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The complex cytologic features of synovial sarcoma in fine needle aspirates,
An analysis of four illustrative cases.

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Abstract

Objective: The cytologic features of conventional monophasic spindle cell and biphasic synovial sarcoma have been defined in detail in several large series. The cytology of rare morphologic variants, especially the subtypes of poorly differentiated synovial sarcoma, are insufficiently evaluated and diagnostically difficult to define. The objective of the present study was to call attention to the variable cytology of rare variants of synovial sarcoma. Furthermore, adjunctive diagnostic methods, necessary for a correct diagnosis are discussed.

Methods: Aspirates from four synovial sarcomas, with cytologic features, which differed from those of conventional synovial sarcoma and from each other, were retrieved from our files and re-evaluated.

Results: In three of the cases a correct diagnosis was not obtained from routinely stained aspirates. In the fourth case, the correct diagnosis was established by a combination of cytomorphology, immunocytochemistry and fluorescence in situ hybridisation (FISH) performed on the aspirated material.

Conclusions: Ancillary diagnostic methods are necessary in the examination of aspiration smears from synovial sarcoma, especially of morphological variants with a cytomorphology, which differs from conventional spindle-cell monophasic and biphasic tumours. Immunocytochemistry and molecular genetic examinations (reverse transcriptase polymerase chain reaction or FISH) are the methods of choice.

Keywords: Synovial sarcoma, fine needle aspiration, cytology, cytodiagnosis cell block, immunohistochemistry, molecular genetics.

Introduction

Synovial sarcoma accounts for 5-10% of soft tissue sarcomas. Most tumours appear between the ages of 10 and 35 years, but they may occur at any age. The majority of synovial sarcomas are deep-seated and arise in the extremities and trunk. Cases have also been reported in the head and neck region, mediastinum and abdominal wall.

Synovial sarcoma is histologically classified as monophasic spindle cell, biphasic, and poorly differentiated. The latter variety has been defined as synovial sarcoma in which at least 25% of the tumour is poorly differentiated [1-3]. Three morphologic variants of poorly differentiated tumours have been described: 1, undifferentiated, small round cell tumours resembling Ewing's sarcoma/PNET; 2, highly atypical spindle cell tumours resembling fibrosarcoma or malignant peripheral nerve sheath tumour (MPNST) and 3, pleomorphic epithelioid cell tumours with occasional rhabdoid features [1-3]. A small subset of tumours is of monophasic epithelial type. Apart from this basic classification, however, other patterns appear in a minority of cases. Synovial sarcoma with a prominent myxoid matrix resembling other myxoid sarcomas has been reported [4], as has synovial sarcoma with more or less extensive cystic changes [5-7] and synovial sarcoma with extensive calcification [8].

The cytologic features of the monophasic spindle cell and biphasic subtypes of synovial sarcoma in fine needle aspirates (FNA) have been thoroughly described in several large series. Altogether 105 tumours have been evaluated in these series and the results are on the whole in agreement [9-12]. In Table 1 a summary of pertinent cytologic features of mono- and biphasic synovial sarcoma is presented.

Poorly differentiated as well as myxoid and cystic variants of synovial sarcoma have only been described in case-reports (4,6,7,13,14).

We have encountered four cases of primary synovial sarcoma, all of which showed cytologic features definitively unlike the typical pattern and, in addition, quite different from each other.

Methods

Four cases of primary synovial sarcomas with unusual cytologic features were retrieved from our files. The patients who presented between the years 1996 and 2005 were examined by FNA before definitive treatment.

Aspirations were performed in the usual manner with a disposable 10cc syringe in a syringe holder. In all cases aspirates were stained with haematoxylin-eosin (H&E) as well as May-Grünwald-Giemsa (MGG). Ancillary diagnostic methods were performed in three cases. The subsequent histologic specimens were all stained with H&E and expression of immunoreactivity against EMA, keratins (CK7 and CK19), S-100 protein and BCL-2 was investigated in all cases. Cytogenetic/molecular genetic analyses were performed on fresh tissue specimens from all four cases.

Results

Pertinent clinical and morphological data are summarized in Table 2.

Case 1. In the first patient FNA smears showed groups of tumour cells mixed with dispersed cells and numerous stripped nuclei. The nuclei were round to ovoid or fusiform with a moderate to marked anisokaryosis, coarse nuclear chromatin, prominent nucleoli and numerous mitoses (Figure 1). Histological sections displayed features of high-grade sarcoma composed of spindle cell and polygonal cell areas

with high-grade nuclear features and an admixture of pleomorphic tumour cells. The histologic specimen was diagnosed as a poorly differentiated synovial sarcoma.

