Fine needle aspiration in the diagnosis of bone tumors

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Fine needle aspiration (FNA) is an important part of the preoperative diagnosis in bone tumors. The diagnosis must be based on clinical, radiologic and morphologic correlation. In palpable lesions, FNA is performed on the most accessible part of the tumor. Deep-seated and/or non-palpable lesions need radiologic guidance. Material from the FNA can be used for additional examinations, i.e. electron microscopy, immunocytochemistry, cytochemistry, DNA-ploidy analysis, chromosomal analysis and molecular genetics. Those examinations are of particular importance in the primary and differential diagnosis of Ewing sarcoma, osteosarcoma and chondrosarcoma. The majority of tumors in FNA aspirates can be classified as primary (benign or malignant) and metastatic tumors. Cellularity, pleomorphism, chromatin pattern, nucleolar structure, mitotic figures and necrosis are parameters of malignancy.

FNA is indicated for the preoperative diagnosis of primary tumors and when recurrent tumor or metastatic lesions are suspected. FNA has the same diagnostic accuracy for the majority of lesions. FNA minimizes the risks of tumor spread associated with open biopsy. FNA does not require hospitalization, and rapid staining techniques permit a preliminary diagnosis within 15–20 minutes after needling, allowing the clinician to inform the patient and plan further preoperative evaluation during the first hospital visit (Willén et al. 1995).

Compared to open biopsy used in the preoperative bone tumor diagnosis, FNA can provide the same diagnostic accuracy in the majority of lesions.

In order to increase the diagnostic accuracy in malignant tumors, ancillary methods like electron microscopy, immunocytochemistry, cytochemistry, DNA-ploidy analysis and cytogenetics can be used. The combination of FNA, electron microscopy and chromosomal analysis is considered to be adequate for the preoperative diagnosis of Ewing's sarcoma and chemotherapy without open biopsy (Åkerman et al. 1996, Åkerman et al. 1986). Applying FNA on bone tumors is cheaper compared to the cost of open biopsy.

This presentation is a review of our FNA-experience in primary and metastatic bone tumors and is based on 1553 FNA's of bone lesions between 1979 and 1996.

Role of cytopathology

The main task for cytopathologists is to decide whether the lesion is a primary or metastatic bone tumor and if primary, whether benign or malignant.

The cytologic diagnosis of malignancy is based on: cellular yield, presence and abnormality of mitotic figures, the cellularity in tumor clusters, fragments or dispersed cells, cellular pleomorphism, nuclear or nucleolar structure and necrosis.

The use of four-grade malignancy-grading, as in histopathology, is not possible with FNA. Cytological diagnosis can, however, indicate low or high grade malignancy.

Aspiration technique, stainings and material sampling for ancillary examinations

The aspiration technique employed is similar to that used for epithelial tumors or soft tissue tumors (Willén et al. 1995). Local anesthetic (ointment) is used only on special occasions. Thus, the radiologists use more often local anesthesia, particulary in cases without complete cortical destruction.

The most useful needles are 22 G (0.7 mm) and needles with stylets. In tumors with preserved cortices, a coarse biopsy needle can be used before the FNA. All passes with the needle are through the same channel, but the direction is changed with each pass in order to sample from a larger part of the tumor. The orthopedic surgeon and/or radiologist decide and mark the most suitable part of the tumor for FNA. At request, the insertion point is tattooed and at surgery the needle track is removed en-bloc with the tumor.

