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Chondroblastoma of bone

A clinical, radiological, light and immunohistochemical study

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Summary. The clinical and morphological findings of 53 chondroblastomas in the files of the Bone Tumour Registry of Westphalia are presented. The mean age of all patients was 19.2 years. The male-to-female ratio was 1.5:1. Forty-two of the tumours (79.8%) were located in the long tubular bones and short tubular bones of the hands and were closely related to the growth plate. Six cases (11.3%) were found in the flat bones, 4 cases (7.5%) in the tarsal bones and 1 case (1.9%) in the craniofacial bones. The characteristic radiological feature of 44 investigated lesions was a mostly eccentric radiolucency with a geographic pattern of bone destruction and matrix calcifications. Periosteal reaction was evident in 9% of the cases. Most tumours demonstrate the typical morphological features of chondroblastoma, but 3 cases resembled a giant cell tumour. In 2 cases a haemangiopericytoma-like growth pattern was observed. Nine of the tumours had an aneurysmal bone cyst-like component. Vascular invasion was seen in 1 case. Immunohistochemically most cells in 30 of the cases and fetal chondroblasts in 3 cases were strongly positive with vimentin and S-100 protein. Collagen type II was positive in the chondroid matrix of the tumours and in fetal cartilage tissue; collagen type VI was present focally around individual tumour cells and was always seen in the chondroid matrix of the lesions and in fetal cartilage. These findings support the cartilaginous nature of these tumours. In paraffin sections, 46.6% of the cases revealed a distinct positive reaction of some tumour cells with the monoclonal cytokeratin antibody KL1 (molecular weight 55–57 kDa). Only 4 of them demonstrated a co-expression with the other monoclonal cytokeratin antibody CK (clone MNF 116, molecular weight 45–56.5 kDa). In paraffin sections all fetal chondroblasts were negative with both cytokeratin antibodies. Frozen sections of 3 tumours showed a strong positive reaction with both cytokeratin antibodies in many chondroblasts, indicating an "aberrant" cytokeratin expression. Osteoclast-like giant cells stained positive with leucocyte-common antigen (LCA) and with the macrophage-associated antibody KP1, but were negative with the other macrophage-associated antibody MAC 387. Recurrence rate was 10.7%. The clinical course of all tumours was benign.

Key words: Chondroblastoma – Bone tumours – Immunohistochemistry

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Introduction

Chondroblastoma of bone was first described by Codman in 1931 as an epiphyseal chondromatous giant cell tumour. In 1942 Jaffe and Lichtenstein introduced the currently used term benign chondroblastoma to emphasize its distinction from giant cell tumour of bone. Chondroblastoma is rare, representing about 1% of all primary bone tumours (Dahlin and Unni 1986; Kurt et al. 1989; Mirra 1989; Huvos 1991) although more than 1400 cases have been reported in literature (Bloem and Mulder 1985; Kyriakos et al. 1985; Springfield et al. 1985; Kurt et al. 1989; Brower et al. 1990). It is usually benign, generally arising in the epiphysis or apophysis of long tubular bones (Schajowicz 1981; Dahlin and Unni 1986; Mirra 1989; Campanacci 1990; Huvos 1991). We present our experience of 53 cases of chondroblastoma from the files of the Bone Tumour Registry of Westphalia, with special reference to immunohistochemical findings.

Materials and methods

All cases with the diagnosis of "chondroblastoma" from 1975 to 1992 in the files of the Bone Tumour Registry of Westphalia, a

Table 1. List of primary antibodies used in immunohistochemistry

Antibodies	Clone	Dilution	Main immunoreactivity	Source
Vimentin (m)	V9	1:30	Mesenchymal cells	Dako
S-100 protein (p)		1:1200	Neural cells	Dako
Collagen type II (p)		1:20	Chondrogenic tissue	Ueda and Nakanishi (1989)
Collagen type VI (p)		1:4000	Most interstitial tissue	Oda et al. (1988)
Cytokeratin (m)	KL1	1:80	Cytokeratins of molecular weight 55–57 kDa; epithelial cells	Dako
Cytokeratin CK (m)	MNF116	1:150	Cytokeratins of molecular weight 45–56.5 kDa, including keratins 10, 17, 18; epithelial cells and a small number of non-epithelial cells (smooth muscle cells, dendritic cells in lymph nodes, syncytiotrophoblasts, cortical neurons and plasma cells)	Dako
CD 45 (LCA) (m)	2B11+PD7/26	1:30	Leucocytes, lymphocytes, macrophages	Dako
CD 68 (m)	KP1	1:100	Macrophages, granulocytes	Dako
Myeloid/histiocyte antigen (m)	MAC 387	1:400	Macrophages, granulocytes	Dako

m, monoclonal; p, polyclonal

total of 60 cases, were reviewed. After reclassification of 4 cases as giant cell tumour, 1 case as an aneurysmal bone cyst, 1 as a chondromyxoid fibroma and 1 as clear cell chondrosarcoma, we found 53 cases in which complete clinical data including follow-up were available for 28. Two of these have recently been published (Edel et al. 1992). The data of age, sex and the tumour localization were known from all cases, radiographs were present from 44 cases and routinely processed paraffin blocks were available in 49 cases. From 3 cases fresh tumour tissue was also snap frozen in liquid nitrogen, but in 4 cases, sections stained with haematoxylin and eosin (H & E) only were available for review. From all paraffin blocks, 4–5 µm sections were prepared and stained with H & E, periodic acid-Schiff (PAS), reticulin and von Kossa. Immunohistochemistry was performed on paraffin sections of 30 representative tumours and on cryostat sections of 3. We also investigated 3 cases of paraffin embedded embryonic hyaline cartilage tissue from aborted fetuses of 8–18 weeks' gestation.

