A case of Ewing´s sarcoma diagnosed by fine needle aspiration

Light microscopy, electron microscopy and chromosomal analysis

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A Ewing´s sarcoma of the thigh in a 9-year-old boy was diagnosed by light microscopy, electron microscopy, and chromosomal analysis performed on fine needle aspirates.

Open biopsy of a musculoskeletal sarcoma more or less increases the extent of surgery necessary for a later radical operation. Soft tissue sarcomas can be accurately diagnosed by a combination of clinical, radiologic, and cytologic examinations obviating the need for open biopsy. Although the exact histogenetic type of sarcoma cannot always be determined by these examinations, they are in most cases sufficient for the decision of appropriate surgery (Angervall et al. 1986).

By contrast, the histologic diagnosis of Ewing´s sarcoma of bone is sometimes difficult even by an open biopsy. We report a case of Ewing´s sarcoma diagnosed by radiology and light microscopic, electron microscopic, and chromosomal examinations of a fine needle aspirate.

Case report

A 9-year-old boy was admitted because of pain in his left thigh of 6 weeks´ duration. A mass could be palpated in the midthigh. Radiologic evaluation was suggestive of a Ewing´s sarcoma in the femur with soft-tissue extension. (Figure 1). Whole-body 99m Tc-MDP scintigraphy showed a highly increased uptake in the femoral diaphysis, but was otherwise normal. CT of the lungs was normal. Bone marrow aspirate and trephine biopsy from the posterior iliac spines were normal. There were no malignant cells in the cerebrospinal fluid.

Fine needle (0.7 mm) aspirates (three passes) from the soft-tissue mass were processed for light microscopic examination, immunohistochemistry, electron microscopy, and chromosomal analysis.

The light microscopy of the first aspirate, stained with hematoxylin and eosin and May-Grünwald-Giemsa, showed two types of tumor cells. One cell type was large with a moderate amount of cytoplasm often containing multiple vacuoles or clear spaces: the nuclei were round to oval with slight polymorphism, finely dispersed chromatin, and one or two small nucleoli. The other cell type was smaller, with a narrow rim of cytoplasm and ovoid or irregular nuclei with evenly distributed dense chromatin (Figure 2). Immunostaining showed that the tumor cells were positive for vimentin (Dakopatts, Copenhagen), but negative for the leucocyte-common antigen (Dakopatts, Copenhagen) by the APAAP technique (Cordell et al. 1984).

The electron microscopy of the second aspirate, fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer with 0.1 M sucrose, showed tumor cells with a moderate amount of cytoplasm and rounded or ovoid nuclei. The cytoplasm was poor in organelles, but contained abundant deposits of glycogen aggregated in large pools. Microtubuli, neurosecretory granulae, or signs of myogenic differentiation were not observed (Figure 2).

The third aspirate was cultured in 10 ml McCoy´s 5A medium supplemented with glutamine, antibiotics, 0.4 µl/ml heparin, and 20 percent fetal bovine serum. The cells were incubated at 37° C for 24 hours. Two hours prior to harvest, 50 µl Colcemid (10 µg/ml)

