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Diagnostic Value of Electron Microscopy in Rare Malignant Musculoskeletal Tumors

Experience from an orthopedic tumor center

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ABSTRACT

Rare musculoskeletal tumors can be difficult to diagnose by light microscopy or immunohistochemistry. Electron microscopy can be of diagnostic assistance especially if histotype specific ultrastructural features exist. In particular, electron microscopy for uncommon sarcomas such as alveolar soft part sarcoma, parachordoma, atypical Ewing's sarcoma and epithelioid sarcoma may be the diagnostic modality of choice.

INTRODUCTION

A musculoskeletal tumor center was established in 1972 at the Lund University Hospital which acts as a referral institution for patients from the Southern Swedish Healthcare Region (population 1.5 million) with proven or suspected musculoskeletal malignancies. All patients with a malignant soft tissue or bone tumor are registered in a Regional Cancer Registry. By centralization, diagnoses are made or confirmed by our pathologist and cytologists.

Sarcomas are a heterogeneous group with over thirty different histotypes. The annual incidence is approximately 17 per million population underscoring the rarity of these tumors which account for less than one percent of all malignancies. Modern diagnostic techniques include immunohistochemistry, electron microscopy and cytogenetics which have helped to refine the diagnosis and classification of sarcomas. Electron microscopy is one of several routine investigations performed on every tumor referred to our institution, and material for this is obtained from both fine needle aspirates as well as surgical or open biopsy specimens. Although rare, the features of many sarcomas are now well-recognized. Nevertheless, diagnostic dilemmas do arise with tumors which are particularly infrequent, those that closely mimic a variety of other histotypes and when the presentation is so atypical as to exclude the likelihood of the correct

diagnosis. This paper presents 8 cases to demonstrate the value of electron microscopy in the management of musculoskeletal tumors.

MATERIALS AND METHODS. RESULTS

There were 4 soft tissue and 4 bone sarcomas (Table 1). The median age was 40 (range 8-73) years. All except one tumor (rib) involved the extremities, and the median tumor diameter was 8.5 (4-16) cm. Material for electron microscopy was obtained from fine needle aspirates in 4 cases and from the operative specimen in 5 cases. Cytogenetic analysis was performed in all cases. In case 8, failure of cultures prevented chromosomal analysis, but reverse transcriptase polymerase chain reactions were used to identify the t(11;22) fusion gene transcript, FLI1/EWS.

Table *1.Clinical data and investigations beside electron microscopy*

Case	Type	Age	Sex	Size (cm)	Site	FNA	OB/SS	IHC	Cytogenetic
1	ASPS	27	M	6	thigh	yes	SS	yes	46, xy
2	Parachordoma	45	F	4	leg	yes	SS	yes	45-46,cx
3	Epithelioid sarcoma	37	F	7	arm	no	OB/SS	yes	46,xx
4	Epithelioid sarcoma	73	M	8	elbow	no	OB/SS	yes	65-75, xy,cx
5	Atypical Ewing's sarcoma	8	F	15	femur	yes	SS	yes	t(11;22)
6	Atypical Ewing's sarcoma	38	M	16	femur	yes	OB/SS	yes	t(11;22) del(22)(q12)
7	Atypical Ewing's sarcoma	19	M	4	foot	yes		yes	t(11;22)
8	Ewing's sarcoma	73	M	8	11th rib	yes	SS	yes	PCR; FLI1/EWS-fusion

FNA: Fine needle aspiration, OB: open biopsy, SS: surgical specimen, IHC: immunohistochemistry

Small samples of fresh tumor tissue was immediately fixed in 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded ethanols, passed through propylene oxide and embedded in Agar 100 resin overnight at +60°. Ultrathin sections of selected areas were stained with uranyl acetate and lead citrate and examined with a Philips CM10 electron microscope at 60 kV. In four cases, in which fine needle aspiration was performed, collection of the aspirate was done according to a method described by Kindblom et al (12).The same timetable and solutions used for surgical specimens were also used for FNA. For cases no 3, 5, 7 and 8, a rapid embedding method was used. The open biopsy of case no 3 was cut into very small pieces (0.5mm^3). Except for shortened times, the same process was used. This made it possible to examine the ultrathin sections and provide a diagnosis on the following day. Cultured cells

from the surgical specimen of case 7 were also examined by EM. The technique has been described previously (33). Glycogen was demonstrated by using 0.5% tannic acid for 2 minutes in room temperature before counter staining with uranyl-acetate/lead citrate.

Immunohistochemistry

Table 2. *Antibodies used in this study*

Antibody	Dilution	Source	Case no.
Vimentin; mAb	1:50	Dako, Copenhagen, DK	1,2,3
Desmin; mAb	1:100	Dako	1,3,5,6,8
Clone DE-R-11; mAb	1:200, 1:50	Dako	1
Smooth muscle alpha; mAb	1:500	Dako	1
Muscle spec. actin; mAb	1:400	Enzo, New York, NY, USA	1
Sarcomeric actin; mAb	1:200 over night	Dako	1
Laminin; polyclonal	1:1200 over night	E.Y. Labs, San Mateo, CA, USA	1
Collagen IV; mAb	1:100 over night	Dako	1
S-100 protein; mAb	1:10000	Dako	1,2,3,4,6,7
Cam 5,2;mAb	1:10	BecktonDickinson, Mountain View, CA, USA	2,3,4,8
AE1/AE3; mAb	1:800	Boehringer, Mannheim, D	2,3
Alpha-1-CT; mAb	1:8000 over night	Dako	2
EMA; mAb	1:20	Dako	2,3
HMB45; mAb	1:300 over night	Dako	3
MNF116; mAb	1:50	Dako	3
PKK-1; mAb	1:50	Labsystems, Helsinki, SF	3
NSE; mAb	1:2000	Dako	5,6,7,8
Chromogranin A; mAb	1:500	Boehringer	5,7,8
HBA-71;mAb	1:20	Signet, Dedham, MA, USA	3,5,7,8

