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CASE REPORT

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Periosteal Ewing-like adamantinoma

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Abstract We report a Ewing-like adamantinoma of the periosteal region of the right tibia in a 15-year-old boy. The tumour was well demarcated but unencapsulated and showed cortical bone erosion. Histologically, the neoplastic cells were arranged in trabecular and cord-like patterns with fibrous, hyalinized, and myxoid stroma. Cellular atypia was mild, and mitotic figures were rarely seen. Many tumour cells expressed wide keratin, epithelial membrane antigen, leu 7, synaptophysin, Ewing's sarcoma-related antigen O13, and some were positive for neuron-specific antigen, vimentin, and CD68. The tumour was negative for S-100 protein, desmin, alpha-smooth muscle actin, and muscle-specific actin. Flow cytometric analysis showed that the tumour was aneuploid. After wide excision the patient has been well for the 16 months since diagnosis.

Key words Adamantinoma · Ewing's sarcoma · Periosteal tumour · Round cell tumour

Introduction

The spectrum of periosteal tumours is wide, spanning periosteal fibroma, periosteal neurofibroma, periosteal chondroma, periosteal lipoma, periosteal fibrosarcoma, periosteal chondrosarcoma, and periosteal osteosarcoma [11]. The differential diagnosis of these lesions does not usually include Ewing's sarcoma and adamantinoma, either clinically or pathologically.

A periosteal adamantinoma showing morphological and immunohistochemical features of Ewing's sarcoma is described, with immunohistochemical and flow cytometric DNA analysis. The problems encountered in the differential diagnosis are discussed.

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Clinical data

A 15-year-old Japanese boy was admitted to a hospital for the removal of a painless, tender, and slow-growing mass that had been present for 1 month in the right leg. The mass was well circumscribed and firm. The boy had no history of antecedent trauma. In July 1996, the lesion, measuring 1.2×0.8×0.5 cm and located between the subcutis and cortical bone of the upper diaphysis of the right tibia, was excised (Fig. 1). The lesion compressed and eroded the cortex of the tibial shaft. It was not associated with any nervous tissue. The cut surface was well demarcated, white, and firm. Laboratory data were unremarkable. An extensive work-up failed to uncover an occult primary neoplasm. In September 1996, the patient underwent wide excision of the upper diaphysis of the tibia; he has shown no evidence of recurrence and no metastatic lesion in the 16 months since the diagnosis.

Materials and methods

The specimens were fixed in buffered formalin and embedded in paraffin. Sections were routinely stained with haematoxylin and eosin (H&E), periodic acid–Schiff (PAS) with and without diastase digestion, Masson trichrome stains, and the argyrophilic staining technique of Grimelius.

Immunohistochemical studies were done on formalin-fixed paraffin-embedded tissues using the aviding–biotin–peroxidase complex method with an ABC kit (Vector Laboratories, Burlingame, Calif.) and the peroxidase–antiperoxidase technique. The commercially available primary antibodies used are summarized in Table 1. Known positive control cases including typical Ewing's sarcomas were utilized for all antibodies. Negative control slides were prepared by substituting a buffer or the primary antibodies.

Flow cytometry was performed on a formalin-fixed, paraffin-embedded tissue block. The technique of Hedley et al. [7] was used for DNA analysis, with some minor modifications [5].

Pathological findings

Histologically, the tumour was well demarcated but unencapsulated. The neoplastic cells were arranged in trabecular or cord-like patterns with fibrous, hyalinized, or myxoid stroma (Fig. 2a, b). The cells had round or oval nuclei with abundant fine chromatin, single small nucleoli, and a moderate amount of clear or lightly eosinophil-

