Chapter 6
A Histological Study of the Barrier Effect of the Physis Against Metaphyseal Osteosarcoma

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Abstract In about half of the children affected by metaphyseal malignant bone tumors, the growth plate and epiphysis are not compromised by the tumor. Invasion of the epiphysis by the tumor seems to occur eventually but takes time.

Keywords Physis • Osteosarcoma • Barrier effect • Histology • Growth plate • Chemotherapy • Radiology • Invasion

6.1 Introduction

Osteosarcoma is a primary malignant bone tumor usually located in the metaphysis. It tends to infiltrate adjacent bone as well as soft tissue. Traditionally, the physis has been regarded as a barrier capable of blocking tumor extension [1, 5], and this idea has been strengthened by experimental studies carried out *in vitro,* which suggest

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M. San-Julian (ed.), *Cañadell's Pediatric Bone Sarcomas: Epiphysiolysis before Excision*, DOI 10.1007/978-3-319-24220-0_6

the barrier effect is due to certain proteins in the physis that are inhibitory of angiogenesis [2, 10, 13, 15, 18]. In this respect, several molecules of possible relevance are different growth factors, such as fibroblast growth factor (FGF) and insulin-like growth factors (IGF) – which control growth of the epiphyseal growth plate growth – and bone morphogenetic proteins and the parathyroid hormone-related peptide [7, 14]. Doubts about the barrier function, however, have been raised by the fact that, in skeletally immature patients with osteogenic sarcoma, physeal invasion is observed to occur [4, 9, 11, 17, 19].

Knowledge of the frequency with which osteosarcoma invades the physis is important in assessment of tumor extension and in planning surgical resection.

In this study, the cases of a large series of skeletally immature patients with osteosarcoma were reviewed with the objective of clarifying how effective the physis is as a barrier to tumor spread. A particular objective was to assess any correlation between the pathological evaluation of the osteosarcoma in its relationship with the growth plate and the corresponding radiological findings. A principle observation made as a result of the study was that there were three different types or stages of behavior of osteosarcomas with regard to physeal invasion.

6.2 Materials and Methods

The series included 450 patients from whom a bone osteosarcoma was surgically removed and diagnosed by biopsy between 1979 and 2013. In order to ascertain tumor extension reliably at the time of diagnosis, all cases had been studied by conventional radiology and digital angiography, In the 1980s, the imaging method used was computerized axial tomography (CT); from the beginning of the 1990s, magnetic resonance imaging (MRI) was used. After histological diagnosis and previous to surgical resection, all patients but one received neoadjuvant chemotherapy, with intravenous doxorubicin, methotrexate, and cisplatin, which was administered intra-arterially.

In order to evaluate the reliability of basing the surgical decision about physis distraction on radiological imaging results, we studied in greater depth the cases in which the relationship of the tumor respect to the growth plate was doubtful. There were 170 such cases, of which a random selection of 38 were studied pathologically. This sub-sample was split into two groups according to whether or not it was considered appropriate to rule out a surgical approach of epiphyseal preservation after physeal distraction because the likelihood of physis invasion, as determined by the radiological evaluation, was too high.

Group I (n = 19):

Patients who received conservative surgery without physeal preservation. In 16 cases, the primary tumor was located in the distal *femur*, in two cases in the proximal *humerus* and in one case in the proximal *femur*. There were 12 girls and 7 boys. The mean age was 13 years, with a range between 9 and 17 years.

Group II (n = 19):

Patients who received conservative surgery with physeal and epiphyseal preservation after physeal distraction. In 13 cases the tumor was located in the *femur* and in 6 cases in the *tibia*. In all cases, on the basis of the above-mentioned imaging methods and prior to surgery, the epiphysis was deemed to be unaffected by the tumor. There were 4 girls and 15 boys. The mean age was 10.5 years (ranging from 4 to 15 years).

The specimens obtained by resection were studied macroscopically and microscopically. In all cases the histological stains applied were the H&E and Masson's trichrome. Multiple sections were taken from the metaphyseal area and, when included in the resection, from physeal and epiphyseal areas.