Alveolar soft part sarcoma. Alveolar soft part sarcoma (ASPS), was first described in 1952 by Christopherson et al. (1), and its ultrastructure elucidated 10 years later by Shipkey et al. (27). ASPS accounts for less than 1% of soft tissue sarcomas and has a predisposition for young adults (2nd and 3rd decades) with a slight prevalence in female patients. ASPS is a slowly growing tumor of uncertain histogenesis and a poor prognosis. It arises in the extremities, trunk, head and neck and apart from metastases to lung and bone, it is also notable for brain secondaries. Surgery is the mainstay of treatment. Adjuvant radiotherapy (25) has also been reported to be effective.

Light microscopy. Light microscopy of FNA and surgical speciment from case 1 revealed a rather monotonous picture with nest-like formations of polygonal cells with eccentric nuclei, some with one or two large nucleoles. The elongated cytoplasm had a granular PAS positive appearance. The cell aggregates were surrounded by thin-walled sinusoidal vessels with flattened endothelial cells. A granular cell tumor was suggested on frozen section.

Immunohistochemistry. The tumor cells stained positive for vimentin, desmin, clone DE-R-11, muscle specific actin, sarcomeric actin, mimicking muscular tumors.

Electron microscopy. Light microscopy of the semithin sections from the surgical specimen revealed tumor cells situated in a compartmental lobular pattern separated by fibrovascular septa. When examined in the electron microscope, the granular material seen in the cytoplasm were rhomboid and rodshaped crystalline structures surrounded by a membrane. The crystals consisted of parallel rigid 6 nm fibrils with a periodicity of 10 nm (5) (Fig 2). Apart from these crystalline structures, small dense Golgi-associated granules with an average diameter of 90 nm were seen. In connection with the small granules were larger granules ranging from 200-400 nm. Some of these latter granules showed partial crystallization of their granular contents. Close to the periphery of the nucleus were ribosomal lamellae (Fig 1). Glycogen and swollen mitochondria were dispered throughout the cytoplasm.

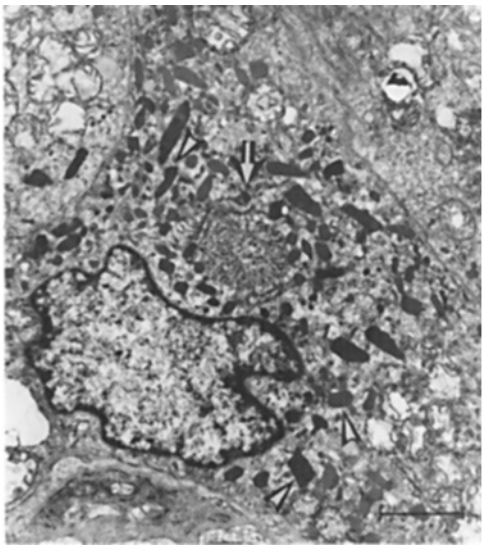

Fig 1. Low magnification of tumor cell filled with rhomboid and crystalline structures (arrowheads). Close to the nucleus is ribosome lamellae (arrow). Original magnification x 6600. Bar 2 μm.

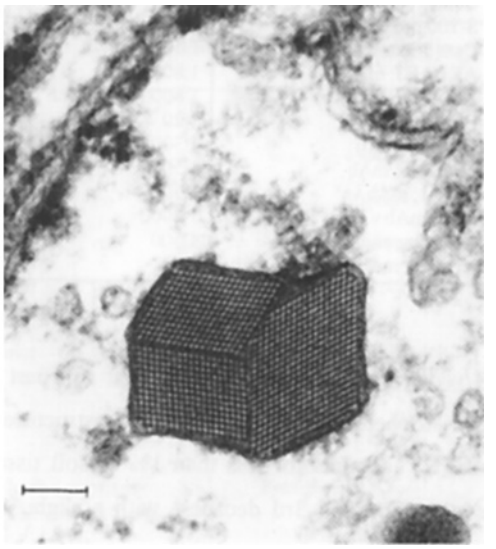

Fig 2. A crystal with parallel fibrils measuring 6 nm and with a periodicity of 10 nm. Original magnification x 105000. Bar 0.1 μm.

Short segments of rough endoplasmic reticulum and polyribosomes were also spread throughout the cytoplasm. Occasional densities were seen along the cell membrane. The cells had a basal lamina. These ultrastructures are specific for ASPS, having never been reported in any other cell type, benign or malignant. Cultured cells from this ASPS were also studied by EM. Crystalline structures situated close to the nuclei were detected.

