Review paper

# Ancillary tests in diagnostic cytology. On the example of the recommendation given by the World Health Organization in soft tissue tumors

Jerzy Klijanienko

Institut Curie, Paris, France

Fine needle aspiration despite being an inexpensive, rapid, accurate and non-invasive diagnostic practice, particularly when used in oncology, is frequently underutilized. The World Health Organization – International Academy of Cytology – International Agency of Research on Cancer initiated a series of Blue Books devoted solely to cytology and reporting methods in particular entities of different organs. The use of ancillary techniques such as immunocytochemistry, molecular biology and flow cytometry was defined in detail. In this review, we have focused on the role of ancillary techniques in the cytological diagnosis of soft tissue tumors. Combination of cytological and histological cellular material with ancillary techniques increases the number of accurate diagnoses and improves clinical management of patients.

**Key words:** fine needle aspiration, cytology, ancillary techniques, soft tissue tumors, WHO classification.

## Introduction

Cytology, considered as an inexpensive, rapid, accurate and non-invasive diagnostic practice, gained acceptance in the pathologist community, particularly when used in oncology. However, as a clinical practice and reasoning based on isolated tissue elements, it requires specialized training and specific morphological analysis. This particularity may make it more difficult and less accessible to pathologists outside of a hospital setting. Moreover, the rarity of certain pathologies (such as soft tissue tumors or salivary tumors), the publications of large, but older series, and changes in international terminology have made cytological analysis more complex.

Aware of this weakness, the World Health Organization (WHO) in collaboration with the International Academy of Cytology and International Agency of Research on Cancer (WHO) initiated a series of Blue Books devoted solely to cytology and reporting methods in particular entities of different organs. Similarly to the classical histology Blue Books, cytology books also contain chapters devoted to ancillary techniques such as immunocytochemistry, molecular biology and flow cytometry.

Currently (February 2026) four *Blue Books of Cytology* have been published online and in print [1]. Four more are in the drafting stage at various degrees of completion. To better illustrate the application of cytological cellular material in ancillary techniques we have taken the example of soft tissue tumors as presented in the Reporting System for *Soft Tissue Cytopathology Blue Books* [2].

Cytological material in soft tissue lesions is generally abundant. Tumors composed of spindle cells, epithelioid cells, polymorphous and round cells usually yield hypercellular cellular material, which may successfully be used in ancillary techniques such as immunocytochemistry, molecular studies and flow cytometry. Depending on the practices of the hospital center, immunocytochemistry may be performed on suspensions, smears or paraffin-embedded cell

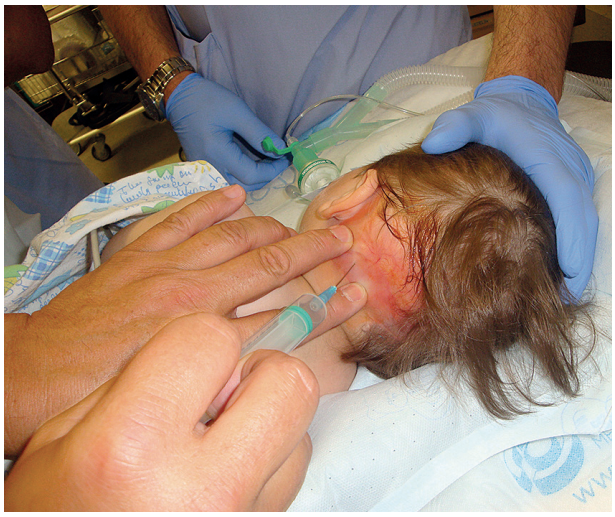

**Figure 1.** Fine-needle aspiration on upper, posterior cervical mass in infantile fibrosarcoma

blocks. Molecular studies can be performed on fresh, paraffin-fixed, or frozen material. Flow cytometry can be performed on cell suspensions. This variability of techniques means that a specific sampling modality between the pathologist and the radiologist must be established. In addition, the use of rapid-on-site-evaluation (ROSE) technique (usually 30 seconds) allows for better targeting of the sampling procedure and ensure the production of good quality smears for morphological study but also for material intended for immunocytochemistry, molecular biology and flow cytometry if necessary. It is very useful, especially in the pediatric population, where high efficiency is required (Figure 1).