Case 2. FNA smears from the second patient exhibited an unusually organoid pattern although true glandular spaces were not found. The typical mixed pattern of cohesive groups and dispersed cells was not present in the smears. Tumour cells were quite large with abundant cytoplasm and large nuclei with macronucleoli. Intercellular strands of a hyaline-like material similar to that described by Dharan et al 1998 [15], were observed in both FNA smears and histologic sections (Figure 2A-2D). In addition, tumour cells surrounding a fibrohyaline matrix appeared in one of the smears (Figure 2A inset). The histology of the excised neoplasm corresponded to the features found in the FNA smears and the sarcoma was classified as a monophasic synovial sarcoma (Figures 2C-2D).

Case 3. In the third patient FNA smears were haemorrhagic and cell-poor, containing scattered spindle cells with fusiform nuclei showing slight to moderate atypia. In addition a few small sheets of moderately atypical tumour cells with abundant cytoplasm in a haemorrhagic background and scattered histiocytes (siderophages) were seen (Figure 3A). A core needle biopsy performed simultaneously demonstrated fibrous tissue with calcifications probably arising from the fibrous capsule but sarcoma cells were not seen. In contrast, the operative specimen showed a predominantly cystic tumour with central necrosis and areas of haemorrhage. In small areas viable tumour composed of moderately atypical tumour cells was present. The tumour was diagnosed as a cystic monophasic synovial sarcoma.

Case 4. FNA smears from the fourth lesion were highly cellular showing a mixture of cohesive and dispersed cell groups of medium round to ovoid cells, which focally

resembled the cellular population typical of Ewing's sarcoma/PNET. Several rosette-like formations were seen in the aspiration smears (Figure 4A). In addition, many tumour cells had abundant cytoplasm containing dense "rhabdoid inclusions" (Figure 4B). A cell block prepared from the FNA specimen and a core needle biopsy performed in conjunction with FNA showed both poorly differentiated spindle cells and a small round-cell pattern focally resembling Ewing's sarcoma/PNET. The immunophenotype was typical for synovial sarcoma: positive staining with EMA and both cytokeratin 7 and 19 (Figure 4C and insets). Furthermore a FISH-analysis of an unstained ThinPrep slide showed separate red and green signals indicative of a rearrangement of one copy of the SYT gene region (Figure 4D) i.e. fusion transcript between the SYT gene located on chromosome 18 and the SSX gene located on the X chromosome.

Discussion

In addition to the three main types of synovial sarcoma such as monophasic spindle cell, biphasic and poorly differentiated other rare variants have been described: so-called myxoid, predominantly cystic and calcified. Poorly differentiated tumours as well as these rare histologic variants may be difficult to diagnose in operative specimens and ancillary diagnostic techniques are often necessary before a correct diagnosis can be rendered. FNA smears from these subtypes are even more difficult to diagnose correctly, mainly because much smaller parts of the tumours are sampled (although the cellular yield often is rich) compared to the operative specimens. In FNA from "conventional" monophasic spindle cell and biphasic synovial sarcoma, cytological criteria pointing towards a correct diagnosis have been defined in a

number of published series. If core needle or open biopsy is omitted before the definitive treatment then we must emphasize that the definitive diagnosis of synovial sarcoma in FNA smears should include the use of ancillary methods (immunocytochemistry and/or cytogenetic/molecular genetic analyses).

Our aim in reporting these four cases is to illustrate the complex appearance of synovial sarcoma in FNA when uncommon or rare variants are sampled.

In Case 1 (1996) a synovial sarcoma with extensive poorly differentiated areas was needed. Compared to the typical features in “conventional” tumours the pattern at low power was the same as in the “typical” smear; a mixture of highly cohesive cell groups and dispersed cells many of which appeared as stripped nuclei. However, anisokaryosis was marked, the chromatin texture coarse, nucleoli prominent and mitoses were common.

In Case 2 (2003) the FNA smears exhibited an unusually organoid pattern with large, atypical tumour cells having abundant cytoplasm and large nuclei with macronucleoli. The typical mixed pattern of smears with cohesive groups and dispersed tumour cells was not present. Another observation was the presence of intercellular strands of a hyaline-like material similar to that described by Dharan et al [15].