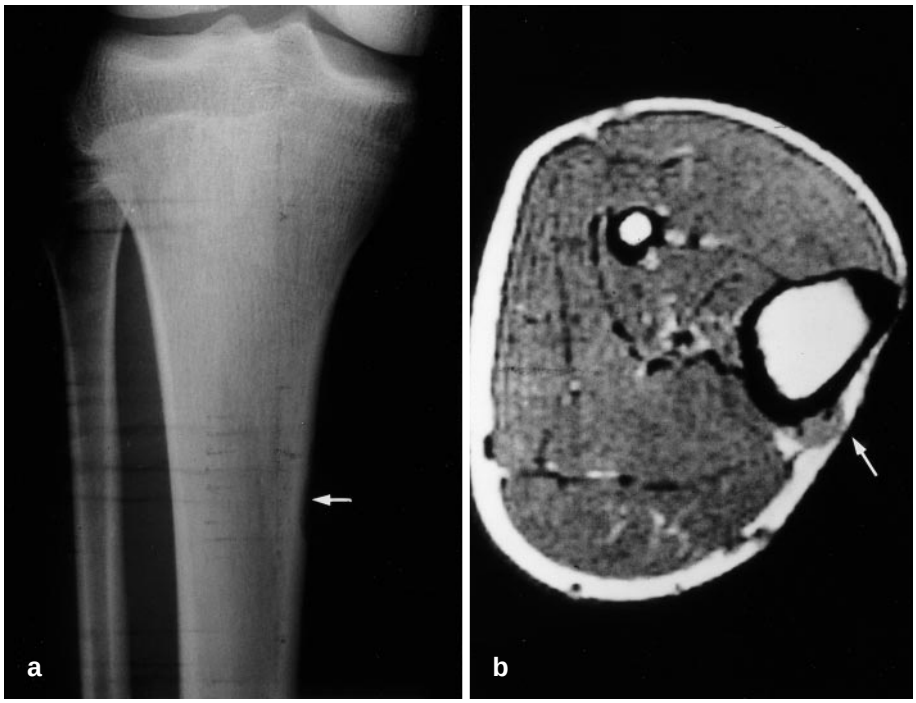

Fig. 1 **a** X-ray of the right tibia shows a periosteal mass (arrow) with cortical erosion. **b** A T1-weighted magnetic resonance imaging shows a soft tissue mass (*arrow*) located between the subcutis and cortical bone of the right tibia with cortical erosion

Table 1 Results of immunohistochemical studies [(*m*) mouse monoclonal, (*p*) rabbit polyclonal, – no staining, + some tumour cells staining, ++ many tumour cells staining]

Antibody	Result	Dilution	Source
Anti-cytokeratin AE1/AE3 (m)	–	1:50	Boehringer-Mannheim, Mannheim, Germany
Anti-cytokeratin CAM 5.2 (m)	–	1:1	Becton Dickinson, San Jose, CA, USA
Anti-cytokeratin 903 (m)	–	1:1	Enzo Diagnostics, New York, NY, USA
Anti-wide keratin (p)	++	1:300	Dakopatts, Glostrup, Denmark
Anti-epithelial membrane antigen (EMA) (m)	++	1:100	Dakopatts
Anti-carcinoembryonic antigen (m)	–	1:30	Dakopatts
Anti-vimentin (m)	+	1:40	Amersham, Little Chadfont, UK
Anti-desmin (p)	–	1:50	Bio-Science, Emmenbrucke, Switzerland
Anti-alpha smooth muscle actin (m)	–	1:50	Dakopatts
Anti-muscle-specific actin, HHF35 (m)	–	1:50	Enzo Diagnostics
Anti-myoglobin (p)	–	1:400	Dakopatts
Anti-S 100 protein (p)	–	1:200	Dakopatts
Anti-neuron-specific enolase (NSE) (m)	+	1:!50	Dakopatts
Anti-neurofilament (m)	–	1:100	Immunobiology Laboratory, Gunma, Japan
Anti-Leu 7 (m)	++	1:200	Becton Dickison
Anti-glial fibrillary acidic protein (p)	–	1:200	Dakopatts
Anti-chromogranin A (p)	–	1:500	Incstar, Stillwater, MN, USA
Anti-synaptophysin (m)	++	1:1500	Euro-Dianostica, Apeldoom, Netherlands
Anti-O13 (m)	++	1:100	Signet Lab, Dedham, MA, USA
Anti-CD34 (m)	–	1:25	Becton Dickinson
Anti-CD31 (m)	–	1:40	Dakopatts
Anti-CD68, KP1 (m)	+	1:100	Dakopatts
Anti-alpha-1 antichymotrypsin (p)	–	1:500	Dakopatts

ic cytoplasm. The cell borders were relatively distinct. Basement membrane surrounded the nests of the tumour cells. No glandular structures were observed. There was mild to moderate nuclear pleomorphism, and the mitotic rate was 0–1 per 10 high-power fields. There was neither definite neoplastic osteoid nor catilage formation. A moderate number of tumour cells showed PAS-positive cytoplasmic granularity, which was removed by diastase digestion (Fig. 2c). The basement membranes were also positive for PAS. The tumour cells were negative for Grimelius staining. The wide excision specimens contained no residual tumour and showed only scar tissue.