According to our experience, the best diagnostic information is given by a combination of air-dried smears stained with May-Grünewald-Giemsa (MGG) and wet-fixed smears with HE staining which provides good nuclear morphology. MGG staining is invaluable not only for cytoplasmatic details but also for identification of myxoid and/or chondroid matrix

Table 1. Cytological features of common bone tumors

Tumor type	Cytology	Ancillary tests
Ewing's sarcoma	dark and light cells, rosette-like structures, vacuolated cytoplasm	EM, t11;22 HBA 71 glycogen
Atypical Ewing's sarcoma and PNET	one cell type, large cytoplasm with cytoplasmic processes	EM, t11;22 HBA 71
Osteosarcoma	pleomorphic cells, spindle cells, atypical mitosis, chondroid and/or osteoid matrix	Alkaline phosphatase EM DNA
Giant cell tumor	multinucleate giant cells, mononuclear spindle cell population, regular mitoses	DNA
Chondromatous tumors	chondromyxoid matrix, hyaline cartilage, binuclear cells	DNA, S-100
Chordoma	large, physaliferous cells, small epithelial-like cells, myxoid matrix, spindle cells	S-100, CAM 5.2 CEA
Histiocytosis X	histiocytes, eosinophils	EM, S-100
Plasmocytoma/myeloma	plasma cells with eccentric nuclei, clear perinuclear zone, rich cytoplasm	EMA, Kappa/Lambda

and osteoid material.

Hank solution or other balanced salt solutions are used for sampling for immunocytochemistry and/or electron microscopy (EM). Fixation in glutaraldehyde is required for EM. Special tubes and material sampling for EM make the technical EM procedures easier (Walaas 1990, Carlén 1996). For DNA-ploidy analysis, both flow and static analysis can be used. Air dried smears, unstained or destained are suitable for static DNA-analysis (Åkerman et al. 1987).

For cytogenetic analysis, aspirated cells are immediately transferred to Mc Coy's 5 A or RPMI medium supplemented with fetal bovine serum (FB5), glutamine and antibiotic (Åkerman et al. 1996).

1–3 aspirates are necessary for each technique. In order to minimize number of passes and utilize the remaining cells in the needles, the needles are rinsed by glutaraldehyde or Hank solution after FNA for routine smears.

Diagnosis of bone tumors in FNA

Cytological criteria for bone tumors which can be diagnosed by FNA (Table 1) are available (Walaas 1990, Dahl et al. 1986). The tumor cell morphology has to be related to the normal cells which can be present in FNA: cartilage, osteoblasts, osteoclasts and bone marrow population, like megakaryocytes.

Ewing's sarcoma

Ewing's sarcomas are easy to aspirate and the FNA morphology is excellent compared to the common artefacts which are common in specimens from open biopsy.

The most prominent feature is a double population of large pale cells with vacuolated cytoplasm and dark small cells with minimal cytoplasm (Figure 1). Both single cells and microbiopsies are common. The cytoplasm is glycogen rich. The nuclei are rounded with fine chromatin and rare atypical mitotic figures. Occasional rosette-like structures are more prominent in HE staining and can mimic neuroblastoma (Figures 2 and 3). Atypical Ewing's sarcoma shows only one cell type with pleomorphism, more cytoplasm containing long cytoplasmic processes (Figures 3 and 4) and frequent atypical mitotic figures. Immunocytochemistry and EM are of great importance for the diagnosis of atypical Ewing's sarcoma and PNET and in the differential diagnosis of metastatic neuroblastoma or alveolar rhabdomyosarcoma (Carlén 1996). B-cell lymphoblastic non-Hodgkin lymphoma or T-cell lymphoma show more prominent nuclear pleomorphism and atypical mitotic activity.

Osteosarcoma

Osteosarcoma morphology is highly variable, depending on tumor type (Walaas and Kindblom 1990). Only high grade osteosarcoma can be diagnosed on

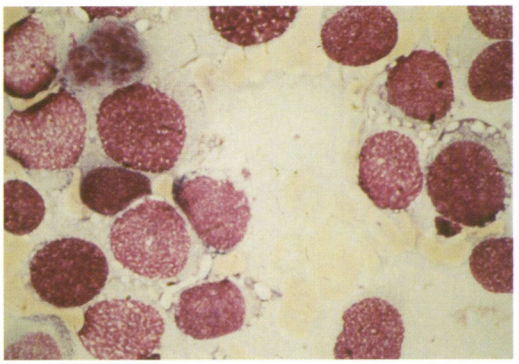

Figure 1. Ewing's sarcoma with a mixture of dark and light cells and vacuolated cytoplasm. HE, ×1250.

