or perhaps palisades reminiscent of schwannoma. Mitoses vary from very occasional to abundant. Nuclear pleomorphism is more common in epithelioid tumours, with abundant myxoid stroma. Chondroid metaplasia or calcifications may also occur, and some tumours show rhabdoid morphology. Most sporadic GISTs are caused by the constitutive activation of KIT, a type III receptor tyrosine kinase, which is encoded by the KIT (c-kit) gene located on chromosome 4q12. The mechanism of activation in most sporadic GISTs is an alteration of the structure of the KIT gene.
of the receptor’s extracellular or cytoplasmic domains caused by somatic mutations of the c-kit gene, which leads to dimerisation and autophosphorylation of KIT with subsequent activation of signal transduction cascades in the absence of ligand binding. Inhibition of KIT activity by a specific tyrosine kinase inhibitor, imatinib, often results in dramatic clinical responses. In contrast to GISTs associated with somatic mutations, GISTs caused by germline mutations are extremely rare. Most GISTs lacking KIT mutations harbour mutations in platelet-derived growth factor receptor alpha polypeptide (PDGFRA), a tyrosine kinase receptor that is highly homologous with KIT. PDGFRA mutations are more common in tumours with gastric localisation, which often display a myxoid and epithelioid phenotype. KIT and PDGFRA mutations appear to be mutually exclusive. We report a rare case of GIST with histological osseous metaplasia and mature bone formation.

Case report

A 60-year-old man presenting with abdominal pain was referred to our hospital. His medical and family history was unremarkable. He had no history of previous abdominal surgery. On physical examination, he showed mild tenderness in the upper abdomen quadrant area. Oesophagogastroduodenoscopy showed in the gastric fundus, below the cardias along the greater curvature, a large ulcer crater with raised edges and bottom covered by fibrin with signs of recent bleeding. Following biopsy, histological examination detected the presence of a GIST. Twenty days later, the patient underwent surgery. No evidence of local invasion or distant metastasis was found during surgery. At gross examination, the tumour was 7.6 cm in maximum diameter, projected into the gastric lumen as an endophytic polypoid submucosal growth and prone to surface ulceration and bleeding. It was a well-circumscribed, nodular mass that lacked a true capsule. The cut surface was grey to pink with an increased consistency. Histological examination revealed a proliferation of spindle cells with a mitotic count < 5 mitoses/50 HPF with areas showing focal osseous differentiation. Interlacing bundles of uniform spindle-shaped cells with ovoid or elongated nuclei and fibrillary eosinophilic cytoplasm were observed. The stroma was hyalinised and with focal calcification with areas of metaplastic ossification. Osteoblasts and osteoclasts surrounded the surface of the heterotopic bone. Haversian canals were occasionally identified in the bony trabeculae. Immunohistochemical studies revealed positive staining for CD34 and CD117 (c-KIT). Based on the above findings, the tumour was diagnosed as a GIST with osseous differentiation and low-grade malignancy. The follow-up period was 12 months, and there was no recurrence.

Discussion

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the gastrointestinal tract, arising from the interstitial cells of Cajal, primarily in the stomach and small intestine. They manifest a wide range of morphologies, from spindle cell to epithelioid, but are immunopositive for KIT (CD117) and/or DOG1 in virtually all cases. GISTs may be divided into three main histologic categories: spindle cell type, epithelioid type and mixed type. Spindle cell GISTs are composed of short fascicles and whorls, characterised by spindle-shaped cells with ovoid or elongated nuclei and paranuclear vacuolisation; the stroma may be variable and show areas of myxoid change, calcification or metaplasia. Epithelioid GISTs are arranged in nests or sheets, characterised by rounded cells with abundant clear cytoplasm and vesicular nuclei. A lower percentage of GISTs shows both histological patterns in different areas of the tumour. The degree

Fig. 1. Histological examination revealed proliferation of spindle cells with a mitotic count < 5 mitoses/50 HPF with areas showing focal osseous differentiation.

Fig. 2. The stroma was hyalinised and extensive calcification with areas of metaplastic ossification was present (A). Cells showed strong staining for CD34 (B) and moderate staining for CD117 (c-kit) (C).
of cellularity and nuclear pleomorphism of GISTs vary considerably and do not correlate directly with a worse prognosis. The three main parameters that correlate with prognosis are the tumour location, size and mitotic rate.

Although most tumours are localised at presentation, up to half will recur in the abdomen or spread to the liver. The growth of most GISTs is driven by oncogenic mutations in either of two receptor tyrosine kinases: KIT (75% of cases) or PDGFRA (10%). Treatment with tyrosine kinase inhibitors (TKIs) such as imatinib, sunitinib, or regorafenib is effective in controlling unresectable disease; however, drug resistance caused by secondary KIT or PDGFRA mutations eventually develops in 90% of cases. Adjuvant therapy with imatinib is commonly used to reduce the likelihood of disease recurrence after primary surgery, and for this reason assessing the prognosis of newly resected tumours is one of the most important roles for pathologists.

Approximately 15% of GISTs are negative for mutations in KIT and PDGFRA. Recent studies of these so-called wild-type GISTs have uncovered a number of other oncogenic drivers, including mutations in neurofibromatosis type I, RAS genes, BRAF and subunits of the succinate dehydrogenase complex. Routine genotyping is strongly recommended for optimal management of GISTs, as the type and dose of TKI used for treatment is dependent on the mutation identified.