6.3 Results

Pathologically, the 38 osteosarcomas studied were of the following histological types: osteoblastic (31), chondroblastic (5), and fibroblastic (2). In all cases, as a result of the pre-operative chemotherapeutic treatment, the tumor tissue presented a highly altered histological picture at the time of resection. Most osteosarcomas showed a percentage of necrosis greater than 90 %. Only in cases in which osteosarcoma showed chondroblastic differentiation was the amount of tumoral necrosis lower. In some cases, there was only a dense mass of post-necrotic connective scar tissue within which histologically normal bone *trabeculae* could sometimes be seen (Fig. 6.1).

Physeal invasion was observed in 13 of the 38 resection specimens (34 %). Note that in all of these cases, on the basis of imaging the occurrence of physeal invasion had been considered to be uncertain. Of the 19 cases in Group I, 12 showed physeal invasion (63 %). There was one case (5 %) of physeal invasion in Group II. In the

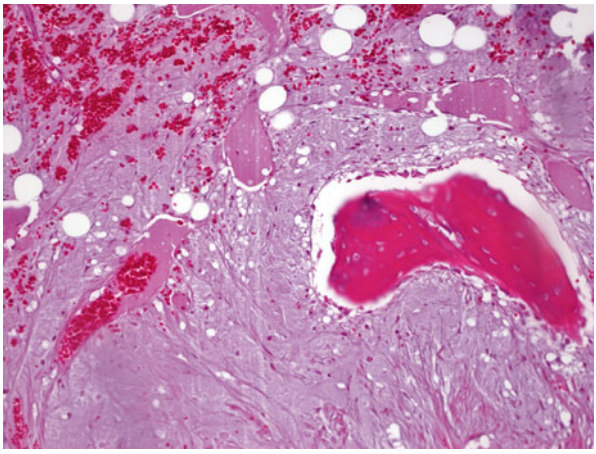

Fig. 6.1 A necrotic osteoblastic osteosarcoma post-chemotherapy can be observed in the border next to the physis (H&E, ×200)

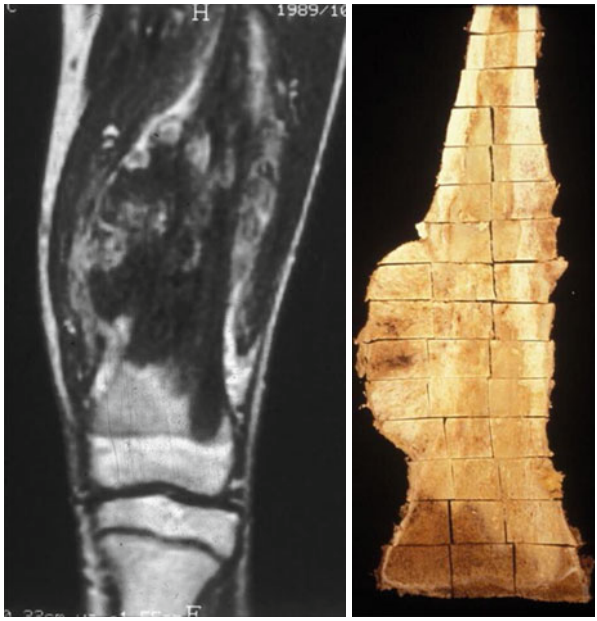

Fig. 6.2 *Left* MRI of an osteosarcoma that is apparently close to the physis. *Right* Macroscopically, an osteoblastic osteosarcoma that is destroying cortical bone and invading the medullar bone. The tumor is located several millimeters away from the growth plate

rest of the cases of Group I, tumors had a large contact area with the physis and had eroded it to some degree, but there was no demonstrable invasion. In Group II, most of the tumors were located at some distance from the physis; in two cases the tumors were close: at 3 mm and 5 mm from it; and in a further two cases the tumors were in contact with the growth plate, one of them with a large base of contact area but without invasion.

The average age at the time of tumor resection was 14.5 years for patients with epiphyseal involvement but 12.5 for patients without.