Case 3 (2003) illustrates the diagnostic problem when tumours are predominantly cystic. In spite of several passes only a few moderately atypical tumour cells or small cell-sheets were present in a haemorrhagic background. Although several passes with the needle in peripheral parts of cystic tumours is recommended, this method may not yield diagnostic cells. A core needle biopsy performed at the same time as the FNA provided no additional diagnostic information as the cores represented only the

fibrous capsule. We have previously demonstrated that simultaneously performed FNA and core needle biopsies enhance the diagnostic accuracy in selected cases of musculoskeletal tumours [16]. However this case illustrates how difficult it may be to obtain diagnostic material from predominantly cystic tumours.

Case 4 (2005) exemplifies a poorly differentiated synovial sarcoma composed of small to medium-sized round and spindle cells, which resemble the cellular population typical of Ewing's sarcoma/PNET. In this case the first diagnostic consideration was whether the tumour cell population represented Ewing's sarcoma/PNET or a poorly differentiated synovial sarcoma. The immunohistochemical stains on a cell block specimen clearly demonstrated the typical immunophenotype of synovial sarcoma: focally positive staining with EMA, cytokeratins 7 and 19. Furthermore a FISH analysis of a ThinPrep slide showed the typical fusion transcript between the SYT gene located on chromosome 18 and the SSX gene located on the X chromosome (SS18/SSX).

Cases 1, 2 and 4 emphasize the importance of supplementing light microscopic evaluation with ancillary diagnostic methods in cases where the evaluation of routinely stained smears limits a specific diagnosis. Furthermore, these cases demonstrate the variable cytologic features of the more uncommon variants of synovial sarcoma in aspirate smears.

In our opinion a technically satisfactory cell block preparation is the method of choice for immunohistochemical investigation of suspect synovial sarcoma. A cellblock preparation is comparable to a micro biopsy; the same immunolabeling procedures are used as for conventionally formalin-fixed tissue specimens. It is easy to compare the various staining results in the cellblock and tissue specimen, and

negative controls are evaluated on the same cellular population that is immunostained.

Electron microscopic examination is a valuable diagnostic tool in the diagnosis of poorly differentiated synovial sarcoma especially when the immunophenotype is inconclusive [9,17]. However electron microscopy as a diagnostic adjunct has been more or less replaced by cytogenetic and/or molecular genetic analyses. As the translocation $t(X;18)(p11,2;q11,2)$ is found in >90% of synovial sarcomas including tumours with poorly differentiated areas [2,17], cytogenetic and molecular genetic analyses are very important diagnostic adjuncts. Molecular genetic analysis (reverse transcriptase polymerase chain reaction (RT-PCR)) has proved to be a suitable method to use on FNA [18] and this method has the advantage that the two most common variants of the SSX gene, SSX1 and SSX2 may be detected. This might be of clinical prognostic importance, as tumours harbouring SS18/SSX1 seem to be more aggressive than those with SS18/SSX2 [19-21]. It is important to note that the RT-PCR analysis from the first patient as well as from fourth patient showed the SS18/SSX1 transcript. The first patient died of tumour and the fourth patient had pulmonary metastases at the time of primary diagnosis, while the second patient has no signs of tumour spread after three years of follow-up; that tumour showed a SS18/SSX2 transcript.

FISH analysis is another suitable method to apply to FNA smears [22-25] as demonstrated in Case 4.

Based on this re-evaluation of four uncommon variants of synovial sarcoma we conclude that the cytologic features in FNA-smears from such cases are very variable and are markedly different from those defining the monophasic and biphasic types.

Poorly differentiated synovial sarcoma is an important differential diagnosis in the evaluation of small cell and spindle cell malignancies. This diagnosis should be considered in cases where evaluation of routinely stained material fails to suggest the specific histologic type and ancillary diagnostic methods should be applied in order to reach a specific diagnosis.

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Legends

Figure 1. (Case 1) FNA smears containing dispersed tumor cells with round to ovoid or fusiform nuclei with a moderate anisokaryosis and coarse chromatin (H&E stain X 25).

Figure 2. (Case 2) (A) Low power view showing loosely cohesive sheets and dispersed tumour cells with abundant cytoplasm and intercellular hyaline basement membrane-like material; Inset: Cells surrounding a fibrohyaline matrix (MGG stain X 25). (B) High power view: note mast cell (MGG stain X 100). (C-D) Corresponding histologic section: thick collagen deposition intermingled among tumor cells (C) and epithelial-like epithelium (H&E stain, x 100).