The immunohistochemical findings are summarized in Table 1. The cytoplasm of many tumour cells were strongly positive for wide keratin (Fig. 3a), EMA, synaptophysin (Fig. 3b), and Leu 7. The tumour also showed diffuse and strong membrane staining of O13 (Fig. 3c). Some cells were positive for vimentin, NSE, or

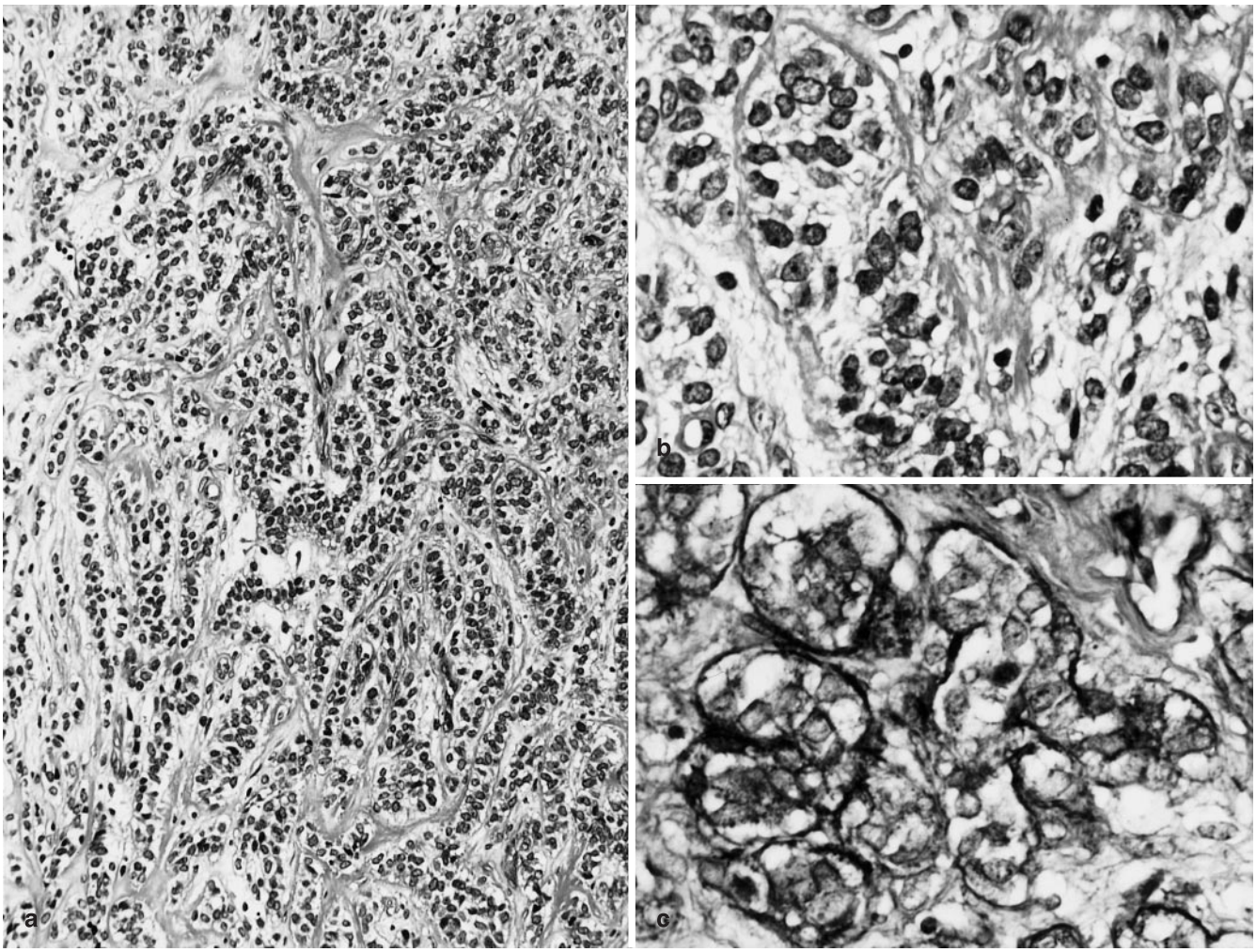

Fig. 2 **a** Neoplastic cells are arranged in trabecular or cord-like patterns with fibrous or myxoid stroma. H&E, ×200 **b** Neoplastic cells have round or oval nuclei with abundant fine chromatin, single small nucleoli, and moderate amount of clear or pale eosinophilic cytoplasm. H&E, ×450 **c** Cytoplasm of neoplastic cells and basement membrane are positive for periodic acid-Schiff staining. ×450

CD68. The tumour was uniformly negative for the other markers.

In the flow cytometric study, the tumour was found to have an aneuploid DNA content with a DNA index of 1.4. The coefficient of variation was 6.5%.