With regard to pathological findings, we observed the following three morphological growth patterns of tumor in relationship with the physis.

1. In 18 cases (47 %), between the tumor and the physis, there was a metaphyseal band of variable width (3–10 mm) within which there was no detectable neoplastic disease. Macroscopically, it was clear that the tumor was at a distance from the growth plate. In these cases, magnetic resonance images also indicated that the tumor was separate from the physis (Fig. 6.2). This disease-free metaphyseal zone had increased vascularization, which consisted of ectatic capillaries, and numerous osteoclastic cells flanking bone *trabeculae*, whose surfaces appeared undulate (Figs. 6.3 and 6.4). In these areas, there was often pronounced VEGF expression in both osteoblasts and osteoclasts (Fig. 6.5).
2. In eight cases (21 %) the tumor was in contact with the physis on the metaphyseal side. This contact without invasion was also suggested by MRI (Figs. 6.6 and 6.7). In the zone of contact, the physis appeared uniformly thinned, the hypertrophic and calcification zones having practically disappeared. To confirm non-invasion, a larger tissue sampling was obtained from these patients, but in no case was tumor observed in the epiphysis.

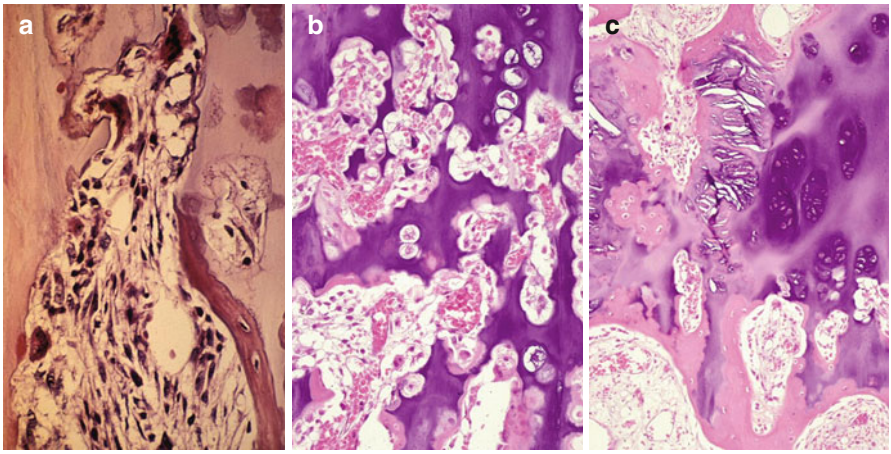

Fig. 6.3 (**a**-**c**) Osteoclastic activation accompanying fibrovascular proliferation in the femoral physis of a 10-year-old patient affected by osteosarcoma. The front edge of the tumor extends upwards to within 4 mm of the physis, which remains un-invaded (H&E, ×200)

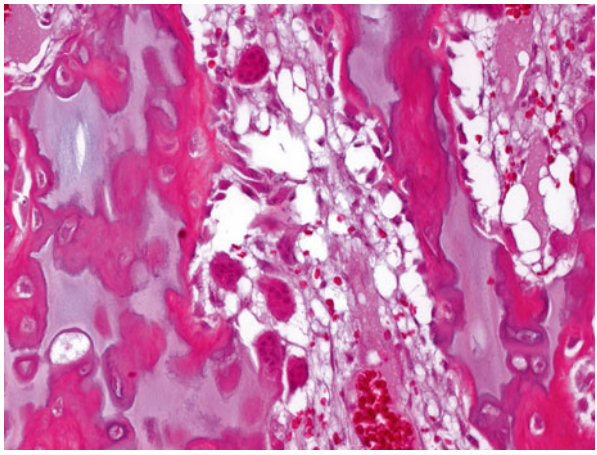

Fig. 6.4 A number of osteoclasts and osteoblasts are seen in the bone trabeculae in the growth plate. The osteoblasts have a hypertrophic aspect (H&E, ×200)