Differential diagnoses. By light microscopy metastatic renal cell carcinoma is the most common differential diagnosis (5). This diagnosis was excluded by EM because of the absence of

epithelial features. Other tumors such as clear cell sarcoma, granular-cell tumor, paraganglioma have an architecture on histology that can lead to misdiagnoses of ASPS. These tumors have specific ultrastructures which are helpful for differentiating between the tumor types (Table 3).

Table 3. *Diagnostic ultrastructures in ASPS and its differential diagnoses.*

Tumor type	Ultrastructure
ASPS	Rodshaped crystalline structures
Renal cell carcinoma	Glycogen deposits, lumen, microvilli, desmosomes
Clear cell sarcoma	Premelanosomes
Granular-cell tumor	Lysosomal inclusions
Paraganglioma	Neurosecretory granules

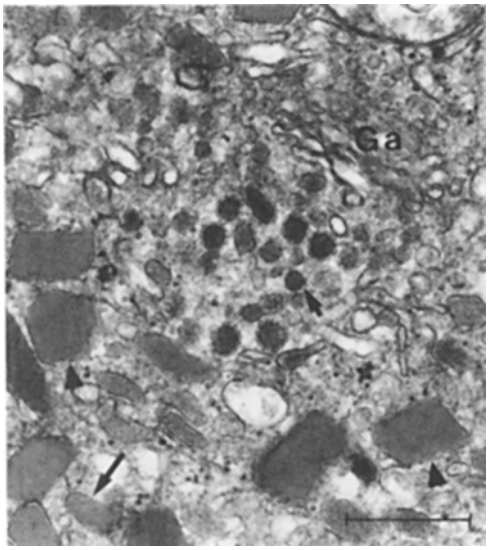

Fig 3. Electron micrograph with the two types of granules, small 90 nm (small arrow) close to the Golgi apparatus (Ga) lager 200-400 (large arrow) and crystallline structures (arrowheads). Original magnification x 39000. Bar 0.5 μm.

Comments. The distinctive crystalline structures of ASPS appear to arise from small Golgi-associated granules that pinch off from the ends of the Golgi saccules, and transform into larger glycoprotein-containing secretory granules whose contents then crystalize (15) (Fig 3).

These two types of membrane limited granules are found in all cases of this tumor studied to date. The theory of crystallization is attractive and seems to be corroborated by the observation of partially ctystalized granules. Immunohistochemistry has helped to resolve aspects of histogenesis and differentiation. Mukai et al. (20) and Foschini (9) found that the intra-cytoplasmic crystals are composed of actin. Others have found desmin, actin (9,19), vimentin and Z protein (20) in ASPS and therefore favored muscular differentiation, while Cullinare et al. suggested a neurogenic origin (2). There has been no ultrastructural evidence of myogenic (skeletal or smooth muscle) differentiation in any of the cases examined by transmission electron microscopy (6). ASPS has an intricate immuno-histochemical pattern which can be difficult to interpret. EM must therefore be regarded as the preferred choice for complementing fine needle aspiration cytology and histopathology.

Parachordoma. Laskowski (14) gave the first of only 20 reported cases of parachordoma (3,10,11,23, 26,30) of which, only three (11,26,30) have provided ultrastructural descriptions.

Light microscopy. FNA of case 2 revealed a tumor consisting of medium sized or large slightly irregular cells, arranged in clusters or cords in a PAS-positive myxoid background. The tumor consisted of lobules surrounded by a fibrous stroma. The nuclei were vesicular and the cytoplasm was large and vacuolated. The light microscopic findings suggested a juxta cortical myxoid chondrosarcoma.

Immunohistochemistry. The tumor cells were reactive against monoclonal antibodies to CAM5.2, AE1/AE3, S-100 protein, vimentin, alpha-1-CT and negative for EMA.

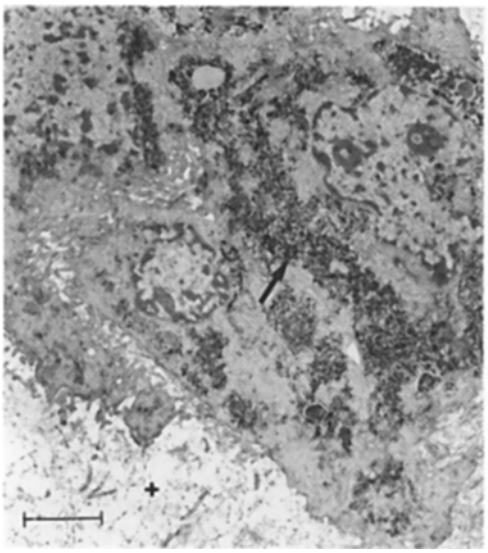

Fig 4. Low magnification of parachordoma cells with large gycogen deposits (arrow). In the matrix proteoglycan aggregates (+). Original magnification x 3900. Bar 3 μm.

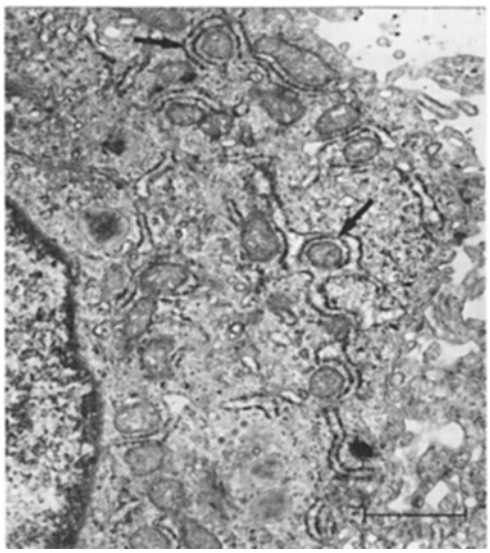

Fig 5. Part of a tumor cell with mitochondria- rough endoplasmic reticulum complexes (arrows). Original magnification x 15500. Bar 1 μm.

