Case Report:
Thin-Layer Cytopathology of a Gastrointestinal Stromal Tumor (GIST) in Effusion: Diagnostic Dilemmas

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Abstract. Although gastrointestinal stromal tumors (GISTs) are uncommon, they represent the most frequent mesenchymal neoplasms of the gastrointestinal tract. During recent years, considerable information has been published about the pathogenesis, molecular biology, histological criteria, surgery, and adjuvant pharmacological treatment of GISTs, but there have been few reports about the cytologic diagnosis of GISTs, particularly in effusions; in such specimens these neoplasms cause a wide range of potential pitfalls. In this case report, we show that by combining morphological and immunocytochemical studies on thin layer slide preparations, the cytologic diagnosis of GISTs can be both accurate and efficient.

Keywords: ascitic fluid, gastrointestinal stromal tumor, immunocytochemistry, cytologic diagnosis

Introduction

Until the late 1970s, there was much misunderstanding about gastrointestinal stromal tumors (GISTs). They were believed to originate from smooth muscle tissue and they were called smooth-muscle tumors of undetermined malignant potential (STUMPs) [1]. In the 1980s, immunohistochemical studies showed variable expression of antigens of muscular derivation together with markers of neural derivation. In 1983, Mazur and Clark [2] reported that gastric stromal tumors lack the ultrastructural features of smooth muscular differentiation as shown by leiomyomas and leiomyosarcomas; they postulated that the stromal tumors originate from mesenchymal stem elements, the latter considered to be progenitors of spindle and epithelioid cells. In 1995, Miettinen et al [3] used the term “GIST” to designate a group of mesenchymal tumors with myogenic or neurogenic differentiation; they emphasized the distinction between stromal tumors that arise from the gastrointestinal tract and such neoplasms at other sites (eg, uterus). In 1998, Kindblom et al [4] reported that GISTs develop from pluripotential stem cells that are programmed to differentiate into intestinal cells of Cajal (ICCs) and smooth muscle cells.

In particular, ICCs represent a network of cells, intercalated between nerve fibers and muscle cells, involved in the generation of electrical pacemaker activity triggering gut contraction. A major advance in GIST knowledge was the discovery of c-kit proto-oncogene mutation in this tumor, as reported by Nakahara et al [5]. The c-kit gene encodes a transmembrane tyrosine-kinase growth factor receptor called kit; the kit receptor can be demonstrated by immunohistochemical staining for CD117. This important finding has led to the classification of GISTs as a separate entity from smooth muscle neoplasms, which are CD117-negative, and to the development of a therapeutic target for GISTs [6].
GISTs represent approximately 1% of all primitive tumors and only 0.1-3% of all gastrointestinal neoplasms, but, at the same time, they are the most frequent mesenchymal lesions of the gastrointestinal tract. GISTs are frankly malignant in 10-30% of cases and cause mortality in 2% of cases. Criteria to predict their tumor behavior include size, necrosis, and mitotic rate. The tumor appears usually as an endophytic submucosal lesion, with diameter ranging from <1 cm to >30 cm. The most frequent locations of GISTs are the stomach (60-65%), small bowel (20-25%), large bowel-rectum (5-10%), and esophagus (<5%). Primary omental or mesenteric localizations are rare; in such cases the correct term is EGIST (extra-gastrointestinal stromal tumor). Mesentery and omentum lack ICCs, which confirms GIST’s origin from multipotential mesenchymal cells. GIST has no preference for gender; its peak incidence is between 40-70 yr, with a broad age distribution [7].

GISTs often become incidentally evident during radiological procedures, during surgery for other lesions, or at autopsy. Histopathologic examination of surgical resection specimens represents the most common method for GIST diagnosis. With regard to morphology, about 70% of GISTs are spindle cell type, 20% are characterized by epithelium-like cells (epithelioid GIST variant), and the remaining 10% have mixed spindle/epithelial phenotype (biphasic GIST) [8,9]. Reports on the cytomorphology of GISTs are sparse and generally based on lavage, brushing, and washing specimens; information about the cytologic aspects of GISTs in effusions is very rare [10,11].