3. The third morphological picture was seen in the remaining 13 cases (32 %), where epiphyseal tumor invasion was observed. Macroscopically, the tumor was seen to be within the epiphysis, and this observation was corroborated by examination of the histological section (Fig. 6.8). Morphologically there were two patterns of physeal invasion. In the first pattern, epiphyseal areas were in close contact with the tumor but not completely invaded by it: tumor cells could be seen permeating the spaces between cartilaginous matrix columns next to dilated capillaries (Fig. 6.9). In the second pattern, in addition to the changes indicated above, there was perforation and thinning of the growth cartilage. The perforation was multi-focal, leaving dispersed islands of highly disorganized cartilage

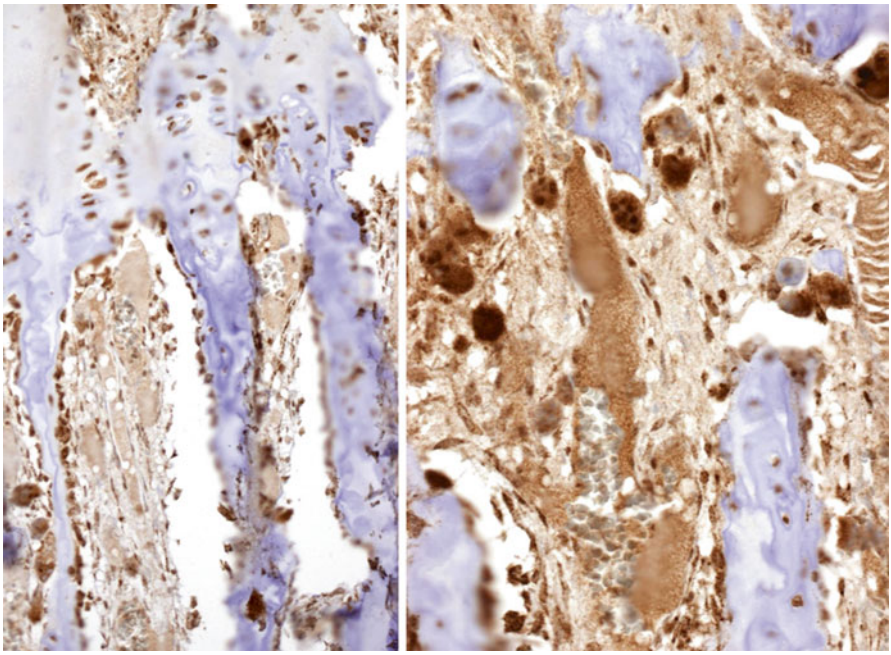

Fig. 6.5 A strong immunoreactivity against VEGF can be observed in the osteoclasts and osteoblasts in the physis. A high density of vessels can be distinguished (Immunohistochemistry, ×200)

within the tumor tissue (Fig. 6.10). In general, the extensions of tumor into the epiphysis appeared to have expanded evenly (Fig. 6.11). Some sections revealed paradoxical areas in which neoplastic tissue appeared on both sides of uncompromised growth cartilage; the apparent lack of connection can be explained by supposing that the plane of section did not cross the area of physeal involvement by the tumor (Fig. 6.12).

6.4 Discussion

Three types of behavior of an osteosarcoma in its relationship with the physis can be distinguished: tumoral contact with the physis, tumoral invasion of the physis, and tumoral trans-physeal invasion with involvement of the epiphysis. The clinical connotations of these growth patterns of remain to be determined, although it is probable that a large area of contact between a tumor and the growth plate is a risk factor for recurrence of tumor after physeal distraction. However, further studies are required to demonstrate that contact between tumor and physis is a contraindication for the technique of physeal distraction.