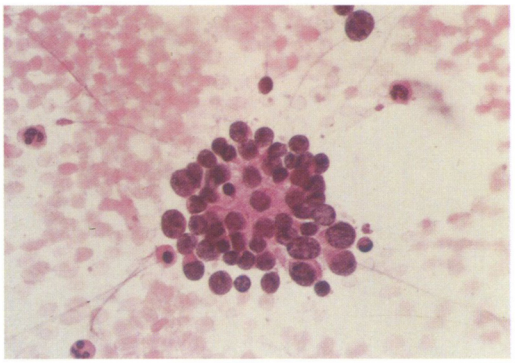

Figure 2. Rosette-like structure in metastatic neuroblastoma. HE, ×600.

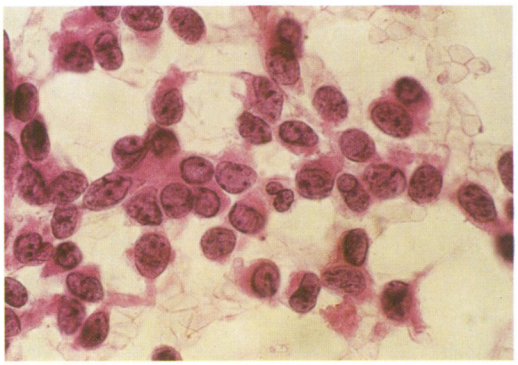

Figure 3. Atypical Ewing's sarcoma: rosette-like structure, irregular nuclei and cytoplasmic contacts. HE, x 600.

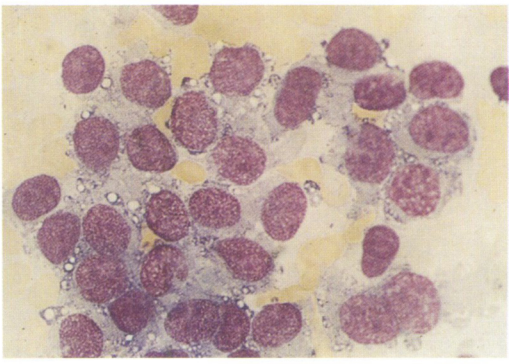

Figure 4. Atypical Ewing's sarcoma: one cell type with atypical nuclei. MGG, ×1250.

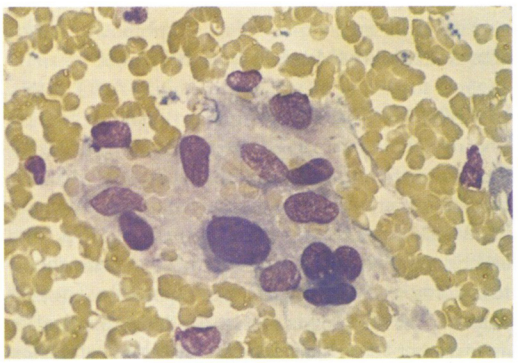

Figure 5. Osteosarcoma with single pleomorphic cells. MGG, ×1250.

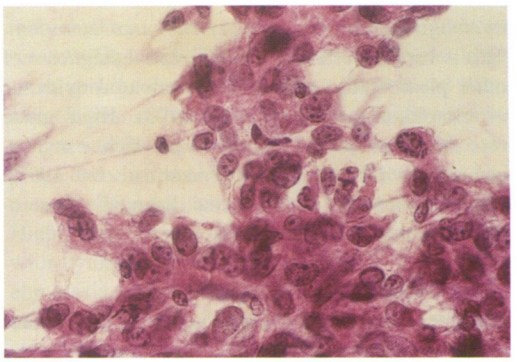

Figure 6. Osteosarcoma: spindle cells with atypical mitotic figures. HE, ×1250.