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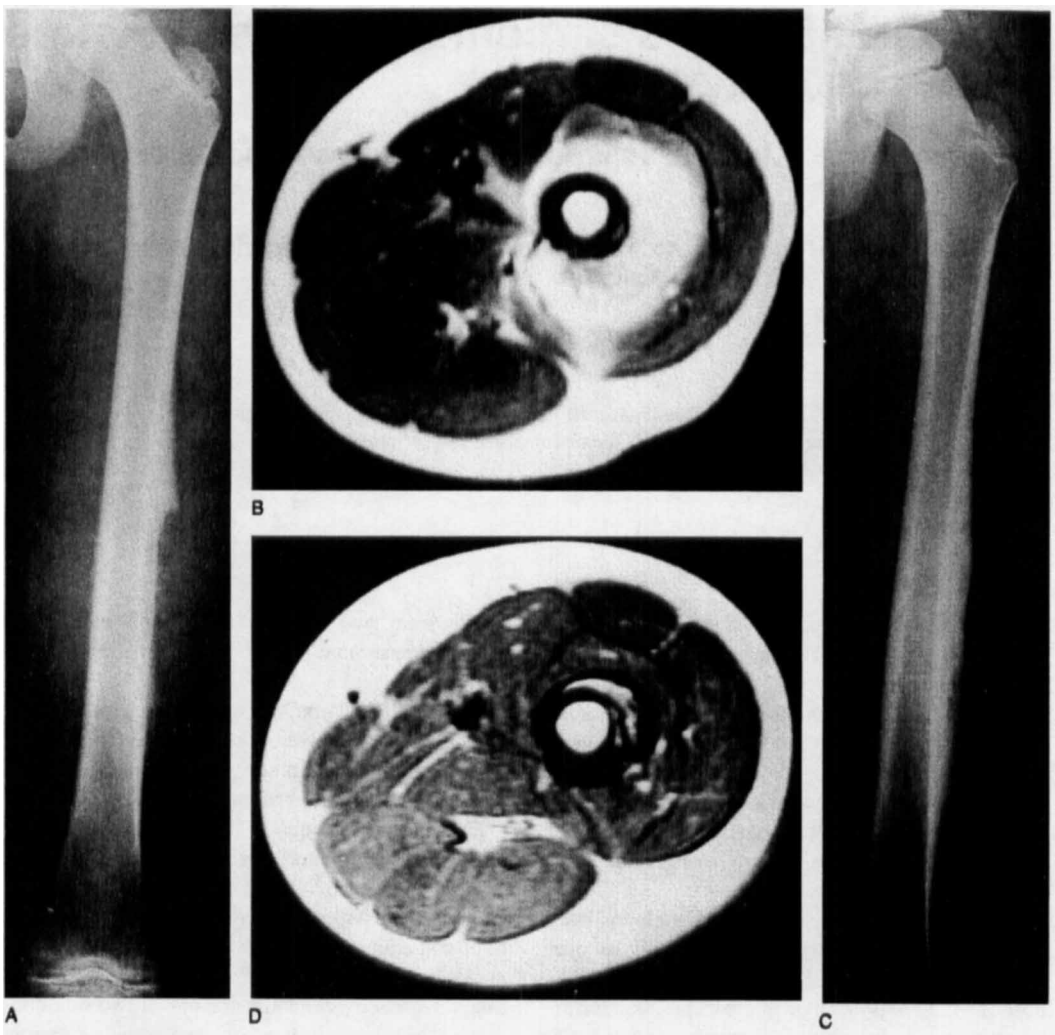


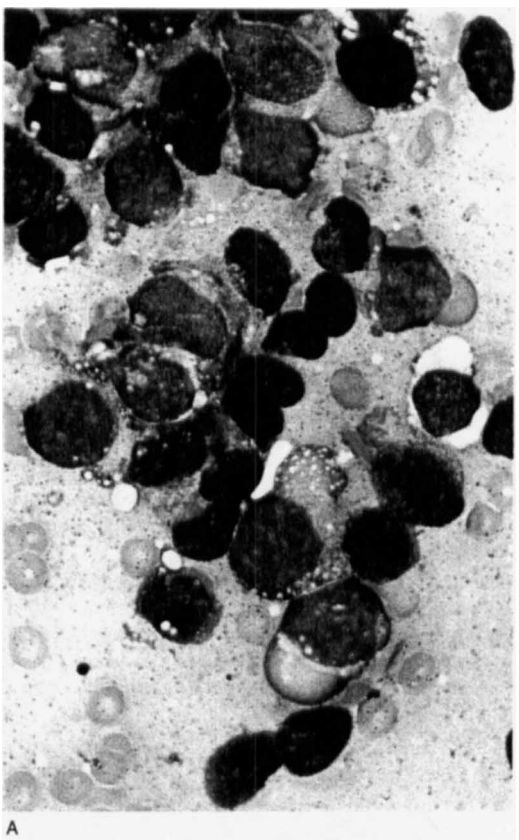
Figure1. A 9-year-old boy with pain in the left thigh of 6 weeks' duration.
A. Intramedullary tumor without cortical destruction.Laminated and spiculated periosteal reaction.
B. MRI. The soft-tissue mass is best seen with T2-weighted images.

After 6 months chemotherapy.
C. Extracortical new bone formation.
D. MRI. T2-weighted images. No soft-tissue mass.

was added to the culture. Chromosome preparations were made by conventional air-drying, including hypotonic shock by 0.075 M KCl for 15 minutes and four changes of fixative (methanol:glacial acetic acid, 3:1). Wright's stain was used for G-banding. The chromosome number could be determined in 12 metaphases; five of these were possible to karyotype. Two related clones were found; both had 50 chromosomes and a translocation between chromosomes 11 and 22 (Figure 2). The karyotypes were 50,XY,+2,+8,+9,t(11;22) (q24;q12),+12 and 50,XY,+2,+9,t(11;22) (q24;q12), +12,t(16;?)(q24;?),+mar.

The light microscopic appearance of the tumor cells, the ultrastructural analysis, and the 11;22 translocation were consistent with a Ewing's sarcoma; an open biopsy would not further support the diagnosis.