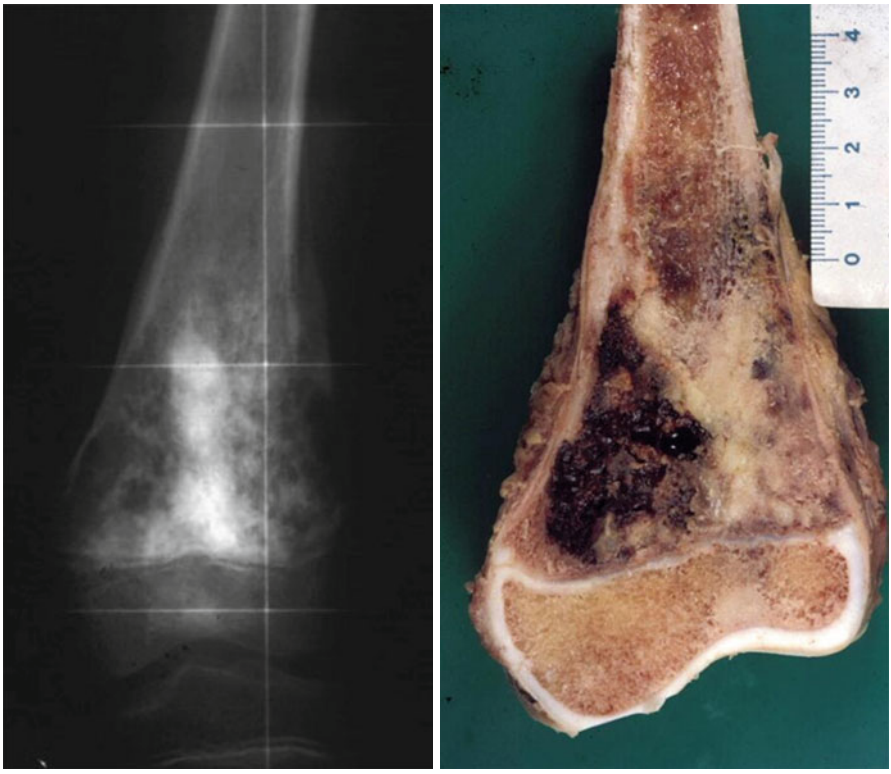

Fig 6.6 *Left* Radiography of a femoral osteosarcoma that shows that the tumor is in contact with the growth plate. *Right* An osteoblastic osteosarcoma in extensive contact with the physis; the histological study determined unequivocally that the tumor had not invaded the physis

The frequency of physeal invasion by the tumor in our series is lower than that in most series previously reported [9, 11, 17, 19]. Because physeal invasion by any metaphyseal osteosarcoma is likely to be a matter of time, differences in the frequency of physis invasion by osteosarcoma in different series can, in part, be explained by differences in the time elapsed between the diagnosis of patients and the evaluation of physeal invasion. In this respect, in our study we had the opportunity to evaluate consecutive MR images from several patients who did not receive any treatment for their tumor at or soon after the time of MRI (Fig. 6.13). These sequences of MR images show progressive osteosarcoma growth and, eventually, invasion of the physis. Conversely, an osteosarcoma's capacity for epiphyseal invasion was not found to have any clear relationship with its histological type.

From our results it can be concluded that it is valid to use radiological imaging as the basis for the decision on whether or not to undertake physeal distraction. In most cases in which conservative surgery was applied without physeal preservation, the resected material showed that the tumor had indeed invaded the physis or to be in extensive contact with it, and consequently the decision not to use physeal distraction