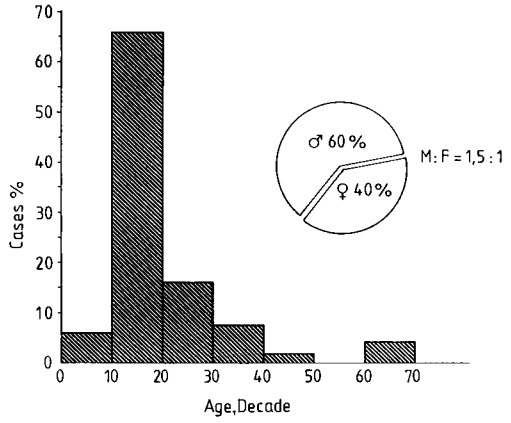
The monoclonal and polyclonal antibodies used are shown in Table 1. All antibodies were diluted in a modified RPMI 1640 medium containing 5 ml RPMI 1640 (Sigma, Deisenhofen, FRG), 45 ml destilled water, 5 ml heat-inactivated bovine serum (Sigma) and 0.05 sodium azide (pH 7.4–7.6). For all immunohistochemical studies we used the alkaline phosphatase anti-alkaline phosphatase (APAAP) method (Cordell et al. 1984). Cryostat sections were mounted on Pritt-coated slides, air-dried for 2 h and then fixed in acetone for 90 s at 4° C. Paraffin sections were mounted on poly-L-lysine coated slides, dried over night at 37° C, deparaffinated and rehydrated in graded ethanols. The slides were incubated in a moist chamber at room temperature for 30 min with the respective primary antibody, with the exception of the antibodies to collagen types II and VI, the antibody to cytokeratin (CK) and the macrophage-associated antibodies KP1 and MAC 387. For the demonstration of the collagen types II and VI the sections were pretreated with 0.01% protease (type XXIV, Sigma) in 0.1 M TRIS-HCl (pH 7.6) at 37° C for 10 min and then with 0.05% hyaluronidase (type IVS, bovine testis, Sigma) in 0.1 M phosphate buffer (pH 5.5) at 37° C for 2 h. They were incubated with the antibodies to collagen types II and VI over night at 4° C. For demonstration of CK, KP1 and MAC 387 the sections were pretreated with 0.1% trypsin (Sigma) for 10 min at 37° C. The sections incubated with the polyclonal antibodies were first treated for 30 min with the bridging antibody mouse anti-rabbit IgG (Dako, Hamburg FRG), diluted 1:100. All sections were then incubated for 30 min with the bridging antibody rabbit anti-mouse IgG (Dako), diluted 1:30 in modified RPMI 1640 containing 10% human serum (Dako). The slides were then treated with the APAAP complex (Dako) diluted 1:50 in modified RPMI 1640 for 1 h. Each step was separated by careful washing in 0.05 M TRIS-NaCl buffer.

For colour development the slides were incubated for 25 min with a modified neofuchsin solution: 0.13 ml neofuchsin (Chroma, Köngen, FRG) dissolved in 2 N HCl were incubated with a freshly prepared 4% sodium nitrite solution for 1 min and mixed with 0.023 g levamisole (Sigma) in 63 ml 0.2 M TRIS-HCl buffer (pH 8.7), finally adding 0.032 g napthol AS-BI-phosphate (Sigma) dissolved in 1 ml *n,n*-dimethylformamide (pH 8.7) (Merck-Schuchard, Hohenbrunn, FRG). The solution was filtered and adjusted with 1 N HCl to pH 8.7.

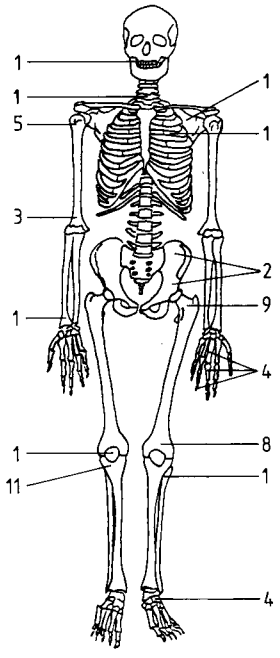
Counterstaining was performed with Mayer's haematoxylin. For controls the primary antibodies were replaced by the TRIS-NaCl buffer mentioned above. Pre-existing tissue and stroma components served as internal positive or negative controls. The interpretation of immunohistochemistry was semi-quantitative.

Results

From 53 cases with the diagnosis of chondroblastoma in our files, 32 were males and 21 females. The male-to-female ratio was 1.5:1. The age of the patients ranged



1a



1b

Fig. 1. Distribution of 53 chondroblastomas by age and sex of the patient (**a**) and site of the lesion (**b**)

from 5 to 64 years with an average of 19.2 years (Fig. 1a). Most of the patients (86.8%) were less than 30 years of age, 5 patients (9.4%) were in the fourth and fifth decade, and 2 (3.8%) in the seventh decade. The localizations of the tumours are summarized in Fig. 1b. Seventeen cases (32,3%) were found in the femur: 9 in the proximal and 8 in the distal end. In 11 cases (20.8%) the proximal end of the tibia was affected. The humerus was involved in 8 cases (15.1%): 5 were found proximal and 3 distal. The distal end of the radius and the proximal end of the fibula were affected in 1 case each (3.8%). Four cases (7.5%) were observed in the hands: 2 in the distal phalanx of the fourth finger, 1 in the proximal phalanx of the third finger and 1 in metacarpal II. In 4 cases (7.5%) the feet were involved with one case each in the cuboid, the talus, the calcaneus and the navicular. Six cases (11.3%) arose in the flat bones: 2 in the pelvis (triradiate cartilage region; os ilium) and 1 case each in the body of the scapula, the left third rib at the sterno-costal articulation, the seventh cervical vertebra and the patella. Only 1 case (1.9%) was found in the craniofacial bones affecting the left temporo-mandibular joint.

The clinical signs and symptoms were mainly characterized by local swelling, pain and limitation of motion of the affected joint. In 10 cases (18.8%) joint effusions were observed. The duration of the symptoms ranged from 5 weeks to 3 years. Laboratory tests were generally within normal limits.

Radiographs were available in 44 cases. In all of them the largest tumour extension was found in the medulla. The long tubular bones were involved in 31 cases (70.5%), the short tubular bones only in 3 cases (6.8%), the flat bones and the bones of the feet in 9 cases (20.4%) and the craniofacial bones in 1 case (2.3%). In the long and short tubular bones, all tumours were always closely related to the growth plate. Twenty-one of them (61.7%) had affected solely the epiphysis, 3 (8.8%) the apophysis, and 7 (20.5%) had involved the epi- and metaphysis. An epi-, apo-, meta-, diaphyseal location was found in one lesion (2.9%) of the proximal humerus. Two of 34 cases (5.9%) in the tubular bones were observed in the meta-diaphyseal region (Fig. 2). An eccentric localization of the lesions was found in 22 of 34 cases (64.7%) in the tubular bones and in all cases of the flat bones and the bones of the feet. The tumour size ranged from 1 to 6 cm. Using Lodwick criteria (1971), all tumours

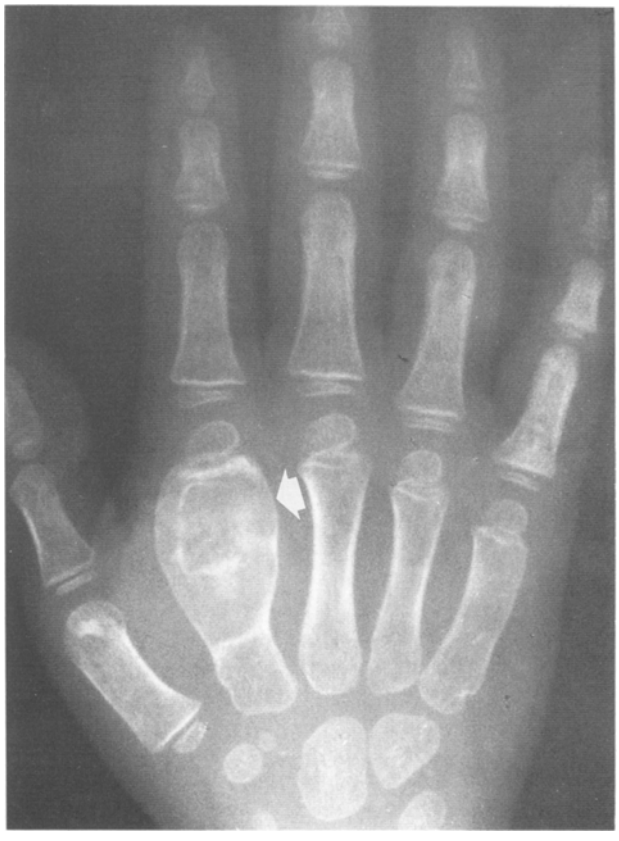


Fig. 2. Conventional radiograph demonstrates an expansile geographic lytic lesion with incomplete enosteal cortical erosion (Lodwck IB) in diametaphyseal region of the second metacarpal bone (*arrow*). Some foci of matrix calcifications are present