Electron microscopy. Material from FNA and surgical specimen were examined by EM. Cords and single cells were embedded in a myxoid matrix of proteoglycan looking aggregates. The cord-cells had centrally situated nuclei. The nuclei were lobulated with evenly distributed hetero-chromatin and prominent nucleoli. The cytoplasm contained large deposits of glycogen (Fig 4) and moderate amounts of lipid droplets. In some cells, mitochondria were surrounded partially or completely by single RER cisternae (Fig 5). Golgi complexes were easy to find, so were lysosomes and filaments of the intermediate type. A few intra-cytoplasmic lumina with a fluccuscent material (not glycogen) resembling those seen in chordomas could be detected (Fig

6). The cell membrane had numerous collections of pinocytotic vesicles, and peripheral microvilli which were on average 680 nm tall and had a diameter of 92 nm and without rootlets. Small dense cell junctions connected the cells. Some isolated single, spindle shaped cells had a cell membrane more similar to that of chondroblasts (Fig 7). These ultrastructural findings are similar to that described by Shin et al. (26) and Ishida et al. (11) and support a diagnosis of parachordoma.

Differential diagnosis. The two most discussed differential diagnoses are chordoma and skeletal or extra skeletal myxoid chondrosarcoma (11,26,30). This parachordoma had the same immunohistochemical pattern as the chordomas we have studied but not in any cases of extra skeletal myxoid chondrosarcoma (Table 4).

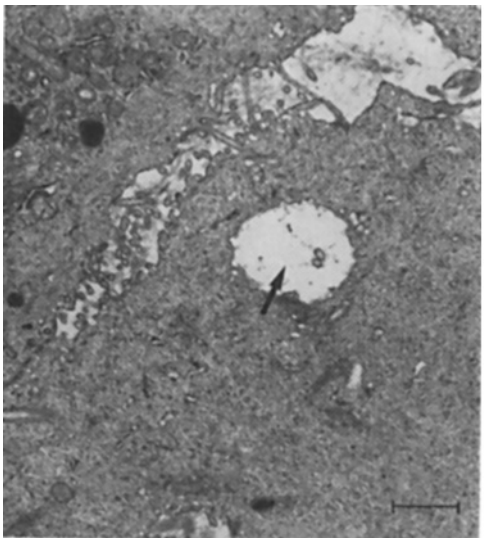

Fig 6. Intracytoplasmic lumens with a fluccuscent material (arrow). Original magnification x 10000. Bar 1 μm.

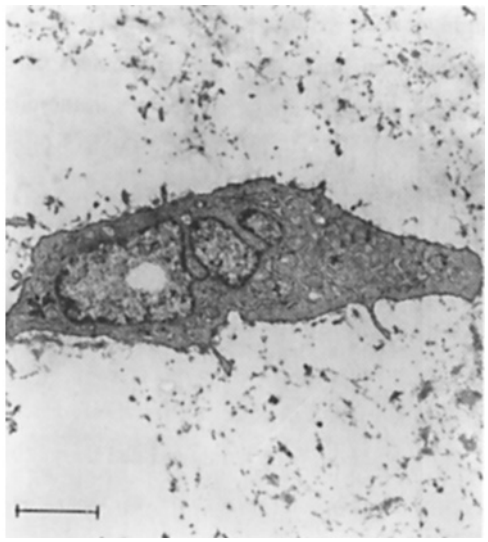

Fig 7. Single cell with chondroblastic appearance in a myxoid matrix. Original magnification x 6610. Bar 2 μm.

Table 4. *Immunohistochemical profile*

	Parachordoma	Chordoma	EMC
Vimentine	+	+	+
S-100 protein	+	+	+
Cytokeratins	+	+	-

EMC: Extra skeletal myxoid chondrosarcoma.

We could not demonstrate any cytogenetic similarities between parachordoma, EMC and chordoma.

*Comments.*Apart from several differences, this parachordoma resembled in EM and in its immunohistochemical profile our other cases of chordoma. The exceptions were 1) the parachordoma had much more glycogen; small lakes of glycogen have been reported (11,26). We did not notise the prominent vacuoles described by Sangueza (23). Instead we found large amounts of glycogen. If tannic acid was not used, washed out glycogen may give an impression of empty spaces. Large amounts of vacuoles may also be due to unstained glycogen and give a false impression of physalipherous cells. 2) more pinocytotic vesicles 3) fewer vacuoles 4) immature cell junctions and 5) no mature physalipherous cells.

Our results concur with others who found ultrastructural and immunohistochemical similarities with chordoma (10,11,23,26). This case of parachordoma had different ultrastructural features to extra-skeletal myxoid chondrosarcoma (33), which had microtubule aggregates in highly dilated RER and a specific cytogenetic translocation t(9;22). The single cells and ground substance in our case were reminescent of extra skeletal myxoid chondrosarcoma, but the absence of microtubular aggregates in the dilated RER excluded this diagnosis. Two types of cells, those forming cords and single cells were also reported by Ishida and differentiate parachordoma from that of chordoma and EMC. The ultrastructural and immunohistochemical results of this parachordoma suggest a mixed differentiation with a predominance of chordoma differentiation.

