lesion we are dealing with, for selection of further, special techniques. We will also get an impression of tumor heterogeneity by comparing aspirates from different parts ot the tumor.

Staining

The air-dried smears are routinely stained according to May-Grunwald-Giemsa and the ethanol-fixed ones according to Papanicolaou. Both types of smears can also be used tor special stains, e.g., Alcian blue at different ionic strength or pH to disclose the nature of myxoid material (2).

Only occasionally do we fix part of the aspirated material in paratormaldehyde for electron microscopy. It has a proven value in demonstrating, for instance, mesothelial and rhabdomyoblastic differentiation (3).

Assessing the specimen

In general, some main features to be noted are listed below.

Background

The background needs to be determined, *e.g.,* necrosis, myxoid, mucoid, chondroid, osteoid material.

Cellular arrangement

It needs to be determined whether cells are arranged in a special way *(e.g.,* tascicular, papillary, alveolar, or only dissociated cells.) and their relation to normal stromal components, including vessels.

Cell size

Cell size is an important parameter, often given in relation to the size ot an erythrocyte or a lymphocyte. Small round blue cell" tumors represent a concept, which comprises important pediatric tumors (neuroblastoma, nefroblastoma), but also rhabdomyosarcomas, especially the alveolar type, and the various types of lymphomas.

Giant cells are seen in many typos ot mesenchymal tumors, both clearly neoplastic ones and reactive ones (foreign body type or osteoclastoma-like).

Cell differentiation

Cell differentiation is very important parameter, since the classification (4) is based on histogenic criteria. The identification of lipoblasts, for instance, in an otherwise undifferentiated sarcoma will have obvious diagnostic impact, as will the identification of rhabdomyoblasts in a small cell sarcoma. A simplified WHO's classification can be used, as follows: tumors of fibrous tissue; fibrous-histiocytic tumors; and tumors of adipose tissue muscle, blood vessels, synovial tissue, peripheral nerves.

Grading

It is often difficult or impossible to grade sarcomas without an identification as to type. There are malignancy-related criteria, however, that support the diagnosis of a malignant tumor, even though the type may not be evident. Lack of differentiation, high nuclear/cytoplasmic ratio, nuclear abnormalities and atypical mitoses belong to this category of cellular changes.

SDecial techniques

Immunocytochemistry

The cytospin specimens are prepared fresh and allowed to air-dry over night, allowing a first micromorphologic assessment on cytology. A potential problem with the cytospin technique for cytochemistry is the fact that the antisera have to be titrated especially for this purpose. It is not always possible to use the same concentrations/procedures as for histopathology. This is particularly true if automated machine is used for immunostaining.

Cvtogenetics

Cytogenics has become an important adjunct in identifying certain SSTs since the tirst specific translocation was described for alveolar rhabdomyosarcomas t(2q; 13q) (5). Further examples are Ewing's sarcoma t(IIq -22q), myxoid liposarcoma t(12q,16p) and synovial sarcoma t(X, 18).

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) is a technique that lends itself to application on cytological material. It is technically easier to use tor detection ot translocations than a cytogenetic analysis, since it can be performed on interphase nuclei. The method also lends itself to detection of amplifications. One example is the N-myc amplification in neuroblastoma. The cytological material, adequately preserved, can also be used tor any other DNA- and RNA-based technique.

Diagnosis

The diagnosis should indicate if the material is sufficient for diagnosis. Only diagnoses from WHO's list should be used. If the diagnosis is one of several possible alternatives, differential diagnoses should be given.

In cases where there is uncertainty as to the benign or malignant nature of the lesion, additional diagnostic suggestions should be given, *e.g.,* type of additional biopsy required, excision, special techniques, etc.

Examples of common soft tissue lesions will be given, both benign and malignant ones.

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Aspiration biopsy of bone tumors

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Cytological diagnosis of bone lesions by fine needle aspiration (FNA) biopsy is by no means a new approach; it is employed to

enhance, rather than to replace, histological examination in the diagnosis of tumors and pseudotumors in bone tissue. Although bone might appear to constitute a natural barrier to FNA, malignant tumors and other nontumor entities often cause destruction and lysis, replacement by soft, fragile tissue, breakdown of bone cortex and pathological fractures, all of which provide fine needle access to the lesion. Even though one of the major uses of FNA in bone pathology is in the diagnosis of tumor metastases, it has also proved a valuable technique for the study of vertebral lesions, pathological fractures and osteosarcomas in which presurgical chemotherapy is indicated. FNA biopsy may also be employed to rule out neoplasia in certain lesions (including osteomyelitis and necrosis).