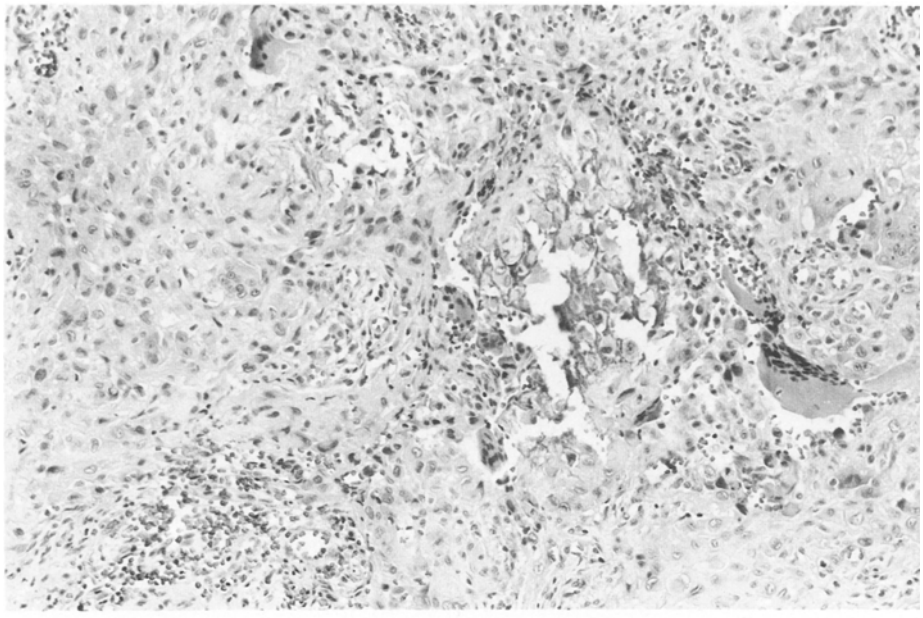


Fig. 3. Typical chondroblastoma composed of polygonal tumour cells with often grooved nuclei, a focus of "chicken-wire" calcifications and osteoclast-like giant cells. H &E, ×160

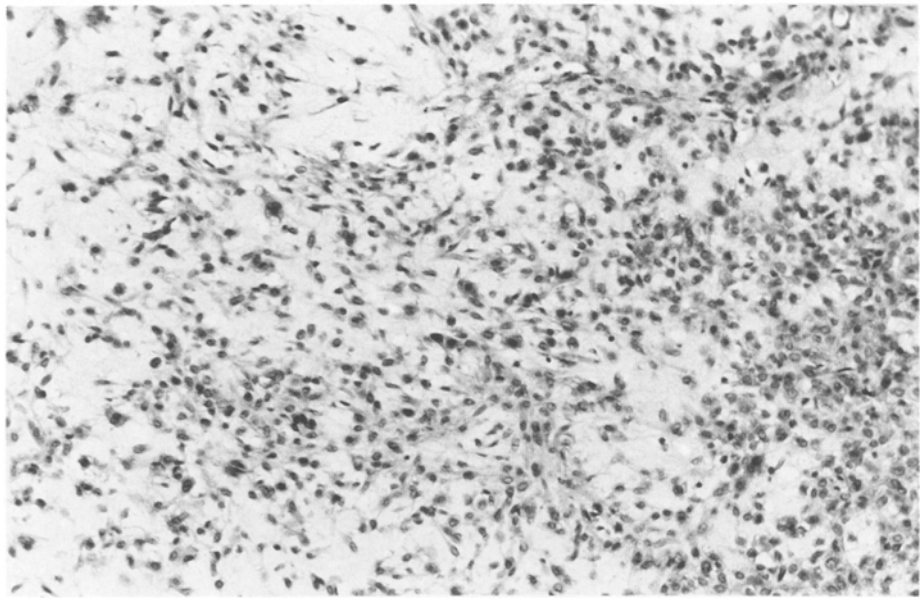


Fig. 4. Chondroblastoma demonstrating areas of fibromyxoid stroma with spindle or stellate cells resembling chondromyxoid fibroma. H&E, ×160

showed a geographic pattern of bone destruction. In 26 of 44 cases (59%) the tumours were characterized by a round or oval osteolysis with a sharp and well-defined sclerotic rim (Lodwick IA). Tumours with incomplete erosion of the cortical bone were seen in 13 cases (29.5%), affecting both tubular and flat bones (Lodwick IB). Four tumours (9%) in the flat bones (scapula, vertebra and pelvic bones) and one lesion in the temporo-mandibular region showed a complete cortical destruction in at least one area and were classified as Lodwick IC. Matrix calcification was present in 21 cases (47.3%). Trabeculation was seen in 12 cases (27.2%) and was more common in the long tubular bones than in any other bone. Periosteal new bone formation was demonstrated in 9 cases (20.4%). Only 1 case was entirely located in the epiphysis.

Table 2. Histological features of 53 chondroblastomas of bone

Features	Total no. of cases (%)
Chondroid	100.0%
Mitotic figures	79.2%
Atypia	–
Calcific deposits	52.8%
Osteoclast-like giant cells	100.0%
Aneurysmal bone cyst component	17.0%
Chondromyxoid areas	7.5%
Hemangiopericytoma-like growth pattern	3.8%
Haemorrhages and necrosis	37.7%
Foam cells and cholesterol clefts	13.2%
Vascular invasion	1.9%