Epithelioid sarcoma. In 1970, Enzinger described a soft tissue neoplasm with epithelioid features (4), which has a predilection for the fingers, hands and forearms. It is the most common soft tissue sarcoma of the hand, and can be confused with a variety of malignant processes such as poorly differentiated carcinoma, malignant melanomas and synovial sarcoma. Its histogenesis is unclear.

Light microscopy. The light microscopic picture revealed a malignant tumor with infiltrative nodular growth. The cells were of medium size, partly spindle-shaped or rounded epithelioid. In case no 3 the cells had a trabecular formation, partly storiform with bizarre nuclei. The tumor cells were surrounded by fibrous tissue; sometimes with hyalinisations. Necrotic and inflammatory changes were present. Both tumors were provisionally diagnosed as epithelioid sarcomas on light microscopy.

Immunohistochemistry. Both cases showed positivity with S-100 protein. Case no 4 also immunostained for CAM5.2, while case no 3 was negative for antibodies against cytokeratin, desmin, HMB 45, EMA and HBA-71.

Electron microscopy. Both cases showed clusters of cells (Fig 8). The cytoplasm contained whirls of intermediate filaments pushing the other organelles towards the periphery

(Fig 9). Intercellular primitive lumina with short filopodia projections were evident. The cells were connected with primitive cell junctions. There was a great size variation between the nuclei of both cases (Fig 10). Case 3 also had a finely granular matrix close to the cell membrane. Besides clusters of cells, short cords of cells were also noticed. Pinocytotic vesicles and both slender and dilated rough endoplasmic reticulum were seen in case no 4. Except for the amount of filaments there was a slight resemblance of this case to synovial sarcoma.

Differential diagnosis. Important differential diagnoses to consider are poorly differentiated carcinoma (7,18), amelanotic malignant melanoma (7) and synovial sarcoma (7,22). Patchefsky stated that epithelioid sarcoma is a variant of synovial sarcoma. We have not noticed the same great amount of filaments he reported among synovial sarcomas (31). The absence of premelanosomes and presence of filament whirls rule out malignant melanoma. Tonofibrils and mature desmosomes are not found in epithelioid sarcomas, which are signs of epithelial histogenesis. Synovial sarcoma do not have the large amount of filament pushing the other organelles aside as seen in these cases.

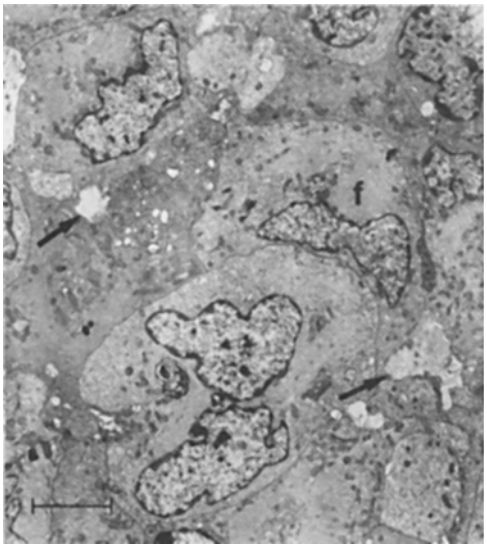

Fig 8. Cluster of cells. Between the cells, small lumens are seen (arrows). The light areas contain intermediate filaments (f). Original magnification x 2950. Bar 4 µm.

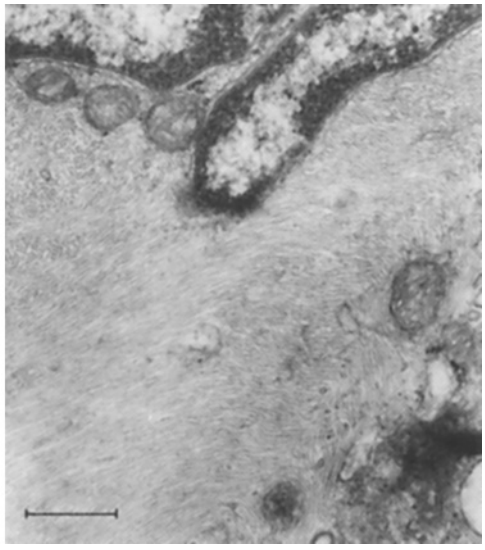

Fig 9. The cytoplasm is filled with filament pushing the other organelles aside against the periphery. Original magnification x 28500. Bar 0.5 µm.

Comments. The ultrastructural observations were much the same as was noted by Meis et al. (18). They also noted an absence of mature desmosomes, only primitive cell junctions. Like our two cases they also showed that the entire cytoplasm was filled with filaments and the organelles were pushed aside. The remaining organelles were scanty. The cells showed a pseudo glandular pattern. Patchefsky (22) reported an outer coat of finely granular matrix as did we.

Patients with lesions situated near dermis and subcutis of the distal extremities should be suspected of having epithelioid sarcoma. Most cases of epithelioid sarcomas reported in the literature show positivity for keratin. While case no 3 was negative for keratin, the ultrastructure was similar to what is described for this tumor.