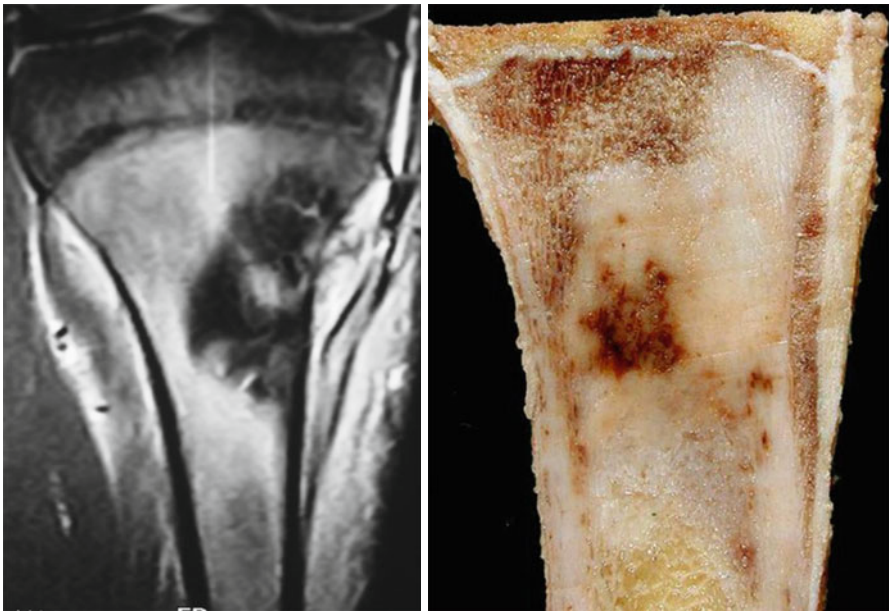

Fig. 6.7 *Left* In the MRI this tibial osteosarcoma seems to be in contact with the growth plate. *Right* However, macroscopically this osteoblastic osteosarcoma is found to be at a distance of 1 mm from the growth plate

was justified. There were, however, some case in which preservation of the physis was erroneously ruled out, while the physis was subsequently found not to have been transgressed by the tumor. From the clinical follow-up of all patients it was clear that only rarely did the tumor recur, and in the few cases when it did, it recurred in the diaphysis, not in the epiphysis. These data show that the radiological approach is valid for the decision on whether or not to carry out physeal distraction for a given patient.

The significant percentage of patients with epiphyseal invasion must surely raise doubts about the traditional notion of the physeal barrier. Two main theories have been proposed to explain how osteosarcoma, in its spread, is able to cross the physis [19]. According to the first, epiphyseal invasion takes place through the pre-existing trans-physeal vascular channels, which communicate the metaphysis with the epiphysis [9, 20]. However, Trueta and Morgan [21] and Brighton [3] observed that, from approximately 1.5–2 years of life until the age of skeletal maturity, the human epiphyseal and metaphyseal circulations are not connected in any way through the physis, which is hypertrophic. Most proliferating cartilage is practically avascular. The second theory is based on the possibility that the tumor induces an intense vascular response at its periphery, which favors its spread [8]. Our findings are concordant with such a mechanism. In fact, it has been demonstrated that osteoblasts [22] can synthesis VEGF, as we have observed in the physis next to the front edge of osteosarcoma. Vascular proliferation of the peritumoral stroma would favor tumor infiltration of the cellular columns of epiphyseal cartilage observed in our