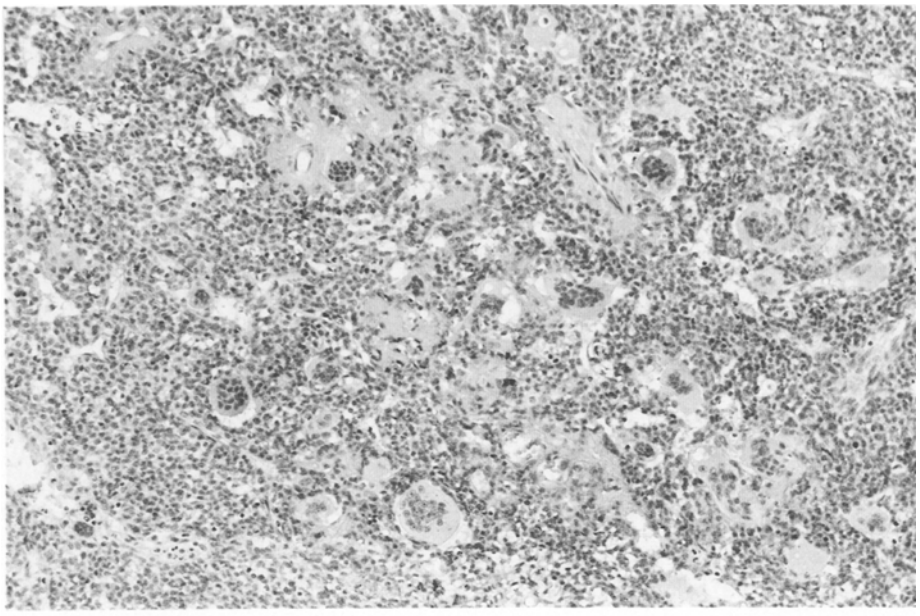


Fig. 5. Chondroblastoma with many osteoclast-like giant cells resembling giant cell tumour of bone. Note small foci of chondroid. H&E, ×160

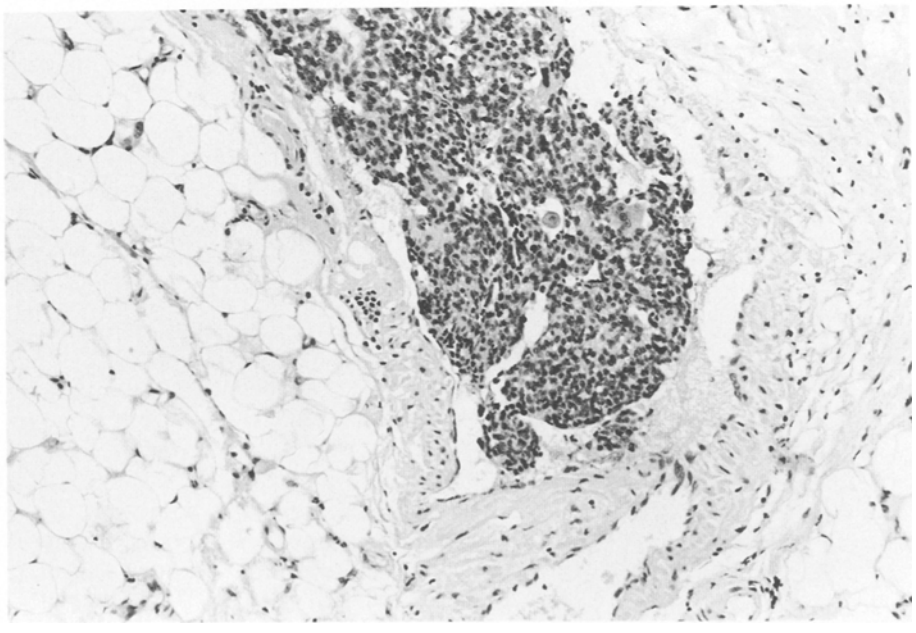


Fig. 6. Chondroblastoma showing vascular invasion of tumour cells at the periphery of the lesion. H&E, ×160

The main histological findings and their frequencies are summarized in Table 2. The basic cell type was a round to polygonal chondroblast with mostly eosinophilic cytoplasm and a distinct cell border (Fig. 3). Occasionally, tumour cells with little or no visible cytoplasm and indistinct cell borders were seen. PAS staining revealed sometimes positive glycogen granules within the cytoplasm of the cells. The nuclei were oval, round or grooved, containing one or two nucleoli and in some cells two or three nuclei were found. Significant nuclear atypia was not found but mitotic figures were seen in 42 cases (79.2%) ranging from one to two per 10 high-power fields (HPF). Two cases (3.8%) showed more than three mitotic figures per 10 HPF. In 48 cases (90%) areas of amorphous pinkish chondroid or fibro-chondroid were found, occasionally smaller than 1 mm in diameter. In the remaining 10% of the cases chondroid differentiation was only weekly expressed. Osseous metaplasia of the fibro-chondroid foci was observed in 4 cases. Small areas of fibromyxoid stroma with spindle or stellate cells (Fig. 4) were also present in 4 cases. Calcific deposits were found in 28 cases (52.8%). Typical "chicken-wire" calcifications were only present in 20 cases (37.7%). Varying numbers of multinucleated osteoclast-like giant cells with sometimes more than 60 nuclei were identified in all tumours, mostly randomly distributed between the chondroblasts or in areas of necrosis. In 3 cases (5.7%) osteoclast-like giant cells were predominant, resembling a giant cell tumour (Fig. 5). Foci of haemangiopericytoma-like growth pattern were observed in only 2 cases. Areas of haemorrhage and necrosis with or without haemosiderin pigment were found

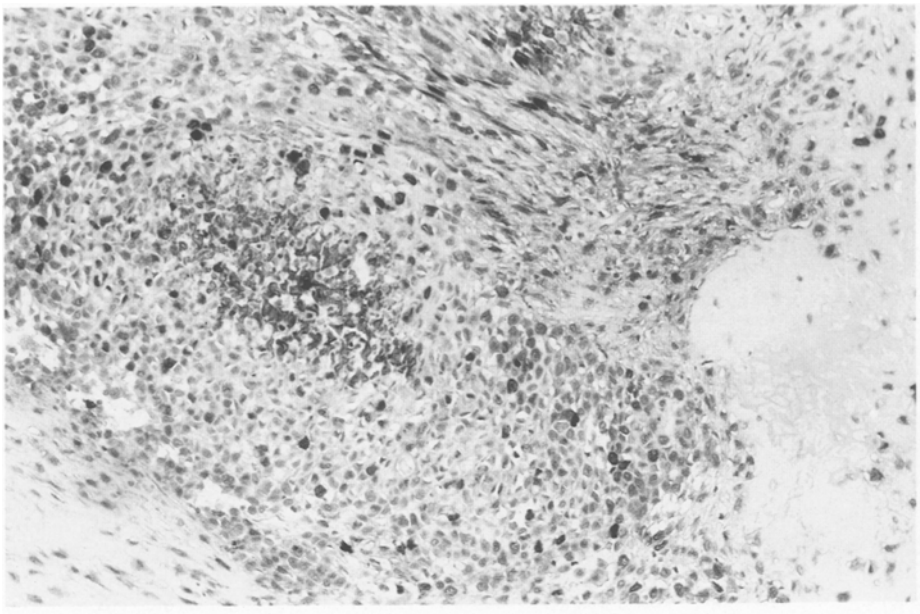


Fig. 7. Chondroblastoma demonstrating strong positive staining of chondroblasts and of mature chondrocytes in fibro-chondroid areas with anti S-100 protein. APAAP method, counterstaining H&E, ×160