Discussion

In the present case, the periosteal location of the tumour was clearly demonstrated by X-ray and magnetic resonance imaging, and intraoperative observation. The cortical bone was eroded. There was clinically no other primary lesion. The distinctive features of the tumour were the trabecular and cord-like arrangements of the small round cells and its epithelial differentiation demonstrated by the positive staining of wide keratin and EMA. The cytological features, the presence of glycogen, the immunohistochemical strong expression of Leu 7, synaptophysin, NSE, and O13 were consistent with Ewing's sarcoma [4]. However, the periosteal location and the epithelial differentiation are not common features of Ewing's sarcoma [2, 6]. The only primary bone or periosteal tumours known to have epithelial elements are adamantinomas of the long bones.

This unusual tumour is very similar to neoplasms variously referred to as ''malignant tumour of humerus with features of adamantinoma and Ewing's sarcoma'' by Meister et al [14], Ewing-like adamantinoma [12], juxtacortical adamantinoma (simulating Ewing tumour) by Ishida et al. [8, 9], and ''adamantinoma of the pretibial soft tissue'' by Mills and Rosai [15]. A similar tumour was recently reported by Schofield et al. [17]. Among the seven tumours, including ours, there were four bone [9, 12, 14, 17] and three juxta- or periosteal lesions [9, 15]. The chief complaint was pain for five patients, and two complained of a mass. The patients ranged in age from 10 years to 38 years (average, 23 years): one died of disease and one had local recurrence despite resection. All these lesions consisted of purely or predominantly epithelial components composed of cells with uniform, round, vesicular nuclei with single small nuclei and clear