Soft tissue lesions are represented by a wide variety of tumors of different tissues. The classical histological classification ranks lesions according to their putative tissue of origin. In this way, WHO classifies the soft tissue lesions as adipocytic, fibroblastic-myofibroblastic, vascular, pericytic, smooth muscle, stratified muscle, peripheral nerve sheath, undifferentiated round cell and uncertain origin [3–5]. Although from an academic point of view this classification is optimal, it may remain quite limited for cytological diagnosis. For this reason, cytology diagnosis consists of two steps. The first step is recognition of the predominant cell type/shape and stroma nature. Tumors are thus classified as mass-forming benign inflammatory processes, adipocytic benign or malignant tumors, myxoid malignant tumors, spindle cell benign or malignant tumors, epithelioid benign or malignant tumors, pleomorphic malignant tumors and round cell malignant tumors [2]. In the second step, ancillary techniques can be applied and will help in more precise diagnosis [6, 7].

We present below the importance of ancillary techniques using cytology material in selected tumor entities in different groups of tumors.

## Adipocytic tumors

As in histology, the differential diagnosis of adipocytic tumors may be difficult. The morphological resemblance between lipomas and liposarcomas may be subtle. *MDM2* gene amplification, along with co-amplification of *CDK4* is diagnostic of liposarcoma. In practice, in a second step, nuclear expression of MDM2 and/or *MDM2* gene amplification detected by fluorescence *in situ* hybridization (FISH) or next-generation sequence (NGS) is necessary to establish accurate diagnosis [6, 7].

## Myxoid malignant tumors

Myxoid liposarcoma, myxofibrosarcoma, and extraskeletal myxoid chondrosarcoma can exhibit a myxoid component. However, a myxoid component can occasionally be present in other benign or malignant entities like dermatofibrosarcoma protuberans, leiomyosarcoma, malignant peripheral nerve sheath tumor (MPNST) and benign fibrous histiocytoma. The myxoid component is easily detected on the smears. Immunocytochemistry and molecular alterations may substantially help in the differential diagnosis of myxoid tumors. Myxoid liposarcoma is characterized by translocation involving the *DDIT3* with either *FUS* or *EWSR1* [8]. Extraskeletal myxoid chondrosarcomas may show immunocytochemical SMARB1 (INI1) loss in tumors with rhabdoid morphology. Some tumors may also show FISH *NR4A3* arrangement or reverse transcription-polymerase chain reaction (RT-PCR) *EWSR1*::*NR4A3* fusion [9, 10]. Finally, myxofibrosarcoma has no specific immunocytochemical or molecular alteration.

## Spindle cell benign and malignant tumors

This is a heterogeneous group of neoplasms with variable clinical behavior. Some benign lesions, such as fibromatosis or nodular fasciitis, may be paucicellular. The clinical presentation is usually characteristic, and cytology confirms only the clinico-radiological diagnosis. This is a relatively difficult group in cytology, where several morphologies can overlap. In the group of spindle cell sarcomas, synovial sarcoma, low-grade fibromyxoid sarcoma, malignant MPNST, infantile fibrosarcoma and leiomyosarcoma are the most frequently diagnosed. Synovial sarcoma is characteristic on morphology by the presence of numerous spindle small-sized cells, isolated or in brunch-like tissue fragments. The diagnosis is confirmed when combination of immunocytochemical positivity for CK7, CK19, and EMA and fusion of *SS18* with *SSX1*, *SSX2*, or *SSX4* is found [11]. Low-grade fibromyxoid sarcoma may show immunocytochemical positivity for MUC4, EMA and

smoth muscle actin (SMA). *FUS* rearrangements (*FUS*::*CREB3L1*, or *FUS*::*CREB3L2*) can be detected using FISH technique. Spindle cell MPNSTs are negative for the S100 and SOX10, while epithelioid MPNSTs are positive. There is no specific molecular marker [12]. Infantile fibrosarcoma has no specific immunoprofile. Interestingly, FISH, RT-PCR, or targeted NGS detection of *ETV6*::*NTRK3* is useful for confirming the diagnosis. Finally, spindle cell leiomyosarcoma stains for muscular markers such as SMA, desmin, and caldesmon, but has no specific molecular markers [13].