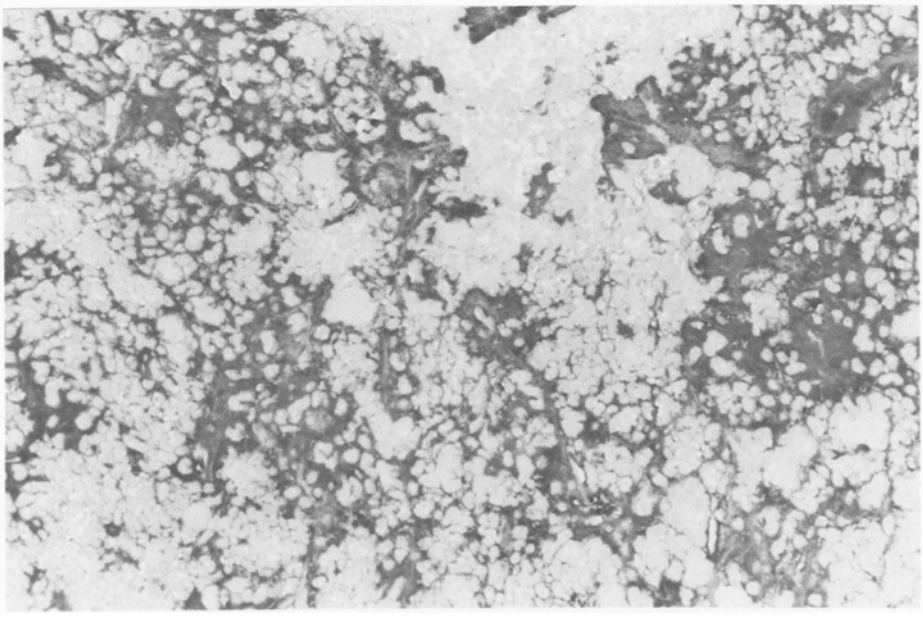


Fig. 8. Chondroblastoma showing moderate to strong positive staining of chondroid matrix with the antibody to collagen type II. APAAP method, counterstaining H&E, ×160

Table 3. Results of immunohistochemical stainings in 30 chondroblastomas and in fetal cartilage

Cell types and stroma component	Vimentin	S-100 protein	Collagen II	Collagen VI	KL1	CK	LCA	KP1	MAC 387
Chondroblasts	+++	+++	−/+ [c]	−	−/+ [a] +++ [b]	−/+ [a] +++ [b]	−	−	−
Fetal cartilage cells	+++	+++	−	−	−	−	−	−	−
Chondroid matrix	−	−	++/ +++	++/ +++	−	−	−	−	−
Osteoclast like giant cells	+/++	−	−	−	−	−	++	+++	−

+++, strong; ++, moderate; +, slight and focal; −, no reaction; reaction of KL1 and CK in [a] paraffin and in [b] frozen sections; [c] positive in the cytoplasm of 2 cases

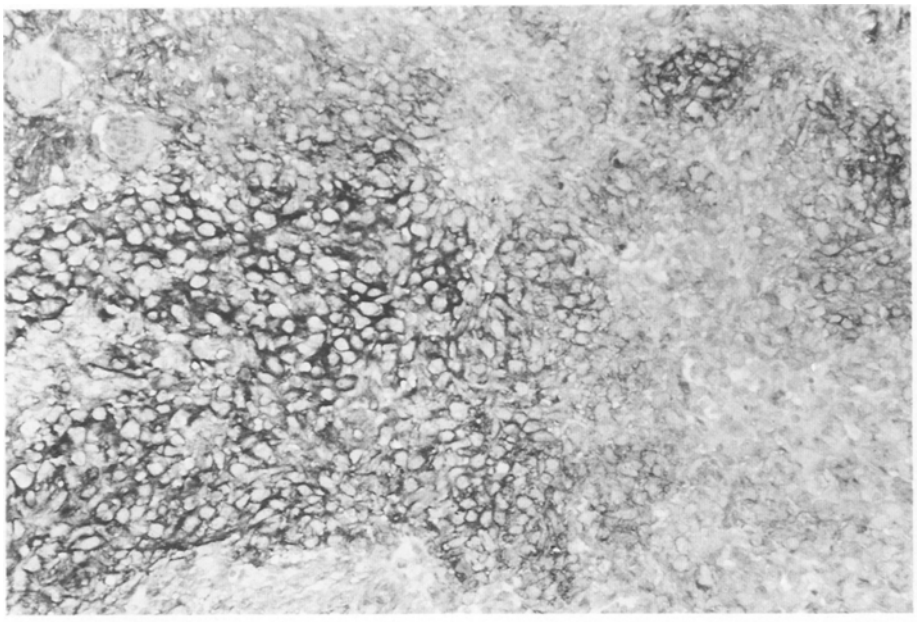


Fig. 9. Chondroblastoma with focally intensive positive staining of intercellular matrix around individual tumour cells with the antibody to collagen type VI. APAAP method, counterstaining H&E, ×160

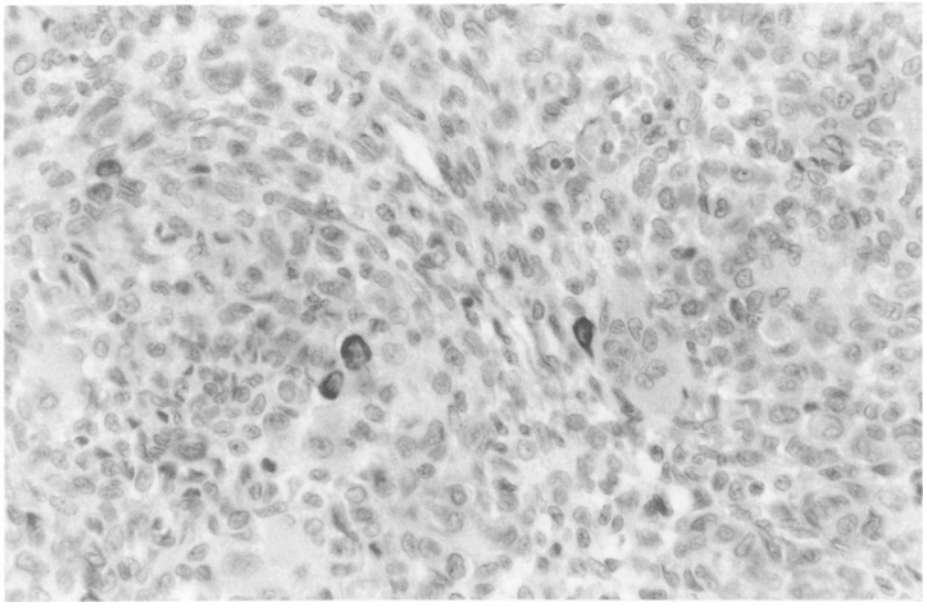


Fig. 10. Chondroblastoma showing a small number of tumour cells stained positive with the antibody to cytokeratin KL1. Paraffin section, APAAP method, counterstaining H&E, ×400

in 20 cases (37.7%) and 7 contained foam cells and cholesterol clefts. Nine cases (17%) were associated with sometimes large blood-filled spaces, simulating aneurysmal bone cyst. Cortical destruction and soft tissue invasion was seen in 5 cases (9.4%). Four were located in the flat bones (pelvis, vertebra, scapula) and 1 in the left temporo-mandibular region. Vascular invasion at the periphery of the tumour (Fig. 6) was observed only in 1 case.