Focal calcification within a large GIST especially within necrotic areas has been reported. Most calcifications in GISTs are usually of a circumscribed patchy type. Rare cases with extensive calcification that constitutes the major portion of the tumour are reported. Calcification has been suggested to indicate less aggressive tumour behaviour, and a slowly progressing tumour shows dystrophic calcification within the area of tumour hyalinisation. Calcification within GIST has been previously reported, but osseous differentiation is an unexpected event. Heterotopic ossification is not usually seen in neoplasms and is more often seen in reparative and degenerative conditions.

In our case, osseous differentiation was defined by the presence of heterotopic normal bony tissue within the GIST probably related to ischaemia, necrosis, or inflammation in the tumour or surrounding tissue. The pathogenesis of osseous differentiation is still unclear and various theories have been proposed. Currently, multiple cell mediators, including platelet-derived growth factor whose receptor is frequently mutated in gastric GIST, are thought to play a role in the regulation of ectopic bone formation. Bone morphogenic proteins (BMP) play an important role in bone formation by inducing local ossification and synthesising ground substance and collagen, but the final step in bone formation depends on adequate concentrations of calcium and phosphate. As reported by morphoproteomic analysis to define the histogenesis of the heterotopic ossification in tumours, this phenomenon could be the result of pluripotent stromal cells that undergo differentiation to form osteoblasts rather than tumour cells undergoing osseous metaplasia.

In conclusion, we present a very rare case of GIST with extensive osseous differentiation. The prognostic significance of ossification in GIST remains unclear because no cases have been reported in the literature. A careful search for cellular areas and the judicious application of immunostaining will thus make it possible to make a correct diagnosis. Therefore, when osseous nodules are encountered in the gastrointestinal tract, the possibility of a GIST should be considered.

References
La responsabilità del medico pubblico innanzi alla Magistratura Contabile dopo un innovativo indirizzo di coordinamento impartito dal Procuratore regionale del Lazio


L’intervento dell’alto Magistrato nel convegno organizzato da SIAPEC-IAP su “Gestione del rischio e responsabilità professionale in Anatomia Patologica”, tenuto il 25 giugno scorso presso la sala conferenza dell’Ospedale S. Eugenio - Azienda USL Roma C, ha suscitato un vasto consenso tra i docenti relatori e gli altri partecipanti all’incontro scientifico.

Il prevalente interesse per l’indirizzo enunciato dal capo della Procura Regionale del Lazio discende dalla nuova impostazione che si è inteso dare alla questione della responsabilità sanitaria, nel quadro del conflitto tra la linea della c.d. “medicina difensiva” ed il “rifiuto di polizza assicurativa” opposto dalle compagnie di assicurazione per esosità dei premi conseguenti all’incremento dei contenziosi civili e penali ed al comunque aumento dei risarcimenti liquidati ai pazienti danneggiati.

Il Magistrato ha fatto un cenno al recente decreto Balduzzi, che ha introdotto una causa di esclusione soggettiva della responsabilità penale per colpa lieve, lasciando, tuttavia, intatto il sistema di risarcimento del danno innanzi al giudice civile.

Il Procuratore de Dominicis ha voluto, altresì, introdurre nel giudizio di responsabilità amministrativa innanzi alla Corte dei Conti un orientamento che potrebbe definirsi di “Giustizia ponderata”, ove la responsabilità del medico viene vista all’interno dell’apparato di cura ed assistenza pubblica e, poi, valutata con criteri equitativi ed umanitaristi.

Il tema che si passa ad illustrare è tra i più ampi e complessi della letteratura giudiziaria e della giurisprudenza elaborata entro i vari ordini magistraturali. Quella della malpractice medica per colpa professionale è, infatti, argomento che coinvolge molteplici profili di interesse, che vanno ben al di là della materia sanitaria, strictu sensu, specialmente quando si prospettino fattispecie illecite idonee a fare emergere un danno pubblico erariale per effetto del quale viene celebrato il giudizio di responsabilità amministrativa innanzi alla Corte dei Conti.

Il tema può dirsi, altresì, complicato dal fatto che nel processo risarcitorio innanzi al giudice (civile) dei diritti patrimoniali la giurisprudenza della Corte di Cassazione è costante nell’affermare che trattasi di responsabilità contrattuale, con onere della prova a carico della U.S.L. chiamata a risarcire il danneggiato, mentre il medico pubblico, lasciato estraneo al processo civilistico – mancando l’istituto del litisconsorzio necessario nei suoi confronti – quando, infine, verrà chiamato dal P.M. contabile a risarcire la U.S.L., preliminarmente invocherà la violazione del suo diritto di difesa!

Questa gravissima situazione non può, invero, essere superata da leggi “salvifiche” o da un’accorta gestione amministrativa, che, ad esempio, accentui la procedimentalizzazione sanitaria a scapito della serenità e della humanitas che devono sempre caratterizzare la nobile arte della scienza medica.

Occorre, infatti, ripartire dal principio, unanimemente riconosciuto, che il diritto alla salute non implica sem...
pre il diritto alla guarigione e che la responsabilità medica resta comunque obbligazione di mezzo e non di risultato.