Case Report

A 53-yr-old male was referred to our hospital in April 2005 for persistent epigastric pain and acute melena. He had a family history of neoplastic disease (lung and bladder cancer in his father). Physical examination showed signs of significant ascites; laboratory tests (ie, blood count and serum electrolyte concentrations) were normal. Abdominal ultrasound confirmed the presence of copious ascitic fluid in the abdominal cavity and demonstrated small peritoneal lesions as well as a solid/cystic mass in the stomach, suspected to be malignant. Evacuative paracentesis was performed to mitigate the patient’s symptoms and to provide specimens for cytological diagnosis. Esophagogastroduodenoscopy showed inflammatory granulation tissue associated with an exuberant connective tissue reaction on the posterior gastric wall, measuring up to 8 cm along the corpus-antrum junction. Multiple gastric biopsies were taken from these sites. Abdominal computed tomography showed multiple, well-defined intramural masses in the mesenteric area. The computed tomography did not reveal liver metastases.

Methods

Fresh ascitic fluid samples (140 ml) obtained by evacuative paracentesis were transported to the cytology laboratory. For slide preparation, we used the filtration-adhesion method (CyroSlip procedure, Bio-Optica, Milan, Italy). Briefly, after centrifugation at 800 rpm for 10 min, ascitic fluid underwent absorption and filtration through a 5 μm polycarbonate membrane; cells captured by the membrane were immediately touch-transferred to slides, in a uniform monolayer of 6 mm diameter. The slides were fixed in 95% ethanol for 30 min and stained by the Papanicolaou and PAS methods for cytochemical studies, and by a panel of 7 mouse anti-human antibodies for immunocytochemical studies: pan-cytokeratin AE1/AE3 (Novocastra Laboratories, Newcastle, UK); monoclonal, 1:100 dilution); vimentin (Novocastra, monoclonal, 1:50 dilution); cromogranin (Novocastra, monoclonal, 1:200 dilution); neuron-specific enolase-NSE (Ylem, Avezzano, Italy; monoclonal, 1:70 dilution); CD34 (Novocastra, monoclonal, 1:70 dilution), S100 (Dako-Cytomation, Carpinteria, CA, USA, polyclonal, 1:100 dilution); and CD117/c-kit (DakoCytomation, polyclonal, 1:100 dilution). Diaminobenzidine was the chromogen. The slides were counterstained with hematoxylin. For negative controls, the slides were incubated with normal goat serum instead of the primary antibody. Positive control slides with tumor cells known to express the tested antigens were included in the immunocytochemical procedure.

Results

Cytology. Papanicolaou-stained slides showed moderate to high cellularity in a bloody background (Fig. 1A). Tumor cells were often arranged in 3-dimensional cluster aggregates with high cell density; rare tumor cells occurred singly and in loosely cohesive groups. Aggregated cells assumed a prevalent gland-like pattern that mimicked adenocarcinoma (Fig. 1B). At high magnification the following details were easier to appreciate: cells were epithelioid in shape with high nuclear/cytoplasmatic ratio; they had indistinct intercellular borders due to inconspicuous membranes and rarefied, lightly stained cytoplasm. Nuclei were large, round or oval, hypercromatic, but rarely pleomorphic; their position within cells was central to eccentric. The nuclear membrane was irregular
Fig. 1. GIST in ascitic fluid shows 3-dimensional sheets of cells (A, Papanicolaou stain, 10X) with a gland-like prevalent pattern (B & C, Papanicolaou stain, 40X and 63X respectively). The tumor cells have epithelioid appearance with high nuclear/cytoplasmic ratio, mild nuclear pleomorphism, indistinct intercellular borders, prominent nucleoli (C) and intracytoplasmic PAS-negative vacuoles (D, PAS-stain, 40X). GIST cells demonstrated strong and diffuse immunoreactivity for c-kit (E, c-kit antibody, 20X) and vimentin (F, vimentin antibody, 63X).

and often thickened, with indentations and invaginations. Cromatin was coarsely granular and nucleoli were frequently prominent (Fig. 1C). Periodic-acid-Schiff (PAS)-negative cytoplasmic vacuoles were often found (Fig. 1D); occasional mitotic figures were seen.

Immunocytochemistry. Cytology specimens showed strong, diffuse immunochemical positivity for c-kit (Fig. 1E) and vimentin (Fig. 1F), focal-weak CD34-positivity, and no staining for cytokeratin AE1/AE3, NSE, S100, or cromogranin. These findings were suggestive of GIST peritoneal metastases.
Histopathology. Cytological identification of epithelioid GIST was confirmed by histopathological analyses of gastroduodenoscopic biopsies. The patient subsequently underwent a distal gastrectomy with perigastric lymphadenectomy and multiple biopsies of mesenteric lesions. Upon gross examination, the stomach showed the presence of a well circumscribed but not encapsulated mass arising from the posterior wall of the lesser curvature and measuring 6 cm in maximum diameter. The tumor cut surface was pink/white and predominantly solid; a central small ulceration was noted in the mucosa; cross-sections exposed a central area of necrosis within the mass. Histopathological analysis revealed neoplastic tissue composed of spindle cells mixed with polygonal cells. Neither of these cell types had significant nuclear abnormalities. The neoplastic cells were arranged in loosely structured tissue fragments that often showed prominent nuclear palisading. Up to 4 mitoses were identified per 50 high-power fields (HPF).