The boy was treated for 6 months with two cycles of preoperative combination chemotherapy according to the Scandinavian Sarcoma Group protocol: "SSG IV, 1983". This regimen is a modification of Rosen's T-11 protocol and includes Cyclophosphamide, Adriamycin, Methotrexate, Vincristine, Bleomycin, and Actinomycin D. The pain disappeared. A repeated radiologic work-up showed signs of tumor healing, and no



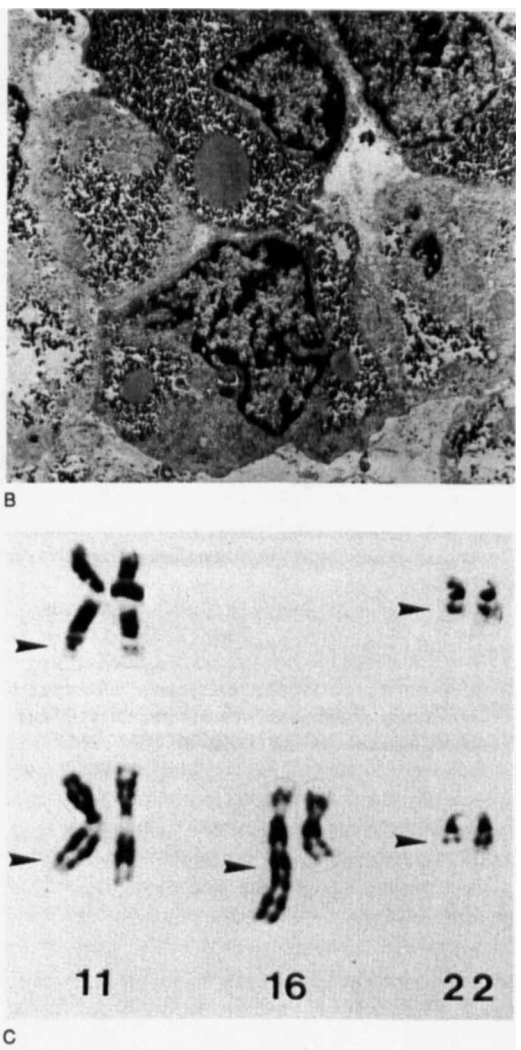


Figure 2. Fine needle aspirate from the soft-tissue mass.
A. Light microscopy with a mixture of large light cells with round to oval nuclei and smaller cells with dark nuclei. In the larger cells, there are vacuoles and large clear spaces in the cytoplasm. MGG, x600.
B. Electron microscopy. Tumor cells with large glycogen pools. x6000.
C. Chromosomes selected from two metaphases demonstrating the structural aberrations encountered in the two clones. In one of them, there was a rearrangement in addition to the 11;22 translocation; a chromosome segment of unknown origin has translocated to the end of the long arm of chromosome 16. The left chromosome of each pair is the deranged one, and the right chromosome is the normal homolog. Arrowheads indicate breakpoints.

metastases could be found (Figure 1) The isotope uptake had decreased considerably, but was still above normal. Radical surgery was performed by hip disarticulation. No remaining tumor could be found at histologic examination of multiple sections from the leg. The chemotherapy continues according to the SSG-IV protocol.

Discussion

The histologic differential diagnosis of Ewing´s sarcoma, metastatic neuroblastoma, rhabdomyosarcoma, and non-Hodgkin lymphoma is difficult, and must in many cases be supported by electron microscopy and immunohistochemistry. The electron microscopic and the cytologic appearance of Ewing´s sarcoma have been reported earlier (Navas-Palacios et al. 1984, Ahktar et al. 1985, Dahl et al. 1986); the findings in our case were consistent with these reports. The negative immunostaining for leucocyte-common antigen excluded a non-Hodgkin lymphoma.

Out of about 40 cases of Ewing´s sarcoma reported in recent years, two thirds had a 11;22 translocation (Mitelman 1985). Most of these analyses have been performed on short-term cultures from tumor tissue obtained at surgery; the use of cell material obtained from a fine needle aspirate has not been reported. Successful chromosomal analyses from fine needle aspi-

rates have, however, been reported in non-Hodgkin´s lymphomas (Kristoffersson et al. 1985)

In our case, hip disarticulation was performed because most of the femur was involved according to the radiologic examinations; an open biopsy for diagnosis had in this case not increased the extent of the definitive surgery. In other cases where limb-sparing surgery is considered, diagnosis without prior surgical biopsy may be even more motivated.

The host of authors for this report demonstrates the need for multidisciplinary management of patients with musculoskeletal sarcomas.

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