Il che non solo sembra ben collegarsi coi fini previsti dal decreto legislativo n. 29 del 1993, per i suoi elementi di risultato, ma richiede la valutazione della colpa soggettiva in concreto.

Ed, infatti, secondo la Consulta, si sarebbe delineato un nuovo modello di responsabilità del pubblico dipendente – da rapportare non ad una valutazione astratta della colpevolezza ma ad una misurazione della responsabilità in concreto.

La valutazione della responsabilità in concreto implica non solo il dovere di accertare l’incidenza dei singoli contributi causativi del danno pubblico, ma richiede che si determini “la porzione di danno addebitabile a carico del presunto responsabile”.

Ed, invero, spetta al giudice contabile – ed al P.M. che è incastrato presso di esso – determinare quan-

do all’attività (dannosa) debba restare a carico dell’apparato e quanto a carico del dipendente.

Nella ricerca del punto d’equilibrio tra danno addebitabile e danno risarcibile – facendo rientrare nella sfera del primo la quota che deve gravare sulla P.A., a titolo di rischio oggettivo, e riversando sul dipendente l’altra quota di danno – ove potrà comunque esercitarsi il potere riduttivo in ragione di eventuali circostanze attenuanti – non appare unicamente evidenziare che la colpa normale o culpa levis, non contestabile al dipendente pubblico, deve ritenersi assorbita nel concetto di rischio oggettivo che è insito in qualsiasi attività o funzione, tanto pubblica quanto privata (cfr. articoli 1228, 2047, 2048 e 2049 codice civile).

Quando il codice civile ipotizza situazioni di danno indiretto e/o di responsabilità oggettiva – così, nel caso del danno cagionato dall’incapace d’intendere e di volere (art. 2047); così, nell’ipotesi della responsabilità dei genitori e dei tutori, dei precettori e dei maestri d’arte (art. 2048, culpa in vigilando); e così, nelle situazioni di responsabilità dei padroni e dei committenti (art. 2049, culpa in eligiendo) – lo fa con riferimento a rapporti di relazione; invece, nelle situazioni scrutinate dalla Corte Costituzionale il quadro è completamente diverso, perché il quesito di fondo è collegato a problemi della buona giustizia. Orbene, ritiene lo scrivente che nel conflitto tra la c.d. medicina difensiva ed il rifiuto di polizza assicurativa la magistratura contabile debba giocare un ruolo equitativo di elevato livello dirimente.

Si tratta, infatti, di applicare ai giudizi sulla responsabilità medica, per malpractice sanitaria, un criterio di quantificazione equilibrato e ponderato, seguendo l’indirizzo della Corte Costituzionale come proclamato con la predetta sentenza additiva n. 371 del 1998.

Infatti, l’orientamento della Consulta è consistito nell’affermare che le finalità di una giustizia monitoria, qual è quella amministrativo-contabile, caratterizzata dalla combinazione di elementi restitutori e sanzionatori, sia quella di accertare “quanto del rischio dell’attività debba restare a carico dell’apparato e quanto a carico del dipendente”.

Ciò, perché, ad avviso di chi scrive, la colpa normale o culpa levis, dopo l’importante riforma del 1994-1996, deve ritenersi integralmente assorbita o traslata nel rischio oggettivo a carico della P.A.

Ed, infatti allorché la legge ha elevato il grado di colpevolezza, dalla soglia della colpa lieve a quella della colpa grave, chi esercita un’attività per un interesse superiore – ad esempio, per la tutela della salute, ai sensi dell’articolo 32 della Costituzione – non può rispondere del risultato negativo nella sua integralità, perché nella vicenda illecita potrebbero coesistere fattori imponderabili, legati, ad esempio, alle scarze risorse tecnologiche o all’organizzazione ospedaliera. Fattori che potrebbero non avere particolare evidenza o incisività nel singolo processo di responsabilità amministrativa.

In nome di una giustizia amministrativa ponderata e – se volete – umanitaristica, si tratterà di stabilire, almeno orientativamente, che il medico pubblico debba essere chiamato a rispondere della metà del danno, rispetto a quanto accertato e liquidato in sede civile o penale, potendosi accollare la restante parte alla USL che avrà poi diritto all’azione di rivalsa nei confronti dell’istituto assicurativo, ove sia stata convenuta apposita clausola contrattuale.

La suddetta ripartizione dell’addebito (metà e metà) non solo costituisce un punto di equilibrio tra apparato sanitario e medico responsabile, ma, nella prospettiva di una giustizia monitoria, ove sia stata convenuta apposita clausola contrattuale.

La suddetta ripartizione dell’addebito (metà e metà) non solo costituisce un punto di equilibrio tra apparato sanitario e medico responsabile, e tra colpa medica e garanzia assicurativa, ma, nella prospettiva di una giustizia monitoria e di indirizzo, mira a proiettare nel giudizio di responsabilità personale e l’intrasmissibilità del danno pubblico e private, ma, nella prospettiva di una giustizia monitoria, mira a proiettare nel giudizio di responsabilità personale e l’intrasmissibilità del danno pubblico e privato, ma, nella prospettiva di una giustizia monitoria, mira a proiettare nel giudizio di responsabilità personale e l’intrasmissibilità del danno pubblico e privato. Fattori che potrebbero non avere particolare evidenza o incisività nel singolo processo di responsabilità amministrativa.