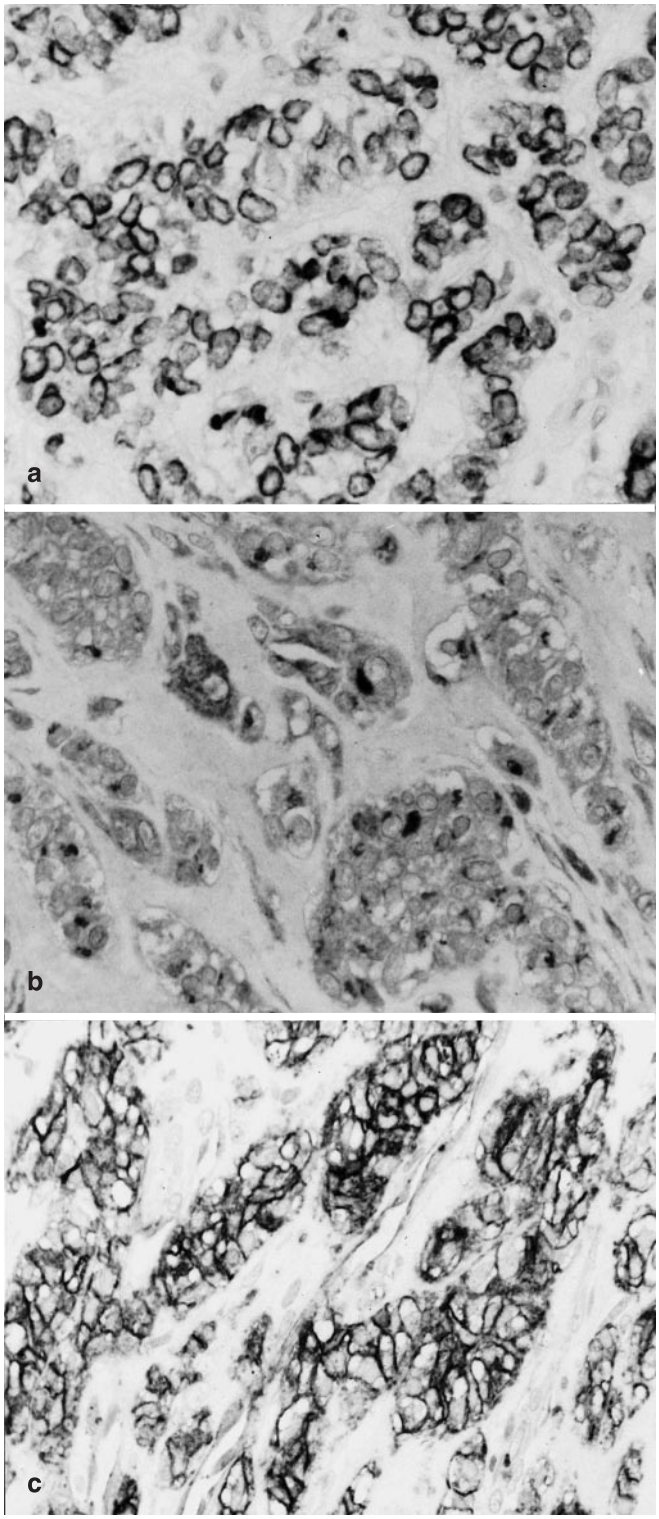

Fig. 3 **a** Many neoplastic cells are strongly positive for wide keratin. Immunostaining, ×300 **b** Many neoplastic cells show perionuclear dot-like staining for synaptophysin. Immunostaining, ×200 **c** Many neoplastic cells show strong membrane staining for O13. Immunostaining, ×400

to eosinophilic cytoplasm, although the present case lacked areas with diffuse proliferation, gland-like structures, and fibroblastic differentiation. The neoplasms in which immunocytochemical analyses were performed showed positive immunostaining of EMA and negative staining of cytokeratin [9, 14]. Ewing's sarcoma-related antigen O13 was not sought, in the other cases.

Conventional adamantinoma of long bones is usually an intracortical or intramedullary lesion, and four basic histological patterns have been described: basaloid, spindled, squamoid, and tubular [3, 19]. Adamantinomas arising in the soft tissue anterior to the tibia without involving the underlying bone have been reported [1, 10, 15]. These cases showed predominantly trabecular or cord-like arrangements. Their EMA-positive and cytoatin-negative profiles are in striking contrast to the fact that conventional adamantinomas are usually positive for cytokeratin, with only occasional expression of EMA [9]. The neoplasm in this case was diffusely positive for the polyclonal wide keratin only among the antibodies directed against several keratins of different molecular weights. O13-positive adamantinoma of long bone or soft tissue has not been reported. All this combines to suggest that these tumours may be a unique variant of adamantinoma or Ewing's sarcoma. The present case showed diffuse and intense O13 positivity, an antibody specificity that has been more and more questioned in recent literature, and this finding does not constitute sufficient evidence that the present case is a Ewing's sarcoma. It should be kept in mind that this type of periosteal tumour with strongly expression of O13 exists.

The main differential diagnoses of the lesion include the monophasic epithelial variant of synovial sarcoma, epithelioid sarcoma, and sclerosing epithelioid fibrosarcoma [13]. The monophasic epithelial variant of synovial sarcoma, which is extremely rare, shows glandular structures and cytokeratin positivity. It usually contains minute foci of spindel cell differentiation. The present tumour did not show any of these features. It lacked nodular arrangement of the eosinophilic cells, with central necrosis and coexpression of cytokeratin and vimentin, findings characteristic of epithelioid sarcoma. The histological features of the present tumour overlapped with those of sclerosing epithelioid fibrosarcoma: both tumours arise primarily in the deep soft tissue, and are frequently associated with the periosteum [13]. They show an epithelioid arrangement of rather uniform small round cells with hyalinized stroma. However, the present tumour did not contain the foci of conventional fibrosarcomatous and benign-appearing fibromatous areas that are always observed in sclerosing epithelioid fibrosarcoma. Focal EMA expression in half the cases of sclerosing epithelioid fibrosarcoma is in contrast to the strong and diffuse EMA positivity in the present tumour.

The histogenesis of this lesion is unclear, and whether it extends the spectrum of adamantinoma or represents an unique entity remains to be determined. It is considered to express both epithelial and neuroectodermal dif-

ferentiations. Immunohistochemical expression of cytokeratin and electron microscopic observation of tonofilaments and intercellular junctions have been described in a few cases of Ewing's sarcoma [6, 16]. The present and the previously reported neoplasms suggest that there may be a close relation between some adamantinomas of long bone or periosteum and Ewing's sarcomas. We agree with Moll et al. [16] that Ewing's sarcoma is derived from a primitive, pluripotential cell that may differentiate, in variable proportions, into cells with mesenchymal, epithelial, and, more rarely, even neural features. Ewing's sarcoma is characterized by the presence of a reciprocal chromosomal translocation t(11;22)(q24;q12) [18]. Fluorescence in situ hybridization may suggest a relationship between this neoplasm and Ewing's sarcoma. The current case showed an aneuploid DNA content consistent with malignancy, but additional cases must be studied to determine whether DNA ploidy status is helpful in predicting the biological behaviour.

The appropriate treatment of this lesion appears to be wide surgical excision. This unusual diagnosis should be considered when small round cell lesions in this anatomical site are investigated, to ensure that the patient receives appropriate prognostic information and therapy.

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