FNA and the morphologic diagnosis must be closely correlated with the radiology. Low grade osteosarcomas must be diagnosed histopathologically. The aspirates are usually cellular with cell groups, fragments and single pleomorphic cells (Figure 5). Cellular and nuclear pleomorphism, multinuclear tumor cells and spindle cells with atypical mitotic figures are common (Figure 6). For osteoid and/or chondroid matrix MGG staining is of eminent importance.

In osteoblastic osteosarcomas, bright red or pink osteoid (MGG) can be found in the background with the cellular fragments (Figure 7) or isolated amorphic acellular material. In telangiectatic tumors the material is blood rich, usually lacking osteoid or chondroid matrix. Also in chondroblastic or fibroblastic osteosarcomas osteoid production can be missing.

Cytochemistry with alkaline phosphatase is strongly positive in all types of osteosarcoma (Figure 8).

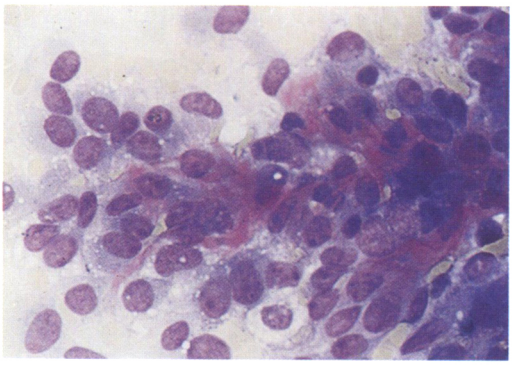

Figure 7. Osteosarcoma: cellular fragment with pink osteoid background. MGG, ×1250.

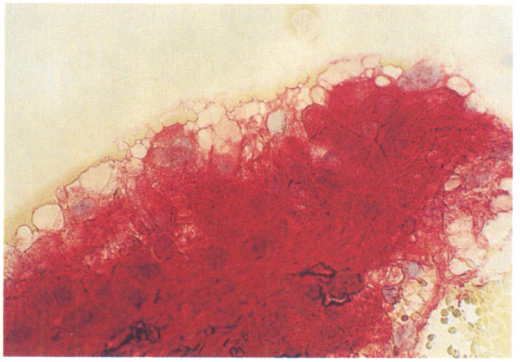

Figure 8. Osteosarcoma: Strongly positive alkaline phosphatase, ×1250.

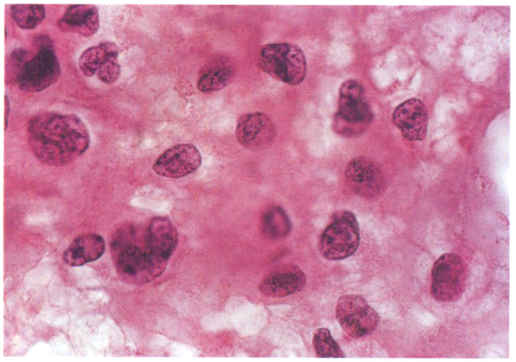

Figure 9. High grade chondrosarcoma with dissociated single cells. HE, ×1250.

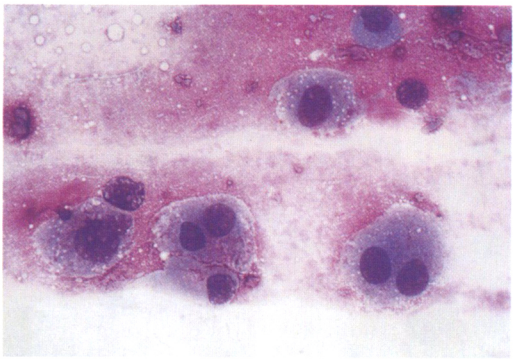

Figure 10. High grade chondrosarcoma: myxoid background, single cells and nuclear hyperchromasia. MGG, ×1250.