La discriminazione tra danno addebitabile e danno risarcibile e la “equa ripartizione del quantum debetur,
potrebbe costituire un criterio giustiziale opportuno e condivisibile. La Corte dei Conti deve raccogliere l’ennesima sfida per il ripristino del buon senso e della ragione su questioni vitali per il buon andamento e l’armonia sociale. Naturalmente l’indirizzo che si sottoscrive necessita di una verifica sostanziale sul piano dell’esperienza giuridica che andrà a maturarsi innanzi alla Sezione Giurisdizionale del Lazio, giudice territorialmente competente.

Prof. Angelo Raffaele de Dominicis
Procuratore Regionale del Lazio
Nella presente ricerca verranno offerte alcune prospettazioni generali sugli errori che possono verificarsi nella diagnosi basata sull’esame di organi, tessuti e/o di cellule ovvero sui preparati istologici e citologici di competenza dell’anatomopatologo. I quadri di valutazione analitica sono riconducibili sostanzialmente a *tre tipi di rischio* dovuti, in via diretta o indiretta, ad errore medico.

Prima di tutto va rappresentato l’*errore diagnostico* che scaturisce da imperizia professionale e/o da negligenza grave ed inescusabile. Questo primo quadro di rischio è quello maggiormente connesso con gli studi di medicina legale.

Il secondo quadro di rischio consegue, invece, ad una situazione di errore che abbiamo definito, per induzione; derivante, cioè, dalla non corretta trasmissione delle biopsie e, quindi, da disfunzioni organizzative del reparto richiedente e nella fase della comunicazione e delle *interrelazioni tra clinico ed anatomopatologo*.

I relativi difetti devono ritenersi “esterni” alle funzioni del Patologo; nondimeno essi ne condizionano l’attività, sviandola ed invalidandola. Il terzo quadro di rischio discende da quello che può definirsi essere l’*errore per deduzione*, a causa di difetti sulla conservazione e/o sull’allestimento o preparazione da parte della equipe tecnica dei reperti istopatologici. Nello scenario suindicato i difetti sono interni e, quindi, di carattere organizzativo.

Si manifesta comunque, di chiara ed immediata evidenza la differenza del grado di responsabilità tra le tre suindicate tipologie di rischio, riconducibili o all’*errore diagnostico* oppure ad altre anomalie esterne o interne al laboratorio di Anatomia Patologica. Prima di affrontare l’analisi delle predette prospettazioni di rischio occorre, tuttavia, fornire una premessa di carattere generale, circa i rapporti tra il clinico ed il patologo e, quindi, fare emergere le connessioni tra referito diagnostico formulato dall’anatomopatologo e decisione conclusiva spettante al clinico.

Infatti, il giudizio definitivo circa la scelta dell’opzione chirurgica più appropriata o della cura che si ritenga meglio adeguata al malato spetta al medico clinico, nel senso di ritenere che la responsabilità del medico pubblico debba essere di *tipo contrattuale* (art. 1218 e 1228 C.C.); per cui, spetta alle ASL, ove il medico trovasi incardinato ovvero allo stesso medico provare che
il danno alligato dal malato non sia dipeso dalla prestazione sanitaria effettuata (medica o chirurgica che sia), ma da altri fattori (imprevedibili o non prevenibili) estranei all’intervento curativo effettuato.

In questo quadro, ove l’obbligazione medica resta, come nel passato, obbligazione di mezzo e non di risultato – ragione per la quale il paziente non può pretendere la sua guarigione! – un ruolo centrale riveste il c.d. consenso informato (G. Cricenti).

Possono in proposito, richiamarsi due significative sentenze della Corte di Cassazione.

Con la decisione della III Sezione civile, n. 9471 del 19 maggio 2004, si è rappresentata una responsabilità sanitaria di tipo oggettivo, con l’affermazione che essa sia strettamente connessa con la struttura assistenziale entro cui il medico trovasi ad operare. Peralto, la responsabilità della struttura sanitaria è emersa in modo netto allorché si è parlato di trasfusioni di sangue infetto e, quindi, sono fioccate le condanne (Cass. SS.UU. n. 577 dell’11.1.2008).

Con altra sentenza del 2004, Sez. III civ. n. 4400, si è poi molto enfatizzato sull’errore diagnostico, ribadendo la natura contrattuale della responsabilità sanitaria, ma affermando – con poca coerenza – che la diligenza professionale del medico deve trovare il suo parametro normativo nell’articolo 1176, secondo comma, C.C.; ove, al contrario, la valorizzazione del grado di diligenza va a fondarsi “sulla natura dell’attività esercitata”; e, quindi, proprio in virtù della predetta disposizione codicistica, occorre tenere conto delle oggettive difficoltà della professione sanitaria; ed anche per questo non riconducibile allo schema meccanistico della responsabilità contrattuale, come sembra discendere dall’art. 2236 C.C.

Sempre secondo questa sentenza va applicato il c.d. criterio probabilistico che si manifesta, poi, essere estremamente rigoroso nei confronti dei medici.

L’approdo di quest’orientamento giurisprudenziale, molto distante, a nostro avviso, dal predetto art. 1176, secondo comma, C.C. condurrrebbe ad una responsabilità contrattuale, ex art. 1321 C.C., di carattere speciale (contratto di speditalità, ecc.).