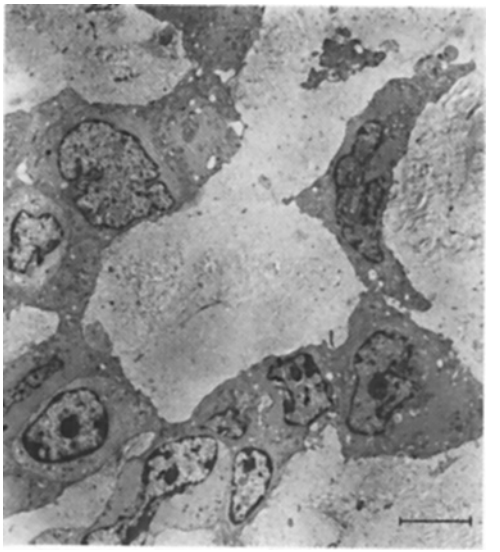

Fig 10. Low magnification of cells with epithelial look in a garland style. A great variation of nuclei size is seen. Original magnification x 2200. Bar 4 µm.

Atypical Ewing's sarcoma of bone. Light microscopic appearances that differentiate small-cell malignant tumors in children can be difficult. A correct diagnosis is important both for treatment and prognosis. Certain diagnoses like rhabdomyosarcoma, metastatic neuroblastoma and malignant lymphoma can be supported by immunocytochemistry and cytogenetic analyses while classical Ewing's sarcoma (EWS), atypical Ewing's sarcoma (AEWS) and peripheral primitive neuroectodermal tumor (pPNET) need additional methods. Among our 36 cases of EWS of bone investigated by EM we have three cases with a divergent picture, so called atypical Ewing's sarcoma.

Light microscopy. The light microscopic picture in all three fine needle aspirates was that of a small-cell malignant tumor with typical Ewing cells but also cells with a variation of nucleus size, form and shape. Some of the cells had more cytoplasm and cytoplasmic processes. A mixture of light and dark cells, characteristic classical Ewing sarcoma, were less prominent or missing. A few rosettes were noticed in cases no 6 and 7. Mitoses were uncommon. Provisional diagnosis was small-cell malignant tumor.

Immunocytochemistry. Cases no 5 and 7 were positive for HBA-71. At the time of diagnosis of case no 6, mAb HBA-71 was not available. Cases no 6 and 7 showed slight positivity for NSE and S-100 protein, respectively. Two cases (5 and 6) were stained for desmin, which was negative and cases no 5 and 7 were stained for chromogranin A with negative result.

Electron microscopy. The main ultrastructural features in all three cases were as follows: Classical Ewing's cells were characterized by compact sheets of small round cells and single cells. The cytoplasm was scarce in organelles and poorly differentiated. Dispersed cell junctions of the primitive type were seen between the cells. The nuclei were round with occasionally one or two invaginations and had sometimes a prominent nucleolus situated in the periphery. The chromatin was finely dispersed. This cell type (Fig 11) had a few mitochondria, abundant free ribosomes and isolated Golgi complexes. Glycogen was abundant and arranged in large aggregates with lipid droplets. In case 7 there were some totally undifferentiated cells with a cytoplasm having almost nothing but free ribosomes and a few mitochondria (Fig 12). No glycogen was seen in these cells. Atypical Ewing's cells were also present, and characterized by a more mature cell type, larger in size and with some of the characteristics of the classical Ewing's cells such as glycogen deposits. The cell organelles were more prominent and numerous (Fig 13). They had abundant mitochondria and Golgi complexes. Cased 6 and 7 also had cytoplasmic filaments. In all three cases some cell processes were seen between the cells.

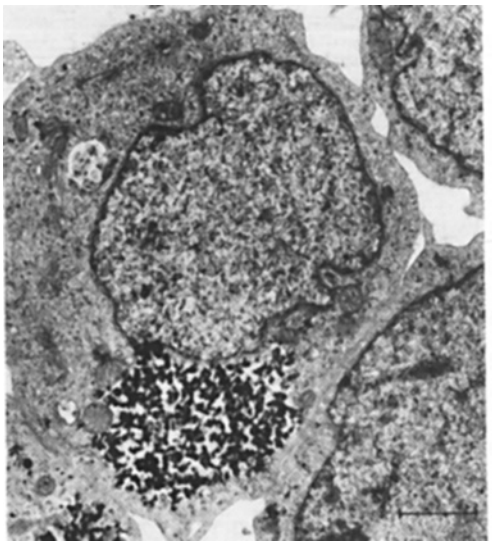

Fig 11. Typical EWS cell with glycogen deposits and few organelles. Original magnification x 11500. Bar 1 μm.

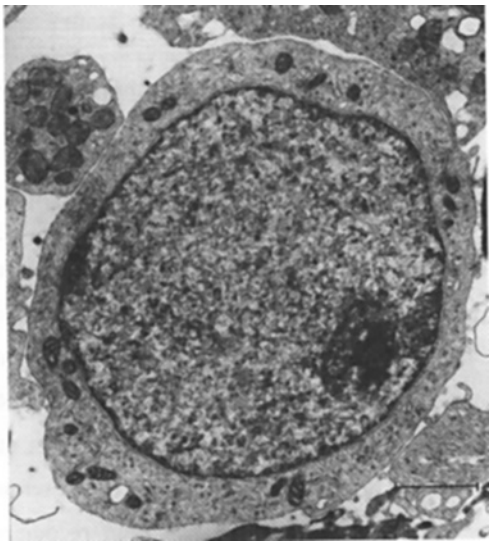

Fig 12. Undifferentiated cell with lots of free ribosomes and a few mitochondria and without glycogen. Original magnification x 11500. Bar 1 μm.