This is very important in the differential diagnoses of other pleomorphic tumors. Ultrastructural evidence of osteoid is helpful (Carlén 1990). High grade osteosarcomas including giant cell rich osteosarcomas show DNA aneuploidy (Åkerman et al. 1990). which is important in differential diagnosis of osteoblastoma or giant cell tumors.

Giant cell tumors

The smears are usually hemorrhagic and multinucleate giant cells are numerous and uniform. The other prominent feature is a mononuclear cohesive spindle cell population with scattered regular mitoses and intercellular collagen. There are several benign bone lesions with similar FNA morphology i.e. osteoblastoma, aneurysmal bone cysts, reparative granuloma and brown tumor in hyperparathyroidism (Walaas and Kindblom 1990, Sneige et al. 1985). Pleomorphism in giant cells or spindle cells and numerous and/or atypical mitotic figures are not present in true giant cell tumors. DNA-ploidy analysis is helpful to confirm malignancy, i.e. giant cell rich osteosarcoma (Åkerman et al. 1987). However, true giant cell tumors can show tetraploidy (Mandahl et al. 1993).

Cartilaginous tumors

The morphological diagnosis of chondromatous tumors is difficult. The sign of malignancy is sometimes more prominent clinically than morphologically (Walaas et al. 1990, Abdul-Karim et al. 1993, Tunç and Ekinci 1996). The diagnosis of low grade chondrosarcoma can be difficult or impossible on FNA alone. MGG staining is a must for the cytological diagnosis, but HE is helpful in the evaluation of nuclear structures.

The chondromyxoid matrix and hyaline cartilage with chondrocytes in lacunary structures are present in chondroma and low grade chondrosarcoma. With the exception of surface chondroma and chondroma in small peripheral bones, the cellularity is low and uniform. In low grade chondrosarcomas, moderate variations in nuclear size and shape and binuclear cells occur, mitotic figures are absent. The high grade chondrosarcomas have myxoid background, high grade pleomorphism, atypical mitotic figures and dissociated single cells (Figures 9 and 10). Spindle cells

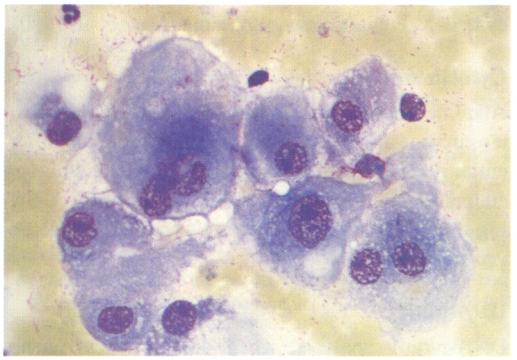

Figure 11. Chordoma: large physalipherous cells with round nuclei and vacuolated cytoplasm. MGG, ×1250.

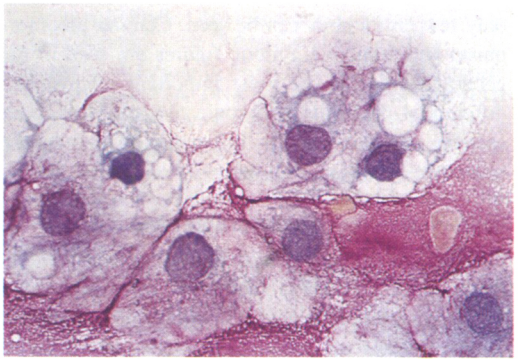

Figure 12. Chordoma: purple stained myxoid background surrounding individual physalipherous cells. MGG, ×1250.

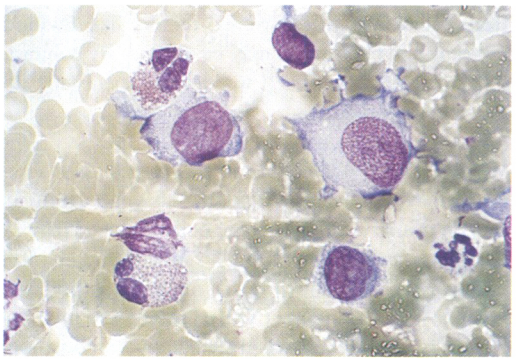

Figure 13. Histiocytosis X: histiocytes with irregular nuclei and eosinophilic leukocytes. MGG, ×1250.