The immunohistochemical findings are summarized in Table 3. Most of the chondroblasts were strongly positive with vimentin in all 30 immunohistochemically investigated cases and in fetal cartilage. Multinucleated osteoclast-like giant cells occasionally failed to stain with that antibody. About half of the polygonal chondroblasts and almost all relative mature chondrocytes in the chondroid areas, including those in fetal cartilage, showed a strong positive reaction with the antibody to S-100 protein (Fig. 7). Type II collagen was strongly positive in the chondroid matrix of all cases and in fetal cartilage tissue (Fig. 8). Two cases revealed a weak to moderately positive intracytoplasmatic reaction with this antibody in some chondroblasts. Type VI collagen was present focally around individual tumour cells (Fig. 9) and almost always in areas of "chicken-wire" calcification and in the chondroid matrix of all cases including fetal cartilage. The chondroblasts, however, were completely negative.

In paraffin sections of 14 tumours (46.6%), a small number of chondroblasts stained positive with the anticytokeratin antibody KL1 (Fig. 10). Only 4 of 30 cases (13.3%) showed a co-expression focally with the other

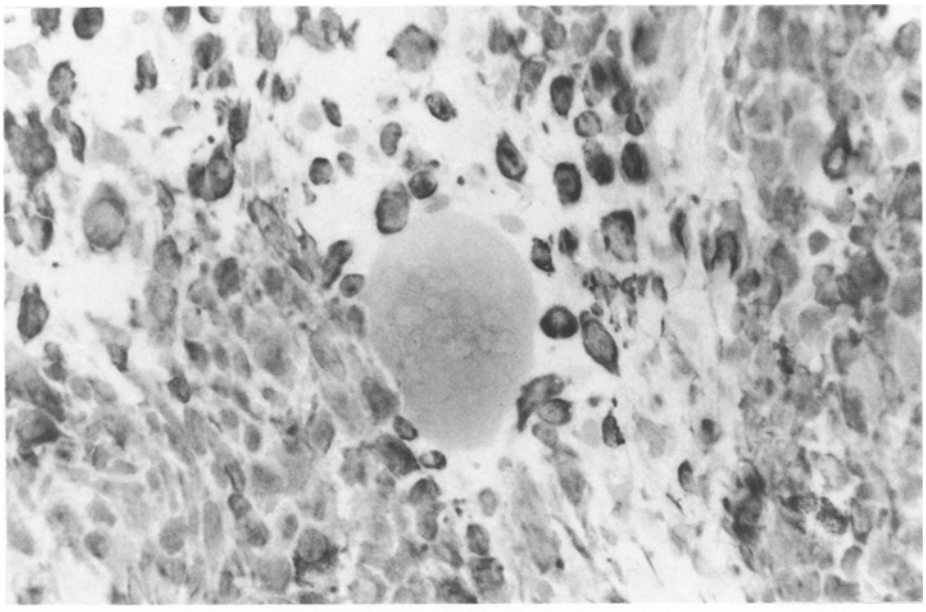


Fig. 11. Chondroblastoma with many CK-positive tumour cells. Note negative reaction of osteoclast-like giant cell in the centre. Frozen section, APAAP method, counterstaining H&E, ×400

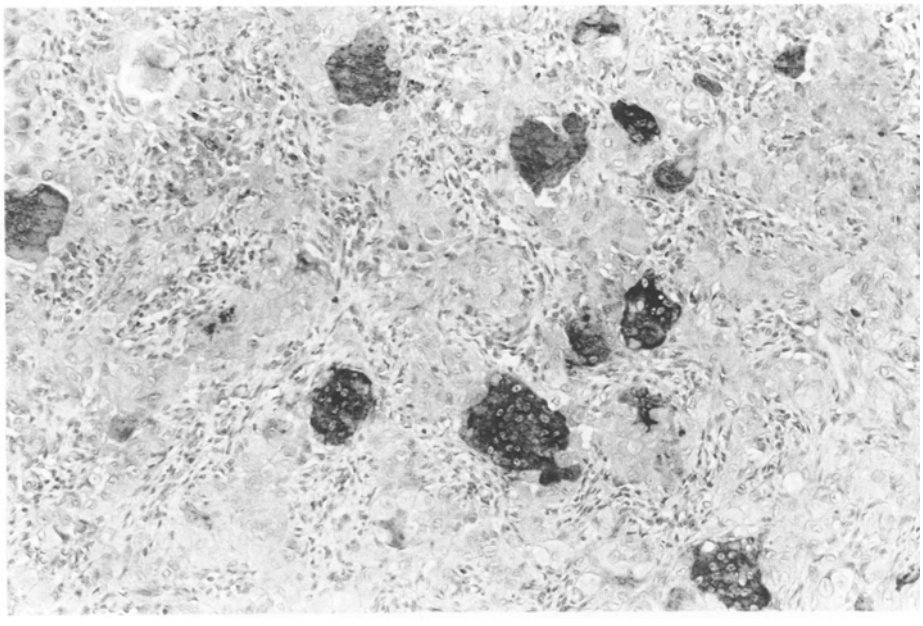


Fig. 12. Chondroblastoma showing strong positive reaction of osteoclast-like giant cells and some histiocytes with the macrophage-associated antibody KP1. APAAP method, counterstaining H&E, ×160

cytokeratin antibody CK used. The fetal chondroblasts failed to stain with both cytokeratin antibodies. A detailed analysis of the 3 tumours studied in frozen sections revealed a strong positive reaction with both cytokeratin antibodies in many chondroblasts (Fig. 11). The antibody CK also stained smooth muscle cells of the vessel walls. Osteoclast-like giant cells and some other mononuclear cells were strongly positive with the macrophage-associated antibody KP1 and with LCA in all cases (Fig. 12). Polyhedral chondroblasts were completely negative with this antibody. Another monoclonal antibody, MAC 387, which also identifies histiocytes, was found to be positive in histiocytes and granulocytes but negative in osteoclast-like giant cells and chondroblasts in all cases.

Data on treatment were available in all 53 cases. The initial treatment was curettage in 50 followed by bone grafting in 45 cases. In 2 cases in which the tumour was localized in the third rib and the left temporo-mandibular joint respectively, the patients were treated by wide surgical resection. In 1 patient with the tumor in the seventh cervical vertebra, partial laminectomy and curettage of the margins was performed. Data on the complete follow-up were available in only 28 cases (52.8%), including the patient with the spinal lesion. Twenty-seven of these patients (96.4%) were initially treated with curettage and bone grafting. Twenty-four of them (85.7%) were free of disease 2 months to 12 years later. In 3 of 28 cases the tumour recurred within 5, 9, and 18 months respectively and in 1 case even 4.5 years after

treatment. Of 3 patients with recurrent disease, 2 were treated by recurettage and 1 who had a large tumour in the proximal tibia by amputation. One patient had a second recurrence 18 months after initial treatment and was again recuretted. All 4 patients with recurrent tumours were free of disease 6–10 years after the last surgical procedure; none of the patients had radiation therapy. Metastatic disease was not observed in any case.