## Epithelioid malignant tumors

Extrarenal rhabdoid tumor, epithelioid sarcoma and alveolar soft part sarcoma are typical epithelioid malignant sarcomas on cytology smears. The epithelioid character and malignancy are cytologically evident, keeping in mind that epithelioid morphology may be simulated by round cell sarcomas and carcinomas [14, 15] (Figure 2). Both, extrarenal rhabdoid tumor and epithelioid sarcoma shows loss of SMARCB1 (INI1) expression, but CD34 positivity is strongly in favor of the latter. Alveolar soft part sarcoma is characterized by *ASPSCR1*::*TFE3* gene fusion. *TFE3* gene rearrangement, which can be assessed by FISH and *ASPSCR1*::*TFE3* fusion using RT-PCR or NGS.

## Round cell tumors

In round cell tumors, the cytological diagnosis of malignancy is evident (there are no benign round cell tumors except round cell component of pilomatrixoma). The different diagnosis between tumor entities is based on round-cell morphology combined with clinical, radiological, and molecular data. Ewing sarcoma, desmoplastic small round cell tumor and alveolar rhabdomyosarcoma are the most common round cell lesions. They should be differentiated with epithelioid tumors, blastemal tumors and certain carcinomas [16–19]. Ancillary techniques can easily discriminate these entities and assist in accurate diagnosis. Ewing sarcoma is usually positive for CD99 and NKS2-2 and show FET::ETS fusions (mainly *EWSR1*::*FLI1*). In desmoplastic small round cell tumor the presence of *EWSR1* and *WT1* gene rearrangements is a useful diagnostic tool. Alveolar rhabdomyosarcomas show immunocytochemical positivity for muscular markers and are fusion-positive *PAX3-FOXO1* or *PAX7-FOXO1*.

## Conclusions

The diagnostic management of soft tissue tumors is complex. The diagnostic process often requires a significant amount of high-quality material. Being cell-rich and stroma-poor, cytological material is particularly well suited for ancillary techniques. By combining cytological and histological morphologies with ancillary techniques, we increase the number of accurate diagnoses. Moreover, having capacity of ROSE, we also improve clinical patient management.

Finally, it has often been said that the difference between fine needle cytology and core needle biopsy is only located in the diameter of the needle, while the cells, genes and proteins, *etc.* remain the same. That is why WHO publishes this series of cytological reporting Blue Books.

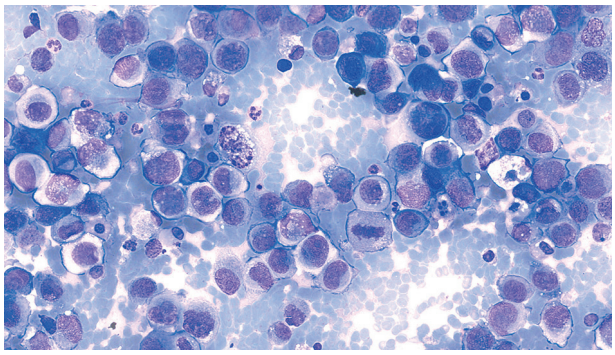

**Figure 2.** Hypercellularity in rhabdoid tumor showing epithelioid morphology (May-Grunwald-Giemsa)

## Disclosures

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4. Conflicts of interest: None.

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## Address for correspondence

**Jerzy Klijanienko**
Institut Curie
Paris, France
e-mail: jerzy.klijanienko@curie.fr