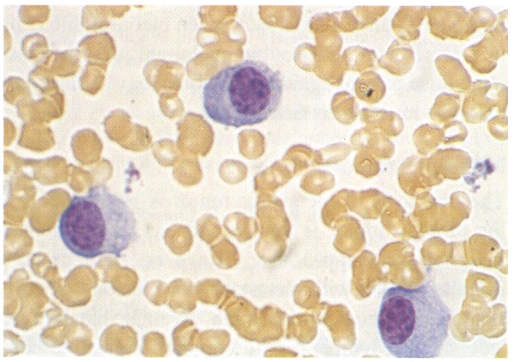

Figure 14. Plasmocytoma: eccentric nuclei clear perinuclear zone and rich cytoplasm. MGG, ×1250.

and/or pleomorphic cells component in adults are signs of dedifferentiated chondrosarcoma (Dee et al. 1991). DNA-ploidy analysis is an important diagnostic tool in chondrosarcoma diagnosis (Åkerman et al. 1987, Mandahl et al. 1993, Kreicbergs 1990).

Alkaline phosphatase staining is important for diagnosing chondroblastic osteosarcoma in children and young. Fanning et al. (1990) and Walaas and Kindblom (1990) described FNA features of chondroblastoma: mononuclear chondroblasts, multinucleated osteoclast-like giant cells and chondroid matrix. A similar FNA morphology can also occur in chondromyxoid fibroma (Fanning et al. 1990).

Chordoma

Cytological features are well described by Plaza et al. 1989, Walaas and Kindblom (1990). Cytologically, a double population consisting of large physaliferous cells with vacuolated cytoplasm (Figure 11) and smaller epithelial-like cells is spread in a network of myxoid background matrix. There are single or double nuclei with small nucleoli and slight to moderate anisokaryosis. The myxoid background is most prominent in MGG, purple stained, fibrillar surrounding individual tumor cells (Figure 12). Mitotic figures are rare. Walaas and Kindblom (1991) also described short spindle-shaped or stellate cells without vacuoles. In the differential diagnosis, chondrosarcoma and metastatic carcinoma must be excluded. Low grade chondrosarcomas produce hyaline matrix with tumor cells in lacunae which is absent in chordoma. High grade chondrosarcoma can mimic chordoma with cytoplasmic vacuoles but shows more hyperchromasia and pleomorphism. This is also valid for metastatic carcinomas having higher atypical mitotic activity than chordoma. In some chordomas, the soft tissue mass can dominate and mimic soft tissue sarcoma i.e. liposarcoma. Immunohistochemistry, EM and cytochemistry are important for the final diagnosis.

Histiocytosis X (eosinophilic granuloma)

The clinical features and radiology, with solitary or multiple osteolytic lesions are often characteristic. However, the appearance of FNA can be variable depending on the stage of the disease. The most characteristic features are histiocytes with irregular lobulated nuclei, histiocytic giant cells and eosinophilic leukocytes (Figure 13). In the late stage of the disease

only few eosinophils can be seen. Cellular pleomorphism and numerous mitotic figures are seen in the generalized form. The most important microscopic differential diagnosis is chronic osteomyelitis, both specific and non-specific inflammation can mimic histiocytosis X.

Solitary plasmocytoma/multiple myeloma

Plasma cells are well recognized by eccentric nuclei, clear perinuclear zones (absent in osteoclasts) and rich amphophil cytoplasm (Figure 14). The diagnosis is easy when only one cell population is present. However, plasmocytomas often bleed heavily and the hemorrhagic aspirates may only contain few plasma cells. Surprisingly often, plasmocytoma is misdiagnosed as another malignancy when pleomorphic cells or plasma cells with double nuclei are present. Plasmocytoma and malignant lymphoma may be differentiated by immunohistochemistry.