Discussion

Chondroblastoma is generally considered to be a benign tumour and occurs predominantly in young males, usually in the second decade of life (Schajowicz 1981; Dahlin and Unni 1986; Mirra 1989; Campanacci 1990; Huvos 1991) as we have found. However, the reported age range has been quite broad, from 2 to 83 years (Kurt et al. 1989). The youngest patient in our series was 5 years old, the oldest 64. The male-to-female ratio was 1.5:1 in our cases and a little lower than in other studies (Spjut et al. 1971; Schajowicz 1981; Dahlin and Unni 1986; Campanacci 1990; Huvos 1991). The long tubular bones were most commonly affected with a predilection for the femur (32,3%), the tibia (20.8%) and the humerus (15.1%), which is in line with most other investigations. One case each was found in the distal end of the radius and the proximal end of the fibula. Both locations are uncommon (Spjut et al. 1971; Schajowicz 1981; Bloem and Mulder 1985; Kurt et al. 1989; Mirra 1989). Four of our cases (7.5%) were observed in the short tubular bones of the hands, which are rarely involved (Schajowicz 1981; Bloem and Mulder 1985; Kurt et al. 1989; Huvos 1991). In an identical proportion of our cases the tarsal bones were affected, as also reported by others (Spjut et al. 1971; Bloem and Mulder 1985; Kurt et al. 1989; Mirra 1989; Brower et al. 1990; Campanacci 1990). Six of our cases (11.3%) arose in flat bones, 2 cases in pelvic bones, 1 case each in the scapula, a rib, the spine and the patella. According to the literature involvement of these bones is rare (Schajowicz 1981; Bloem and Mulder 1985; Kurt et al. 1989; Mirra 1989; Brower et al. 1990; Mayo-Smith et al. 1990; Imai et al. 1991; Resendes et al. 1991). The skull and the jaw bones are also less frequently involved, as recently reviewed by Edel et al. (1992). Chondroblastoma of the spine is extremely rare. Reviewing the literature we have found only 12 cases, including 1 of our own (Edel et al. 1992).

Radiologically, the tumours in the tubular bones of our series were always related to the growth plate, as also demonstrated by others. The affection of the metadiaphysis is extremely rare (Sotelo-Avila et al. 1986) and was observed in only 2 of our cases. In most of the cases the radiographic findings were typical for chondroblastoma, showing an eccentrically located round or oval osteolysis (64.7%) with matrix calcifications (47.3%), trabeculation (27.2%) and a sharp and well-defined sclerotic border (Lodwick IA) or an incomplete erosion of the cortex in some parts of the tumour (Lodwick IB). These findings are in agreement with other studies (Bloem and Mulder 1985; Freyschmidt and Ostertag 1988; Kurt et al. 1989; Mirra 1989; Campanacci 1990). Five of the cases in flat bones and the single lesion in the craniofacial bones were characterized by a focal complete cortical destruction and soft tissue involvement (Lodwick IC). Similar observations were made by Kurt et al. (1989) in their series. Periosteal reaction with new bone formation was present in 20.4% of our cases and most commonly associated with tumours in the long tubular bones (6 cases), which is in line with other investigations (Brower et al. 1990).

Radiological differential diagnosis generally includes only those lesions which occur in the same age group as chondroblastoma (individuals between 10 and 26 years of age). The most important lesions are: giant cell tumour, chondroma, low-grade chondrosarcoma, clear cell chondrosarcoma, chondromyxoid fibroma, purely lytic osteosarcoma and inflammatory disease (Bloem and Mulder 1985; Dahlin and Unni 1986; Freyschmidt and Ostertag 1988; Mirra 1989; Campanacci 1990; Huvos 1991). From this differential diagnosis it is clear that before definitive treatment histological examination of a biopsy specimens is essential.

The histological features of most tumours were characteristic for chondroblastoma as described by most other authors (Jaffe and Lichtenstein 1942; Spjut et al. 1971; Schajowicz 1981; Dahlin and Unni 1986; Kurt et al. 1989; Mirra 1989; Campanacci 1990; Huvos 1991). Amorphous pinkish chondroid or fibro-chondroid, essential for diagnosis of chondroblastoma (Dahlin and Unni 1986), was present in all our cases, but in 10% of them it was not prominent, which is in accordance with other investigations (Bertoni et al. 1987; Kurt et al. 1989). Chondromyxoid areas with spindle or stellated cells, typical for chondromyxoid fibroma, were observed in 4 of our cases and has been reported by others (Schajowicz 1981; Dahlin and Unni 1986; Kurt et al. 1989), but these foci were very small in our material. In 3 of our cases there were areas which contained numerous osteoclast-like giant cells, sometimes resembling a giant cell tumour. Typical "chicken-wire" calcifications were only present in 37.7% of the cases, but calcific deposits are not always found in these tumours (Schajowicz 1981; Kurt et al. 1989). Two cases showed areas with a haemangiopericytoma-like growth pattern, which is occasionally observed in chondroblastomas (Kurt et al. 1989; Mirra 1989), but more common in mesenchymal chondrosarcomas (Schajowicz 1981; Dahlin and Unni 1986; Mirra 1989; Campanacci 1990). Nine cases (17%) contained cyst-like spaces, resembling aneurysmal bone cyst. Such aneurysmal bone cyst-like components have been observed in up to 25% of the reported cases (Schajowicz 1981; Huvos 1991).

Occasionally the differential diagnosis between chondroblastoma and clear cell chondrosarcoma may be difficult, because the latter may show prominent areas identical or nearly identical to those observed in chondroblastoma (Mirra 1989). However, clear cell chondrosarcoma is characterized by clear cell chondrocytes with round vesicular nuclei and a water clear cytoplasm and trabecula of osseous metaplasia (Mirra 1989; Leggon et al. 1990; Huvos 1991; Present et al. 1991). One of

our reviewed cases showed these morphological features and was reclassified as clear cell chondrosarcoma.