Some of the processes contained only glycogen while others contained microtubules and one to two mitochondria together with free ribosomes. Some processes contained neurosecretory-like granules (Fig 14). It was hard to illustrate both microtubules and granula in the same process. A tendency to rosette formation was noticed by EM in cases 5 and 6. These three tumors were diagnosed as atypical Ewing's sarcoma. Our EM findings corresponded with the reported ultrastructural characteristics of atypical Ewing's sarcoma (8,16,17,21).

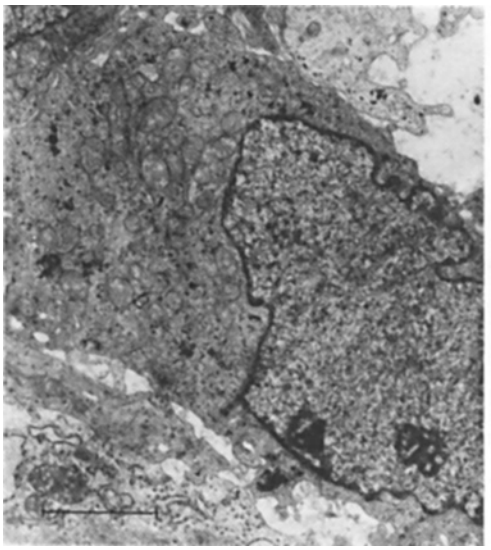

Fig 13. Atypical EWS cell with lots of mitochondria and small glycogen deposits. Original magnification x 8900. Bar 2 μm.

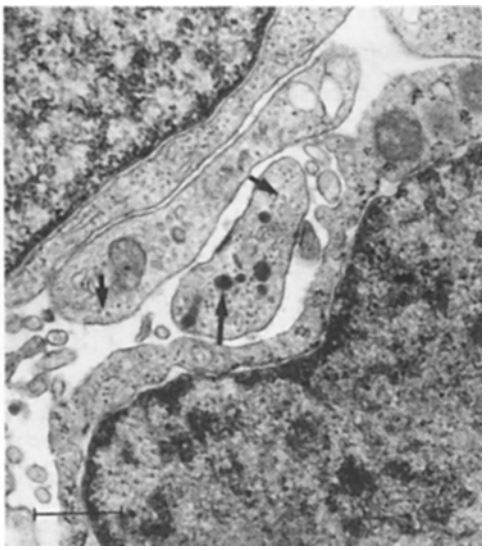

Fig 14. Between two cells, cell processes with neurosecretory-like granules (arrow) and microtubules (small arrow) are seen. Original magnification x 28500. Bar 0.5 μm.

Differential diagnosis. Distinction between Ewing's sarcoma of bone and the other small-cell malignant tumors like metastatic neuroblastoma, embryonal rhabdomyosarcoma and malignant lymphoma can be done since these tumors have distinct EM features.

Comments. It is possible to differentiate atypical EWS from pPNET by two simultaneously occurring cell types which are absent in pPNET, where mostly cells with a pre-neuroblastic appearance are found. Atypical EWS has a mixed differentiation with features of both typical EWS and pPNET and should therefore be termed atypical EWS (Fig 15).

Abundant glycogen has been recognized in some cases of PNET (13,29), and cell processes with neurosecretory granules and microtubules have been seen between tumor cells in cases of atypical EWS although the processes were more prominent in PNET (29). EWS is considered by some to represent the most immature form of neuroectodermal tumor. Schmidt et

al. (24) found no significant differences in terms of survival between typical and atypical EWS but found that prognosis worsened with increasing neural differentiation. They called tumors with no or one neural marker EWS, while PNET consisted of cases with two or more neural markers.

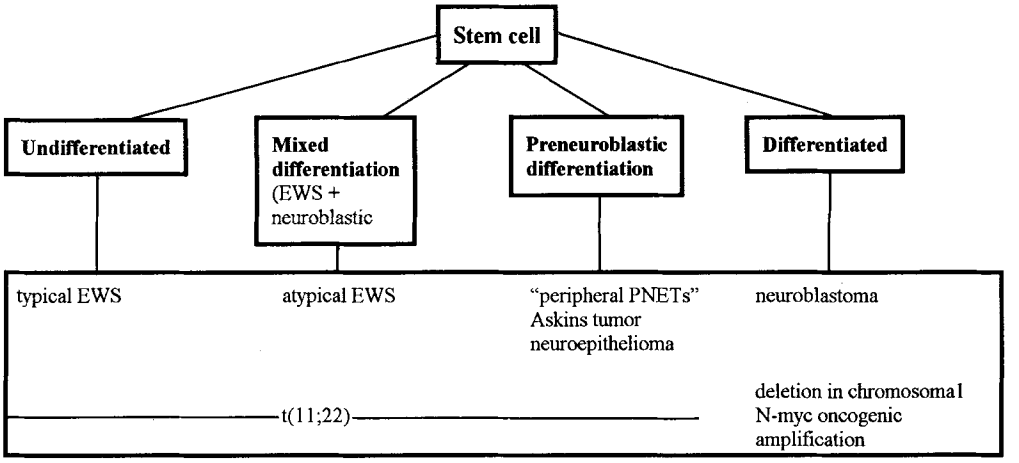

Fig 15. Ewing's sarcoma seen in the PNET perspective (summarized from the literature)