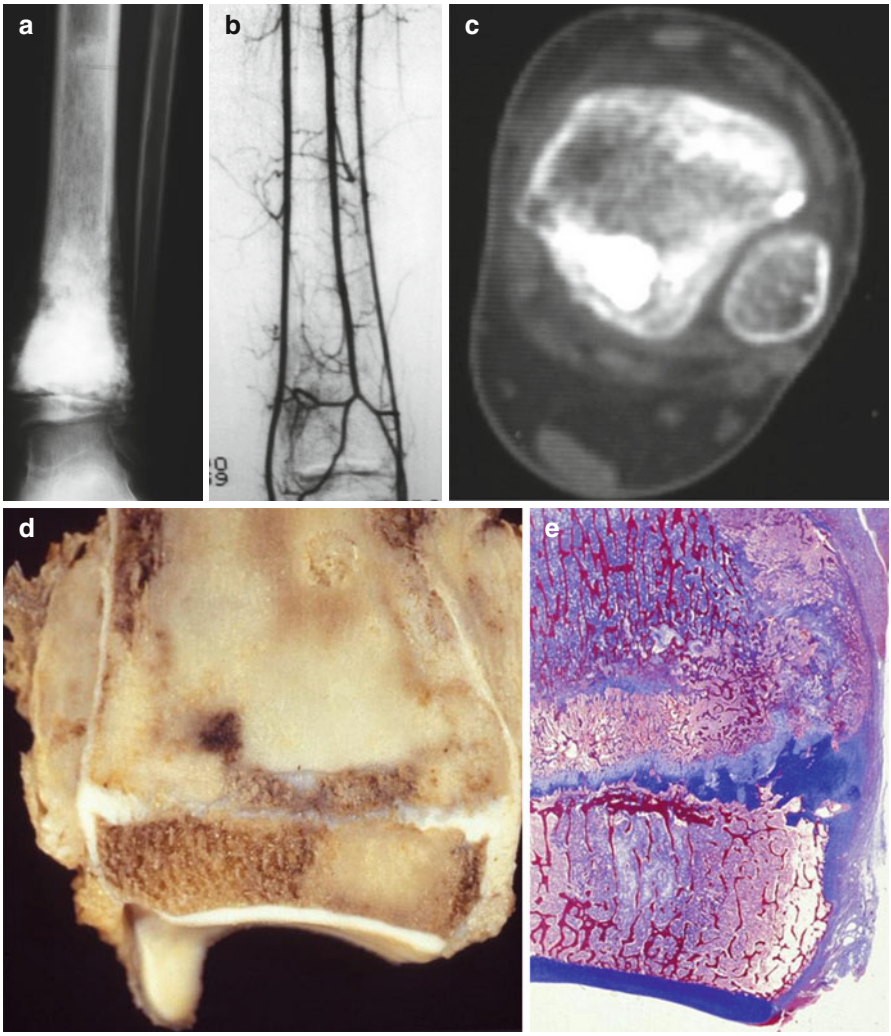

Fig. 6.8 (**a**–**c**) *Left* Osteosarcoma in the distal tibia. Angiography and CT scan showed invasion of the epiphysis. (Note that in the 1980s, MR imaging was not available). (**d**, **e**) This osteosarcoma is eroding the growth plate and invading the physis. In the histological cut stained with Masson trichrome, the osteoblastic osteosarcoma is clearly located in the epiphysis, but the location of physis invasion is not observed in this section

histological studies. The final effect of vascularization would depend on the balance between pro-angiogenic and antiangiogenic factors produced in the environment of osteosarcoma [12]. Several pro-angiogenic growth factors, such as FGF-2 and VEGF, produce their effect by linking to proteoglycans, such as heparan sulfate. Other molecules, such as syndecan or perlecan, regulate the vascular distribution [5, 12, 23]. As well as by vascularization, tumoral infiltration would also be

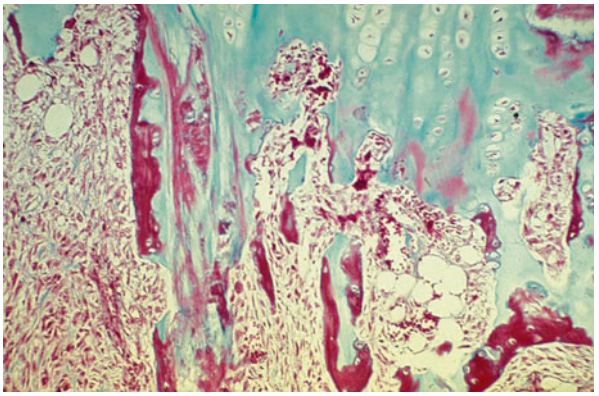

Fig. 6.9 Physeal invasion by an osteosarcoma in a 12-year-old boy. Tumor cells infiltrate as finger-like growths permeating the calcification and hypertrophic zones of the physeal cartilage (Masson's trichrome, ×40)

Fig. 6.10 The remains of physeal cartilage in the femur of an 8-year-old girl with metaphyseal osteoblastic osteosarcoma. The patient had received chemotherapy before resection of the tumor. Several islands of physeal cartilage can be seen surrounded by reparative tissue which occupies the whole thickness of the physis. This organization of tissue has substituted the necrotic tumor (H&E, ×100)