The chondrogenic origin of the tumours was especially stressed by our immunohistological findings. All tumours contained variable amounts of collagen type II, which is characteristic for tumours showing cartilaginous differentiation (Remberger and Gay 1977; Ueda and Nakanishi 1989; Ueda et al. 1990, 1992; Takigawa et al. 1991). Collagen type II was found in chondroid areas of all cases and in fetal cartilage. This type of collagen was also weakly to moderately expressed within the cytoplasm of some chondroblasts in 2 cases. Type VI collagen was present in a strange pericellular accumulation between the tumour cells and also in the chondroid matrix of all cases as well as in fetal cartilage. Similar observations have been made by other authors in normal and neoplastic cartilaginous tissue (Ayad et al. 1984; Ueda et al. 1990, 1992). S-100 protein was expressed in about 50% of the chondroblasts and in almost all mature chondrocytes within chondroid areas, as already demonstrated by others in tumours of chondrogenic origin (Cocchia et al. 1983; Nakamura et al. 1983; Monda and Wick 1985; Weiss and Dorfman 1986; Okajima et al. 1988; Semmelink et al. 1990; Present et al. 1991). Most tumour cells and osteoclast-like giant cells stained positively with vimentin. In a recent study on 7 chondroblastomas including one with lung metastasis, Semmelink et al. (1990) reported that many of the tumour cells showed a moderate to strong positive reaction with a polyclonal antibody to cytokeratin and co-expression of epithelial membrane antigen (EMA) was seen in all but one lesion. A monoclonal antibody CAM 5.2 with specificity to cytokeratin 8 was positive in the tumour cells of 5 cases including the lung metastasis. An analysis of the pulmonary metastasis studied in frozen sections revealed in addition a strong positive reaction of the chondroblasts with antibodies to different cytokeratins (8, 18, 19), while another antibody to cytokeratin 7 only stained the well-differentiated chondrocytes. In a previous paper on two chondroblastomas of the head and spine (Edel et al. 1992) we were unable to demonstrate EMA, cytokeratin Lu5 (specific for cytokeratin 1 and 19) and cytokeratin KL1 (molecular weight 55–57 kDa) in any tumour cell. In the present study on 30 cases of chondroblastomas a small number of cells was strongly positive with KL1 in about half of the cases. Only 4 of them showed co-expression with the antibody CK (molecular weight 45–56.5 kDa with specificity to cytokeratins 10, 17 and 18). The 3 cases of embryonic cartilage failed to stain with both antibodies. Tumour tissue studied in frozen sections of 3 cases, however, demonstrated a strong positive reaction with both cytokeratin antibodies applied, which is in agreement with the observations made by Semmelink et al. (1990). The expression of cytokeratin has recently been reported in an increasing number of other non-epithelial tumours (Miettinen 1990; Swanson 1990) but the results with epithelial markers in the same types of tumours are often controversial. For example some authors have occasionally observed these markers in chondrosarcomas (Dervan et al. 1988; Karabela-Bouropoulou et al. 1988; Weiss et al. 1988; Hashimoto et al. 1990), in classical and chondroid chordomas (Abenoza and Sibley 1986; Salisbury 1987; Meis and Giraldo 1088; Bouropoulou et al. 1989; Swanson 1990; Persson et al. 1991) while others have not found them in these tumours (Lossnitzer et al. 1986; Chu 1987; Wick et al. 1987; Brooks et al. 1989; Swanson et al. 1990). A possible reason for that may lie in the differences in antibody specificity and fixatives used in different studies, since our results clearly indicate that immunoreactivity of keratin antibodies depends on the fixation technique used and that frozen section material seems to be the best method for the demonstration of different types of cytokeratins (Miettinen and Kovatich 1991). Our own findings may be explained by a biphasic or dual differentiation of multipotential stem cells within the bone marrow towards mesenchymal and epithelial cell elements, as recently proposed by Ling and Steiner (1986), who reported a chondrosarcoma associated with a squamous cell carcinoma and discussed the possibility of intermutability or metaplasia between mesenchymal and epithelial tissue. Evidence for the theory of multipotential stem cells come from experimental studies which have shown that cytokeratins 8 and 18 are expressed transiently in murine embryonic multipotential mesenchymal cells (Kulesh and Oshima 1988), in the developing skeletal muscle of the mouse (Kosmehl et al. 1990) and myocardial cells of some vertebrate species (Kuruc and Franke 1988). The same types of keratins have also been observed transiently in human embryonic smooth muscle cells (Gown et al. 1988) and fetal fibroblasts (von Koskull and Virtanen 1987). These findings indicate that cytokeratin expression in mesenchymal cells is probably not only an aberrant phenomenon but an indication of early differentiation stages. Thus it might be speculated that some chondroblasts in developing cartilage tissue may also express some types of cytokeratins, but our preliminary results, as well as those of Swanson et al. (1990), do not confirm this hypothesis. In both studies on paraffin embedded tissue the fetal chondroblasts were negative with the antibodies to cytokeratin used. Our observations and those of Semmelink et al. (1990) indicate that cytokeratin expression in chondroblastoma is obviously an aberrant phenomenon, but further comparative investigations on frozen samples of different embryonic mesenchymal tissues and the corresponding tumours with different cytokeratin antibodies are necessary to distinguish between aberrant and natural keratin expression in mesenchymal tumours.

The positive reaction of the osteoclast-like giant cells with the monoclonal macrophage-associated antibody KP1 and with LCA is in accordance with the findings of Brecher and Simon (1988). It is interesting to note that the other macrophage-associated monoclonal antibody MAC 387 failed to stain this cell type. This observation is in agreement with other investigations, which have shown that this antibody is non-specific as a tumour marker or a marker of histiocytes (Loftus et al. 1991).

The clinical course of chondroblastoma is usually benign. However, there are some reports on metastatic

malignant-behaving chondroblastomas, which differed in no observable way from classical benign chondroblastoma, as recently reviewed by others (Kunze et al. 1987; Semmelink et al. 1990; Van Horn et al. 1990). In addition, a few chondroblastomas have been described showing a clinical course with sarcomatous transformation with or without a history of irradiation (Bloem and Mulder 1985; Kyriakos et al. 1985). Despite these facts radical surgery is not generally necessary, because approximately 90% of the patients were cured by initial curettage or local excision with bone grafting (Kryriakos et al. 1985; Mirra 1989).

Local recurrence rates are mostly considered to be low after adequate treatment, ranging from 5% to 10% (Schajowicz 1981; Bloem and Mulder 1985; Springfield et al. 1985; Dahlin and Unni 1986; Mirra 1989), although a higher recurrence rate in those tumours with aneurysmal bone cyst-like components has been reported (Huvos 1991). The local recurrence rate in our 28 patients with data of complete follow-up was 10.7%, which is in agreement with most other studies. Only one of our patients had a second recurrence. All of our patients including those showing vascular invasion or recurrent disease and further treatment were free of disease up to 12 years later. None of the patients had metastatic disease. Thus vascular invasion is not absolutely correlated with a more aggressive local behaviour and the development of metastasis in chondroblastoma (Kurt et al 1989). Vigorous curettage of the primary tumour may also result in lung deposits through embolization of tumour cells (Kyriakos et al. 1985; Huvos 1991).

The histogenesis of chondroblastoma is still under debate. A histocytic origin seems to be unlikely, as discussed in detail by Brecher and Simon (1988). In our study we clearly demonstrated that collagen types II and VI, both characteristic for normal cartilage and tumours showing cartilaginous differentiation (Remberger and Gay 1977; Ayad et al. 1984; Ueda and Nakanishi 1989; Ueda et al. 1990, 1992), were present in the matrix of chondroblastoma. These findings support further strong evidence for the cartilaginous nature of this tumour, first proposed by Jaffe and Lichtenstein in 1942. The expression of cytokeratin in chondroblastoma seems to be more an aberrant phenomenon than a representation of an early stage of embryonic differentiation.

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