Figure 3. (Case 3) (A) Cell-poor FNA smears of cystic synovial sarcoma showing a few sheets of moderately atypical tumour cells with abundant cytoplasm in a haemorrhagic background and scattered haemosiderin loaded histiocytes (arrow) (MGG stain, x 100). (B) MRI showing partly cystic lesion; Inset: whole tumor section showing cystic neoplasm with central necrosis (H&E stain, X 15).

Figure 4. (Case 4) (A) High power view showing rosettes and (B) occasionally cytoplasmic densities creating “rhabdoid” appearances (arrow) (H&E and MGG stains respectively, x 100). (C) Cell block section with features of spindle cell sarcoma (H&E stain, x 15); Inset: Immunostains with antibody against EMA (upper right) and keratin ck 19 (lower right) performed on the aspirates (cell block) shows focal positivity in the tumor cells (EMA and ck 19 x 25). (D) Separate red and green signals indicative of a rearrangement of one copy of the SYT gene region (FISH, breakapart probe).

Tables

Table 1.

Important cytologic features in fine needle aspirates from mono-and biphasic synovial sarcoma. A summary of cytologic findings in 105 cases.

Cell-rich aspirates

Mixture of (tridimensional) groups or branching tissue fragments and dispersed cells

Often vessel stalks in tissue fragments

Stripped nuclei

Small to medium-sized cells

Cells spindly, rounded or oval

Nuclei ovoid, rounded or fusiform

Bland chromatin

Inconspicuous nucleoli

Acinar structures or epithelioid-like cells in biphasic tumour

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Table 2.

Pertinent data in the four cases

Case	1 (evaluated 1996)	2 (evaluated 2003)	3 (evaluated 2003)	4 (evaluated 2005)
Gender and age	Male, 68	Female, 14	Female, 30	Male, 36
Clinical data	4 cm firm and tender mass in the left hand	3 cm subcutaneous mass in the right groin	Deep-seated 5cm predominantly cystic mass in the right lower leg	Deep-seated mass between the tibia and fibula in the right lower leg, pulmonary metastases
Cytologic features	Mixture of cohesive cell groups and dispersed cells. Numerous stripped nuclei. Moderate to marked anisocytosis, anisokaryosis and numerous mitoses. Scattered mast cells	Predominantly dispersed large tumour cells with abundant cytoplasm, ganglion-like cells. Slight atypia. Focally intercellular hyaline basement membrane like material	Haemorrhagic, cell-poor smears. Oval and spindle cells with fusiform nuclei. Slight to moderate atypia. Presence of haemosiderin loaded histiocytes	Mixture of cohesive cell groups and dispersed small to medium-sized cells. Vacuolated cytoplasm and rounded or irregular nuclei with finely granular chromatin and inconspicuous nucleoli
Ancillary studies on the FNA	IC (cytospin) : Vimentin strongly positive, S-100 focally positive	ND	IC (Liquid based cytology) : failed due to insufficient material	IC (cell-block): EMA focally positive, CK 7 and 19 positive in single cells. FISH-analysis: SS18/SSX transcript (rearrangement of one copy of the SYT gene region)
FNA diagnosis	High grade malignant sarcoma	Mesenchymal tumour NOS; Nodular fasciitis?	Intramuscular vascular tumour Haemangioma with reactive changes?	Synovial sarcoma
Core needle biopsy	ND	ND	Performed in the same séance as the FNA. No diagnostic help: cell poor fibrous tissue with microcalcifications	Performed in the same séance as the FNA IH: EMA focally positive, CK 7 and 19 positive in single cells. RT-PCR: SS18/SSX2 transcript.
Definitive treatment	Primary radical surgery	Primary radical surgery	Primary radical surgery	Radiotherapy

Ancillary diagnostics on the surgical specimen	IH: EMA focally positive Electron microscopy: intercellular microlumina with microvilli. RT-PCR :SS18/SSX2 transcript	IH:EMA strongly positive, CK 19 focally positive. Cytogenetic examination: t(X;18) RT-PCR: SS18/SSX1 transcript	IH: EMA positive. CK 7 and 19 positive in scattered cells Cytogenetic analysis: Failed	ND
Final diagnosis	Poorly differentiated monophasic synovial sarcoma	Monophasic synovial sarcoma	Predominantly cystic monophasic synovial sarcoma	Poorly differentiated synovial sarcoma

IC: Immunocytochemistry; IH:Immunohistochemistry; CK: Cytokeratin; NOS, not otherwise specified;
RT-PCR: Reverse-transcriptase polymerase chain reaction, FISH: Fluorescence in situ hybridisation