Table 5. *Comparison of the ultrastructure in typical and atypical Ewing's sarcoma of bone and pPNETs*

Morphology	Typical EWS[1]	**Atypical EWS**[1]	**pPNET**[2]
Cell shape	regular	irregular	irregular
Nucleus	round-oval	round/irregular	cleaved
Nucleolus	small	prominent	prominent
Glycogen/lipid	abundant	moderate	scant or absent
Ribosomes	abundant	moderate	moderate
Filament	exceptional	common	prominent
Mitochondria	few	abundant	moderate
Cell junction	primitive	primitive	long primitive
Cell processes	absent	few	abundant
Neurosecretory granules	absent	rare	abundant
Microtubules	absent	few	abundant

[1]Own cases [2]References 8,16,17,21

A distinction between EWS and PNET is clearly of clinical relevance. However, Terrier et al (28) recently suggested in a review of 315 Ewing's sarcomas that subclassification has no prognostic significance. Several investigators (8,16,17,21) compared ultrastructural characteristics of typical

and atypical EWS and pPNET and our results of EWS and AEWS correspond with those results shown in Table 5. There is a great quantitative difference between these three entities in expression of the same features and the value of EM detecting these differences is clear. Suspected cases of EWS are always diagnosed in Lund by FNA and ancillary methods. EM is the diagnostic tool of choice. While cytogenetic and molecular genetic analyses are helpful, they do not differentiate EWS from pPNET tumors.

Primary Ewing's sarcoma of bone in a 73-year old man. Ewing's sarcoma of bone is typically an age-related tumor. This rare tumor mainly affects young people in the 1st and 2nd decades with a prevalence of males. Occasionally they can appear as isolated cases in older age groups. When this happens it is of importance to use ancillary methods such as electron microscopy, immunocyto-chemical and cytogenetic analyses.

Light microscopy. FNA revealed the unexpected finding of a small-cell malignancy in the aspirate from a 73-year old man. The cells had sparse cytoplasm with vacuoles, rounded nuclei, small nucleolus, dispersed chromatin, light and dark cells.

Immunocytochemistry. The tumor cells were stained with mAb CAM5.2, NSE, chromogranin A, desmin and 013. Only mAb 013 was positive.

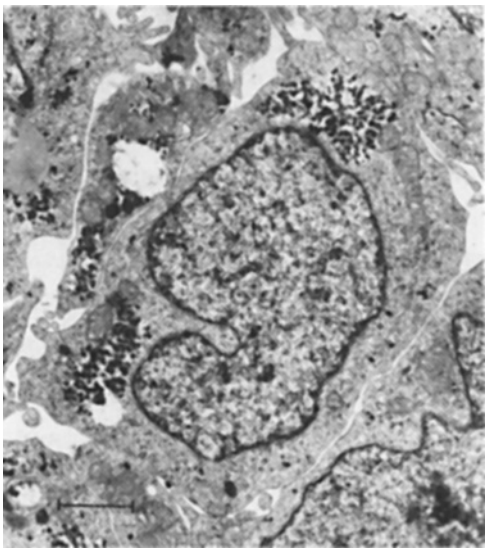

Fig 16. Electron micrograph of a typical EWS cell from case no 8. Original magnification x 11500. Bar 1 μm.

Electron microscopy. The fine needle aspirate consisted of clusters of small round cells alternated with single cells. The sparse cytoplasm contained characteristic glycogen deposits associated with lipid droplets. The cytoplasm also had a few cytoplasmic organelles except for polyribosomes, a moderate number of mitochondria and short strands of rough endoplasmic reticulum. The cells were joined by primitive cell junctions. No neuroendocrine granules, microtubules or filaments were detected. The ultrastructural features were characteristic for classical Ewing's sarcoma of bone (Fig 16).

Differential diagnoses. The combined evaluation of electron microscopy, immunocytochemistry and cytogenetic examination excluded carcinoma, malignant lymphoma and pPNET.

Comments. The finding of Ewing's sarcoma in a 73-year old man is extremely rare. This tumor belongs to a tumor group affecting people below 20 years of age. The diagnosis was also supported with positive reactions for vimentin and the monoclonal antibody 013, recognizing glycoprotein p30/32. RT-PCR (polymerase chain reaction) analysis revealed an amplified product at the 650 bp level representing a FL11- EWS- fusion transcript, characteristic for Ewing's sarcoma and PNET (32).

DISCUSSION

Diagnosis of musculoskeletal tumors are difficult and all ancillary methods must be used to achieve a correct diagnosis. Information regarding a tumor's ultrastructure appears diagnostically important and may be conclusive in the presence of tumor specific changes. Tumors like alveolar soft part sarcoma, parachordoma, epithelioid sarcoma and Ewing's sarcoma with its variants are examples of tumors whose diagnoses are strongly facilitated by ultrastructural examinations. While light microscopy and immunohistochemistry are the foundations for histological diagnoses, the routine use of electron microscopy can significantly enhance diagnostic accuracy and should be included in the armamentarium of orthopedic oncology centers.

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