Com’è noto, nelle ipotesi civilistiche di responsabilità extratratuituale, ex art. 2043 c.c., il danneggiato deve provare che l’evento illecito è dipeso dal fatto ingiusto del danneggiante; invece nell’ipotesi di responsabilità contrattuale, ex art. 1218 cc, il danneggiato deve alligare solo il danno subito, mentre spetterà all’altra parte dimostrare che l’illecito non è conseguenza del suo comportamento bensì di circostanze esterne ed imprevedibili e, comunque, non dipendenti dalla sua prestazione.

Spostando nell’ambito sanitario i due schemi di responsabilità civilistica, nel primo caso (r. extratratuituale) l’infermo dovrà non solo allegare il fatto illecito della sua mancata guarigione o della sua pregiudizievole menomazione, ma dimostrare che la malattia allocata sia stata la diretta conseguenza della incompetenza professionale del medico o della sua colpevole prestazione, intesa come malpractice sanitaria ossia come ingiustifcabile negligenza ed inosservanza dei doveri professionali; nel secondo caso, invece, la posizione dell’infermo (r. contrattuale) è alquanto comoda, siccome egli dovrà allegare e provare soltanto la sua malattia oppure la sua mancata guarigione, incombendo l’oner della prova sul medico o sull’ente ospedaliero nel quale il sanitario trovasi incaricato che il presunto illecito sanitario non sia dipeso dall’intervento chirurgico o dalla cura praticata.

Come si può vedere le due situazioni, di responsabilità extratratuituale o extratratuituale, sono molto differenti tra loro sia per quanto riguarda l’oner della prova, sia in relazione alla dimostrazione del nesso di casualità tra il comportamento attivo-omissivo ed evento dannoso (mancata guarigione, ecc.).

L’aver adoddato sul medico l’oner di provare che il pregiudizio biologico o psicofisico non sia dipeso dalla sua prestazione bensì da fattori imprevedibili ed imprevendibili appare decisione di estrema gravità nei confronti dei dottori della scienza medica.

In passato la Cassazione aveva sempre ritenuto che la responsabilità sanitaria dovesse rientrare nello schema dell’extraitratuitualità, ex art. 2043, ma con l’avvento della medicina pubblica, i cui oneri di spesa sono a carico del Servizio Sanitario Nazionale (SSN), tutto il quadro è cambiato con effetts anche sulla competenza delle magistrature (civile, penale e amministrativa).

Infatti, se i processi per responsabilità sanitaria vengono celebrati innanzi all’Autorità Giudiziaria Ordinaria (AGO), nelle sedi civili o penali, l’azione di rivalsa a difesa dell’economia pubblica – ove la ASL sia stata tenuta a garantire il danneggiato dalla malpractice sanitaria – deve tesserini innanzi alla Corte dei conti su iniziativa del PM contabile.

Va poi messo ben in evidenza che per i fatti dannosi che integrano illeciti penali, colposi o dolosi, si osservano gli orientamenti della giurisprudenza penalistica (cfr. Sez. Unite Penali 12.07.2012 n. 27), inclini ad applicare l’art. 40 C.P. secondo il quale “non impedire un evento, che si ha l’obbligo di impedire, equivale a cagionarlo … qualora il compimento dell’attività omessa avrebbe impedito, con quasi certezza, il verificarsi dell’evento stesso” (teoria della causalità adeguata).

Al contrario, nella giurisprudenza civilistica (cfr. Sez. III Civ. n. 4400 del 2004) si applica il rapporto di causalità ipotizzato dall’art. 41 C.P., secondo cui la condotta omissiva del medico viene vista come condizione necessaria dell’evento (teoria della condicio sine qua non).

L’indicata giurisprudenza civile ha elaborato, altresì, il concetto di probabilità tecnica, per cui l’attività del medico, se intervenuta con tempestività e correttezza, avrebbe evitato, non con quasi certezza!, ma con elevata probabilità, il verificarsi dell’evento pregiudizievole per la salute dell’ammalato.

In sostanza l’orientamento civilistico, ora prevalente (condicio sine qua non) si manifesta più severo di quello penalistico (della causalità adeguata) perché all’ipotesi della quasi certezza dell’evento si è inteso sostituire quello della elevata probabilità dell’evento, sia pure secondo le previsioni tecniche della scienza medica.