The FNA technique used in bone is similar to that employed in other viscera or masses; however, it is sometimes necessary to use needles of greater caliber and length. The biopsy must often be guided by radioscopy or CT scan. The most serious complications have been reported in spinal taps, although their incidence is in fact very low. Material obtained by biopsy should be appropriately processed for hematoxylin and eosin, Papanicolaou and Giemsa staining. Extensions should also be kept for histochemical and immunohistochemical examination, It is also useful to process samples for ultrastructural and microbiological analysis (e.g., identification of primary tumor, or of etiological agent in the case of infections).

Since many bone tumors present in clearly defined age groups and locations, clinical and radiological characteristics should be borne in mind during cytological examination. In terms of morphology, the following six major groups of lesions can be distinguished by reference to the nature of the matrix and to cell shape: i) chondroid-matrix tumors; ii) anaplastic cell tumors; iii) round cell tumors, iv) spindle cell tumors; v) osteoclast-like giant cell tumors; and vi) epithelial cell tumors. Each of these groups involve lesions which may be distinguished from each other by reference either to clinical/radiological characteristics or to additional cytological findings.

Chondroid-matrix tumors stain metachromatically to Giemsa. This category includes cartilaginous lesions (enchondroma, chondrosarcoma, chondroblastoma and chondromyxoid fibroma), notochordal lesions (chordoma) and bone lesions (chondroblastic osteosarcoma). In benign lesions and well-differentiated malignant lesions, cellularity is scarce to moderate and uniform, and located in lacunae; by contrast, in highly malignant tumors matrix is sparse, and cellularity is abundant and pleomorphic. Differential diagnosis of these lesions is required mainly with regard to metastases from mucosecretory or renal carcinomas. The major diagnostic problem, which is more readily solved by histological examination, is to distinguish between highly cellular benign tumors and low-order malignant tumors.

Round cell tumors present a highly cellular, monomorphic aspirate, whose cytological characteristics are generally indicative of a high degree of malignancy. Special techniques are often required to arrive at a more specific diagnosis. This category includes Ewing's sarcoma peripheral neuroepithelioma, multiple myoma plasmocytoma, lymphoma leukemia, round cell osteosarcoma and mesenchymal chondrosarcoma. Differential diagnosis is required with regard to bone metastases from a neuroblastoma, embryonic rhabdomyosarcoma and small cell carcinoma of the lung.

Bizarre and anaplastic cell tumors contain abundant cell debris, scattered and clustered cells and marked pleomorphism and nuclear atypia. The clearest example of these lesions is found in the medullary osteosarcoma, although they may also be present in poorly differentiated forms of tumors such as pleomorphic malignant fibrohistiocytoma, highly malignant chondrosarcoma, pleomorphic myeloma and osteoclastic sarcoma. The chief diagnostic feature of the osteosarcoma is the presence of atypical osteoblasts forming osteoid matrix or immature bone. Osteosarcomas frequently display chondroblastic or fibroblastic differentiation.

Spindle cell tumors display a highly varied histogenesis in which it proves difficult to differentiate – on strictly cytological grounds – between benign processes and low-order malignant tumors. In such cases, clinical/radiological correlation, immunohistochemical techniques and electron microscopy are of considerable diagnostic value. This category includes fibroblastic, myofibroblastic, histiocytic, vascular, muscular and neural lesions.

The presence of osteoclast-type giant cells is a relatively common finding in aspirates from bone tumors and pseudotumors, and is therefore of little diagnostic value. Nonetheless, the abundance of such cells in aspirate may suggest a provisional diagnosis of giant cell tumor, particular if the diagnosis is supported by sufficient clinical and radiological evidence.

Aspirates containing epithelial cells are the most common finding in FNA biopsy of bone tissue, since in most cases neoplasia takes the form of metastases from carcinomas of breast, lung, kidney and thyroid. These lesions should not be confused with primary bone epithelial lesions such as those observed in intraosseous meningiomas, adamantinomas and epidermal inclusion cysts.

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