Metastatic tumors

The diagnosis of metastatic tumor is not difficult if the primary tumor is known and has a documented morphological appearance. However, some tumors initially present with metastatic bone lesions. In children, metastatic neuroblastoma, alveolar rhabdomyosarcoma and lymphoblastic lymphoma must be considered. Metastatic carcinoma is the first choice in adults. Carcinoma of the breast, prostate, lung and kidney are the most common types. Metastatic carcinoma of the kidney is an important differential diagnosis of chordoma. The cytological appearance of metastatic tumors shows general features of malignancy and is recognizable as a foreign cell population. Once again, ultrastructure and immunocytology can contribute to a specific tumor diagnosis.

Ancilliary diagnostic methods (Table 1)

Electron microscopy

EM is essential in the primary diagnosis of Ewing's sarcoma, atypical Ewing's sarcoma and PNET. Ewing's sarcoma is glycogen rich and scarce in organelles. Atypical Ewing's sarcoma and PNET show decreasing glycogen but increasing amount of organelles and neurosecretory granules. In poorly differentiated osteosarcoma, the EM finding of osteoid can support the diagnosis (Carlén 1996). The differential diagnosis of chordoma, metastatic carcinoma and chondromatous tumor can be solved by ultrastructural features. The matrix of chordoma is rich in proteoglycan particles absent in adenocarcinomas. Cartilaginous tumors show chondrocytes and chondroblasts. Birbeck's granula are diagnostic for histiocytosis X, eosinophilic granuloma (Katz et al. 1980).

Cytochemistry

Alkaline phosphatase is positive in all types of osteosarcoma and the staining is important in the differential diagnosis of osteosarcoma. Osteoclast-like giant cells stain negative for alkaline phosphatase. Ewing sarcoma and atypical Ewing's sarcoma are glycogen rich tumors.

DNA-ploidy

DNA-ploidy analysis, when demonstrating aneuploidy is of great importance in differentiating chondromatous tumors like cellular chondroma, chondroblastoma and chondrosarcoma and osteosarcoma from osteoblastoma. However, tumors like Ewing's sarcoma and chordomas may be diploid. By contrast, other pediatric small cell tumors and metastatic tumors are aneuploid.

Immunocytochemistry

Immunocytological examinations may be useful in several primary and metastatic bone tumors. HBA 71 is strongly positive in Ewing's sarcoma and atypical Ewing's sarcoma showing far more reliability than neurone specific enolase (NSE). Desmin and myoglobin are of importance when rhabdomyosarcoma metastases are suspected. The results of immunocytology in neuroblastoma depend on tumor differentiation. S-100 protein and CD1 support a diagnosis of eosinophilic granuloma. S-100 protein is positive in chondromatous tumors and chordoma, but the later is positive for cytokeratin antibodies (Cam 5.2) and is also negative for CEA. These immunostains are important for differentiating chondrosarcoma, chordoma and metastatic carcinoma. Plasmocytoma and myeloma are monoclonal, and also demonstrate EMA membrane positivity. A diagnosis of malignant lymphoma is supported by T- and B-markers and Kappa or Lambda monoclonality. CD 30 is mandatory for the diagnosis of large cell anaplastic lymphoma. Prostatic carcinoma metastasis is proven by PSA staining and thyroid carcinoma metastases by the presence of thyroglobulin. Malignant melanoma metastases stain positive for S-100 protein and melanin associated antigen (HMB 45).

Cytogenetic and molecular genetic analysis

FNA can be used for cytogenetic and molecular genetic analysis. This is of diagnostic importance in Ewing's sarcoma. The pattern of complex aberrations in osteosarcoma is of limited value, but may support a diagnosis of high grade sarcoma (Åkerman et al. 1996).

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