Conclusivamente la Cassazione, nel prendere in esame il
rapporto di causalità relativamente ai fatti illeciti omis-
svi ha richiamato entrambi gli articoli 40 e 41 C.P., e
tuttavia abbiano l’impressione che nel quadro della re-
sponsabilità medica l’indirizzo civilistico sia più severo
ed intransigente di quello penalistico.
Va, peraltro, ricordato che, con la riforma del processo
civile in grado di appello e di legittimità innanzi alla
Corte di Cassazione, il giudizio di danno per respons-
sabilità medica se confermato in secondo grado, diverrà
automaticamente inammissibile innanzi alla Corte di
Cassazione per palese improbabilità di accoglimento.
Differà così improponibile la censura del difetto di mo-
tivazione avverso la sentenza impugnata, ex art. 360,
n. 5 cpc.
La giurisprudenza civile della Cassazione in tema di
responsabilità medica tende ormai a rappresentare uno
spazio di dannosità sanitaria di grande dimensione.
Può darsi comunque, che mentre la responsabilità del
medico è sanzionabile in sede civile, anche solo per
colpa lieve o colpa normale, salvo le garanzie derivanti
dalla polizza assicurativa; invece, innanzi al giudice
contabile la responsabilità sanitaria deve fondarsi alme-
no sulla colpa grave.
Inoltre, secondo la giurisprudenza contabile la colpa
professionale, ex art. 1176, secondo comma, si identi-
fica con la colpa grave tout court, ai sensi dell’articolo 1
La Corte dei Conti, peraltro, non rifiuta gli atti d’indirizzo
giurisprudenziale impartiti dalla Corte di Cassazione né
quelli delle giurisdizioni di merito (per ultimo, senten-
za della Sezione Giurisdizionale del Lazio n. 234 del 6
marzo 2013). Inoltre, devono sempre costituire indirizzi
generali in materia di responsabilità civile del medico
le decisioni assunte dalla Corte Regolatrice, a Sezioni
Unite civili, rattonie materia (cfr. sent. n. 589 del 1999;
n. 21619 del 2007; n. 576 del 2008; n. 16123 del 2010;
n. 17143 del 2012 e n. 19873 del 2013).
In estrema sintesi, può darsi che sembra costante
nell’indirizzo della Cassazione l’orientamento contrat-
trualistico, fondato sull’obbligo della particolare dili-
genza professionale, ex art. 1176, secondo comma C.C.
e con le sole limitazioni dei problemi di speciale diffi-
coltà, ex art. 2236 c.c.
Tuttavia si sta facendo strada nella giurisprudenza di meri-
to l’interpretazione in virtù della quale il paziente-danne-
ggiato-attore non solo deve provare l’esistenza del rapporto
(interferenza o visita ambulatoriale) e l’insorgenza di una
malattia o l’aggravamento di una precedente patologia ma
costituisce presupposto necessario ed indispensabile, non
solamente per il medico o per l’equipe sanitaria, ma, a seguito
delle vaste ed incisive riforme introdotte all’inizio del
XXI secolo, le condizioni del processo contabile appa-
iono, quindi, non più soddisfacenti. Innanzi alla Corte dei Conti
può essere chiamata a rispondere anche una società o
un’organizzazione convenzionato ad es., clinica specialistica
convenzionata.
Tuttavia non è questa la sede per una disamina dei vari
profili di collegamento tra i molteplici procedimenti giudiziari mirati all’accertamento della responsabilità
medica. Si può dire, comunque, che la sentenza pronunciata nel processo civile, per la declaratoria di danno
promossa dal paziente-danneggiato non ha efficacia di giudicato esterno nel giudizio di responsabilità amminis-
trativa innanzi alla Corte dei Conti. È ciò, a prescindere
dalla partecipazione o meno del sanitario nel processo
civile, quale presunto autore del fatto lesivo e come tale
convenuto nel quadro della giurisdizione ove il medico.
In sostanza, può dirsi che sembra costante
nell’indirizzo della Cassazione l’orientamento contrat-
trualistico, fondato sull’obbligo della particolare dili-
genza professionale, ex art. 1176, secondo comma C.C.
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(interferenza o visita ambulatoriale) e l’insorgenza di una
malattia o l’aggravamento di una precedente patologia ma
altri aspetti sono svolti in modo inadeguato, e quindi può
ottenere il titolo di illecito
in modo negativo
secondo una definizione differente.
La A.S.L. o il medico-danneggiante-convenuto ha
l’onere di dimostrare che l’inadempimento o l’inesatto
adempimento non vi è stato e che, pur esistendo, non è
stato eziologicamente rilevante, tale da causare l’evento
(malattia) alligato dal paziente. In buona sostanza, sem-
brerebbe che la più recente giurisprudenza civile di
merito (cfr. Trib. Varese, sentenza 26 novembre 2012;
Trib. Arezzo, sentenza 14 febbraio 2013) voglia alleg-
gerere l’onere probatorio a carico del medico.
In via generale la fonte giuridica della responsabilità
sanitaria innanzi alla Corte dei Conti resta l’art. 28 del-
la Costituzione, cui può aggiungersi l’art. 47 della legge
n. 833 del 1978, il cui regolamento di attuazione appro-
vato con D.P.R. n. 761 del 20 dicembre 1979 prevede
che al personale sanitario si applicano le disposizioni di
characteré generale che concernono il pubblico impiego
in materia di responsabilità amministrativa.
È stato giustamente osservato che nella materia sanitaria
può parlarsi correttamente di danno erariale solo nel caso
in cui una A.S.L. o una Azienda Ospedaliera
convenzionata siano state chiamate a rispondere del danno
causato da un sanitario o da un’equipe medica incordi-
nati nelle relative strutture assistenziali.
Il rapporto di servizio, quale condizione generale per
accedere al giudizio innanzi alla Corte dei Conti, giudicato
di carattere generale che concernono il pubblico impiego
in materia di responsabilità amministrativa, costi-
tuisce presupposto necessario ed indispensabile, non
solamente per il medico o per l’equipe sanitaria, ma, a seguito
delle vaste ed incisive riforme introdotte all’inizio del
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Tuttavia non è questa la sede per una disamina dei vari
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**PROSPETTIVE GENERALI DI RISCHIO IN ANATOMIA PATHOLOGICA**
dei Conti viene riservato il potere di accertare la responsabilità del medico, presunto responsabile.