Tissue specimens were tested with the set of antibodies, yielding the following results: c-kit-positivity, CD34-weak positivity, S100-negativity, cromogranin-negativity, synaptophysin-negativity, vimentin-positivity, actin-negativity, and desmin-negativity. This pattern suggested the diagnosis of biphasic GIST. The same characteristics were found in subserosal mesenteric lesions. Considering the size of the primary tumor (>5 cm), mitotic count (< 5 mitoses/50 HPF), presence of coagulative necrosis, and metastases to lymph nodes and peritoneum, the final diagnosis was biphasic GIST with highly malignant behavior, perigastric lymph node involvement, and peritoneal dissemination [12,13].

Discussion

Although primary GISTs have been reported in the omentum, mesenteries, and retroperitoneum, GISTs at these sites are frequently metastatic from the GI-tract [14]. In such cases, nonspecific symptoms and peritoneal involvement with ascites may be the sole presenting features of these tumors. During the past few decades, a lot of information has been reported about the biology and diagnosis of GISTs; however, little is known about the cytologic appearance of GiSTs, particularly in effusions. In this report, we describe the third case [10,15] of cytologic diagnosis of GIST in ascitic fluid. We confirm that metastatic GIST cells acquire an epithelioid appearance in ascitic fluid and we attest to the diagnostic problems that arise in differentiating GIST from other epithelioid and non-epithelioid neoplasms [16].

In ascitic fluid, GISTs morphologically resemble adenocarcinomas. The most confusing findings are related to cells in a nested pattern and to the occurrence of prominent intracytoplasmic vacuoles. In our case, positive immunoreactivity for vimentin, but not for cytokeratins, within gland-like structures suggested a mesenchymal nature of the tumor; PAS-negativity demonstrating the lack of mucin within cytoplasmic vacuoles also supported the diagnosis of GIST. Mesotheliomas may display PAS-negative, or rarely PAS-positive, intracytoplasmic vacuoles, but certainly, in contrast to GIST, pancytokeratin positivity is a characteristic of mesotheliomas.

Amelanotic melanoma with its large nuclei and prominent nucleoli might be confused with GIST; weak CD117-positivity of its cells has also been reported, as well as vimentin-positivity. However, melanoma cells usually grow as single elements and stain S100-positive; GIST cells aggregate in 3-dimensional clusters and they usually stain S100-negative. Leiomyomas and leiomyosarcomas are the gastrointestinal tract tumors that are most frequently confused with GISTs [17]. In these cases, cytologic samples are often moderately cellular with inconspicuous mitotic features. Smooth muscle differentiation (SMA-positivity, desmin-positivity) is typical of these tumors, but they show nonreactivity for hematopoietic stem cell marker (CD34) and absent or only weak immunoreactivity for CD117.

Schwannomas typically stain positive for CD34 and S100, but negative for CD117. Diagnosis of hepatocellular carcinoma may also be considered. In our case, the lack of endothelial cells surrounding groups of tumor cells, the absence of intracytoplasmic bile pigment, and the CD117-positivity unquestionably excluded metastasis from liver carcinoma.
In summary, the main aims of this case report are: (a) to demonstrate that GIST in ascitic fluid may closely imitate adenocarcinoma cells; (b) to delineate the wide range of other malignant neoplasias that enter into the differential diagnosis of GIST; and (c) to focus attention on the filtration-adhesion technique to obtain cytologic samples with clear cellular details and well-preserved nuclear chromatin patterns. Finally, in agreement with Cheuk et al [18], who stressed the variability of the cytologic presentation of GISTs, we advise caution in making the diagnosis of gastrointestinal stromal tumors in effusions by morphology alone and we emphasize the central role of cytochemistry and immunocytochemistry.

Misdiagnosis of GIST may lead to inappropriate therapy, since conventional chemotherapy and radiotherapy are not effective in the treatment of GISTs. However, these tumors have been found to be responsive to adjuvant treatment with Imatinib/STI571, a potent selective inhibitor of tyrosine kinase activity of the c-kit receptor [19]. In this context, correct diagnosis is essential for proper clinical management, since GIST is the first model for targeted therapy in oncology [20-22].

References