Passando, ora, alla seconda prospettazione di rischio, per errore dovuto ad induzione, occorre premettre che il patologo può essere indotto a sbagliare per fatto esterno al suo laboratorio.

Ciò potrebbe essere riconducibile addirittura al clinico o al suo staff nella fase che si definisce preanalitica; preordinata, appunto, al riferito finale di Anatomia e Istologia Patologica.

Nella circostanza potrebbe trattarsi di rappresentazioni diagnostiche sbagliate o trasmissione di reperti carenti o non appropriati che producono come diretta conseguenza la formulazione di una diagnosi isto-cito-patologica affetta da errore ed, altresì, perfino dannosa.

Può dirsi, perciò, che l’errore per induzione, potendo incidere negativamente sulla diagnosi del patologo, cui incombe l’obbligo di identificare il processo patologico dell’infarmità e valutare i dati morfologici di questo processo, potrebbe avere ricadute devastanti sul malato ed aprire la strada alla soluzione negativa del trattamento curativo prescelto.

Prima di entrare nell’analisi e commento delle possibili disfunzioni che possono avere ingenerato l’errore per induzione occorre preliminarmente affermare che le eventuali responsabilità non possono avere carattere indiretto o oggettivo, dovendo essere imputate a carico di chi, per avventura o per colpa professionale, abbia formulato una diagnosi clinico-chirurgica errata ovvero di chi, per negligenza o imperizia, abbia allestito biopsie inadeguate e non accurate rispetto alle condizioni dell’infarmità.

Nel quadro delle imputazioni di responsabilità non solo valgono i criteri basati sulla soggettività e sulla colpevolezza, ma le deroghe al principio di responsabilità individuale devono essere rigorosamente preordinate dalla legge ed accolte dalla giurisprudenza dei giudici, che devono interpretare ed applicare le norme sia pure con riguardo alle disposizioni-parametro prestabilite in Costituzione.

Incidentalmente ed a titolo di esempio, si ricordi che l’omicidio preterintenzionale, detto anche "oltre l’intenzione", rientrando nello schema della responsabilità per colpa oggettiva, è stato sempre considerato dalla dottrina penalistica come una anomalia giuridica o una fattispecie delittuosa al limite della buona evoluzione giudiziaria.

Analogamente, nel giudizio civile i casi di colpa oggettiva o indiretta sono tassativamente previsti dalle leggi o da specifiche disposizioni del codice civile.

Ad esempio, il danno causato dall’incapace d’intendere e di volere è a carico di chi sia tenuto alla sorveglianza (art. 2047 C.C.); il danno causato dal minore è a carico dei genitori; quello degli allievi è a carico degli insegnanti (art. 2048 C.C.); il danno provocato dai domestici e dai commessi è a carico dei padroni (art. 2049 C.C.); il danno causato da animali è a carico dei proprietari (art. 2052 C.C.). Il codice civile prevede anche altre situazioni di responsabilità oggettiva: nell’esercizio di attività pericolose (art. 2050 C.C.), nella custodia di beni (art. 2051 C.C.), nella rovina di un edificio (art. 2053 C.C.), nella circolazione di veicoli (art. 2054 C.C.) ecc. Dunque, al di fuori dei casi specificatamente stabiliti dalle leggi e dalle norme del C.C. si configura inammissibile una responsabilità oggettiva o indiretta, siccome ciascuno deve rispondere degli atti a lui imputabili in quanto commessi con piena capacità di intendere e di volere.

Ne consegue che nel caso dell’errore diagnostico ad-dossato all’anatomopatologo per induzione e, quindi, causato da errore presupposto posto in essere con piena colpevolezza dal clinico o dal chirurgo o dai loro staff, la responsabilità non può essere presunta ne imputata a titolo formale.

Infatti, chi la contesta ha l’onere di agire e di provare la lesione di un interesse innanzi al giudice competente, ai sensi degli artt. 99 e 100 c.p.c.


Infine, nel concorso di persone nel reato si applica il principio secondo cui ciascuno risponde dell’intero fatto che costituisce reato (art. 110 c.p.), salvo le circostanze aggravanti o attenuanti per ciascuno dei compartecipi.

Il breve excursus normativo, che si è voluto sommariamente descrivere, non fa che confermare il principio generale, valido in tutti i sistemi giuridici, secondo cui la responsabilità si configura, come diretta e personale, salvo le ipotesi di concorso e di corresponsabilità.

Oltre, noi riteniamo che nel caso in cui l’anatomopatologo cada in errore per fatto induttivo le imputazioni di responsabilità devono gravare integralmente sull’autore dell’errore presupposto, da cui il patologo è stato tratto in inganno e sviato nell’esercizio della sua funzione di consulente medico qualificato nel campo della diagnositca cito-isto-patologica.

La letteratura scientifica ha passato in rassegna le circostanze, anche dovute a caso fortuito, che possono dare luogo ad errore per induzione, con diagnosi clinico-chirurgiche difettose o viziate nella fase della rappresentazione e della trasmissione dei reperti: dalle biopsie incisionali, chirurgiche escisionali, alle resezioni chirurgiche, alle biopsie ottenute tramite sottile ecoguidato, il TAC guidato ed ai campioni citologici.

Proporre in questa sede un’esenzione esaustiva è indagine non facile né agevole.

Va però tenuto presente che la letteratura di settore ha sempre segnalato il rischio derivante da rappresentazioni sbagliate e inesatte, denunciandone la pericolosità e le potenzialità negative: come fattori di rischio che condizionano le qualità e la funzione del referito dell’anatomopatologo.

Oltre, nella fase preanalitica, il rischio derivante da er-
Il concetto di rischio nella diagnosi istopatologica comprende l’organizzazione dell’attività dell’équipe tecnica o a del laboratorio di anatomia patologica per fatti legati alle funzioni ab externo dalle altre che si riscontrano all’interno.

Nella terza prospettiva di rischio si è usata la logica dell’allestimento dei preparati istologici e citologici, comunque la successiva fase analitica, che implica la corretta descrizione macroscopica delle biopsie dei pazienti, il campionamento dell’estratto del criostato e l’allestimento dei preparati istologici e citologici, compreso il referto del patologo, possono vedersi compromessi.

In via molto semplificata, tra le operazioni di rischio si annoverano:

1) campioni da pazienti sbagliati;
2) prelievi non idonei (come nel caso di una biopsia superficiale invece che eschissionale);
3) tessuto inadeguato per una diagnosi patologica;
4) campione inserito in liquido fissativo sbagliato o non idoneo;
5) richiesta di un esame cito-istopatologico errato;
6) perdita di un tessuto diagnostico a causa di un esame intraoperatorio non necessario;
7) errore nella etichettatura e registrazione del campione;
8) scambio di campioni tra un paziente e l’altro;
9) informazioni errate o assenti, con conseguente sbagliata formulazione diagnostica nella richiesta di esame;
10) disfunzioni durante il trasporto dal reparto clinico al servizio di Anatomia Patologica che provoca modificazioni dello stato del campione biologico;
11) perdita del campione durante il trasporto.

Nella terza prospettazione di rischio si è usata la logica, errore per deduzione, per separare le disfunzioni ab externo dalle altre che si riscontrano all’interno del laboratorio di anatomia patologica per fatti legati all’organizzazione dell’attività dell’équipe tecnica o a negligenza ed imperizia imputabili ai tecnici.

I tecnici di laboratorio devono ritenersi pienamente responsabili del proprio operato e rispondere di tutta la gestione del laboratorio, della sua sicurezza, della qualità dell’utilizzo dell’hardware e del software, della preparazione delle inclusioni in paraffina, dell’allestimento dei vetrini con sezioni di tessuto o cellule, delle colorazioni di routine e speciali, della conservazione dei vetrini e delle inclusioni. Il compito della equipe tecnica è di annullare le possibilità di confondere i campioni di un paziente e, quindi, garantire che in fase di accettazione, allestimento delle inclusioni in paraffina, taglio delle inclusioni con allestimento dei vetrini con sezioni di tessuto e consegna dei casi per la riferitazione all’anatomopatologo risultati sia sempre attuata una politica coerente che preservi e garantisca l’identificazione univoca del soggetto, dei suoi campioni biologici e dei risultati prodotti (vetrini allestiti con sezioni di tessuto o cellule).

Il che implica non solo un adeguato impegno professionale nel trasferimento delle biopsie nelle biocassette, ma anche nell’assistenza al patologo, sia nelle operazioni di campionamento che in quelle autoptiche in sala settoria. Anche nell’errore per deduzione il rischio dell’anatomopatologo appare legato all’attività di diretta collaborazione dei tecnici di laboratorio ed alla nuova figura del tecnico coordinatore.

La casistica delle disfunzioni comprendono:

1) errori essenziali nella fase di accettazione e della presa in carico dei campioni biologici (biopsie, parti di organo o organo intero);
2) difetti nella etichettatura del materiale e nella identificazione del paziente cui assegnare il campione;
3) errore nella etichettatura della biocassetta e del vetrino;
4) contaminazione dell’inclusione in paraffina o del vetrino allestito con la sezione di tessuto;
5) errori nell’allestimento del materiale citologico;
6) errori nel taglio delle inclusioni (la lesione non viene intercettata o addirittura scartata nella fase di sgrossamento della inclusione);
7) perdita della biocassetta o della inclusione relativamente a campioni che non possono essere successivamente ricostruiti;
8) scambio di vetrini; scambio di biocassette; scambio di inclusioni;
9) errori relativi al paziente (sbagliato) o al clinico (sbagliato);
10) errori nella consegna del referto o per informazioni al clinico sbagliato.

Sulla materia, in disparte il concorso di responsabilità, di cui si è già detto, può ritenersi fondamentale la legge 10 agosto 2000 n. 251 che ha dettato una specifica disciplina sulle professioni infermieristiche e sui tecnici di laboratorio.

Ma di essa dovrà farsi ampio ed analitico valutazione in altra ricerca.
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Pathologica publishes six issues per year. The annual subscription rates for non-members are as follows:
Italy € 105.00; all other countries € 115.00. This issue € 20.00 for Italy, € 25.00 abroad.

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

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Journal printed with total chlorine free paper and water varnishing.

Printed by Pacini Editore, Pisa, Italy - December 2014