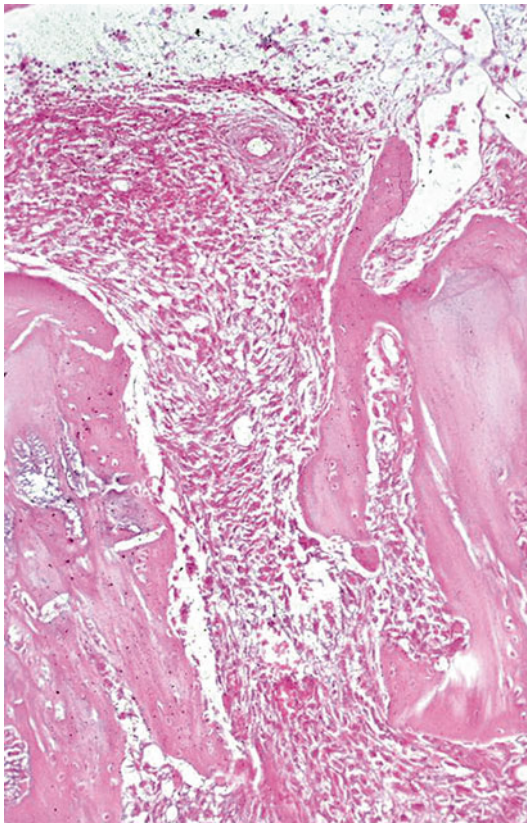

Fig. 6.11 Physeal tumor invasion in the form of finger-like projections in a 9-year-old girl with osteoblastic osteosarcoma. The periphery of the osteosarcoma was not necrotic despite pre-operative chemotherapy. This multi-focal type of growth is more difficult to detect radiologically (H&E, ×100)

enhanced by the osteoclastic reaction as a phenomenon that, hypothetically, could precede the progress of the tumor. This cellular reaction was seen in the free zone between the tumor and the physis. These findings have also been observed in a similar sequence of angiogenesis and osteoclastic activation in the growth plate [11].

Apart from via the trans-physeal route of epiphyseal invasion, another explanation of how the tumor gets into the epiphysis is to suppose that it can establish epiphyseal metastatic foci without alterations in the growth cartilage. This type of metastasis, so-called *skip metastasis*, has been observed to occur between various zones of a single bone [6] and even between adjacent bones of mature individuals without affecting the articular cartilage [16]. However, there was no evidence of such metastasis in our histological study.

Age is a factor which one might expect to influence trans-physeal spread of osteosarcoma. Physeal involution commences shortly before skeletal maturity and, as a result of this, at certain points, meta-physo-epiphyseal vascular communication is re-established. Theoretically, this factor would increase the possibility of an osteosarcoma invading the epiphysis.

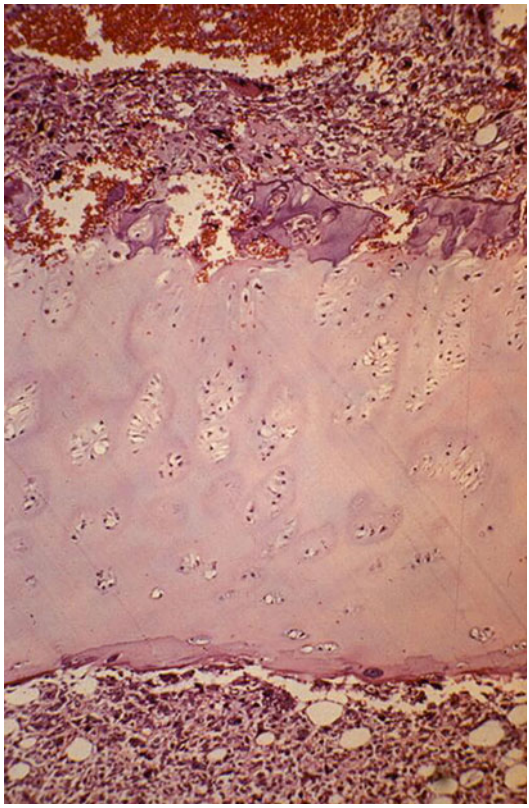

Fig. 6.12 A paradoxical pattern of metaphyseal-epiphyseal infiltration is shown in which there is apparent preservation of the physis, at least on the plane of this section. The case concerns a 15-year-old boy with osteosarcoma (H&E, ×100)

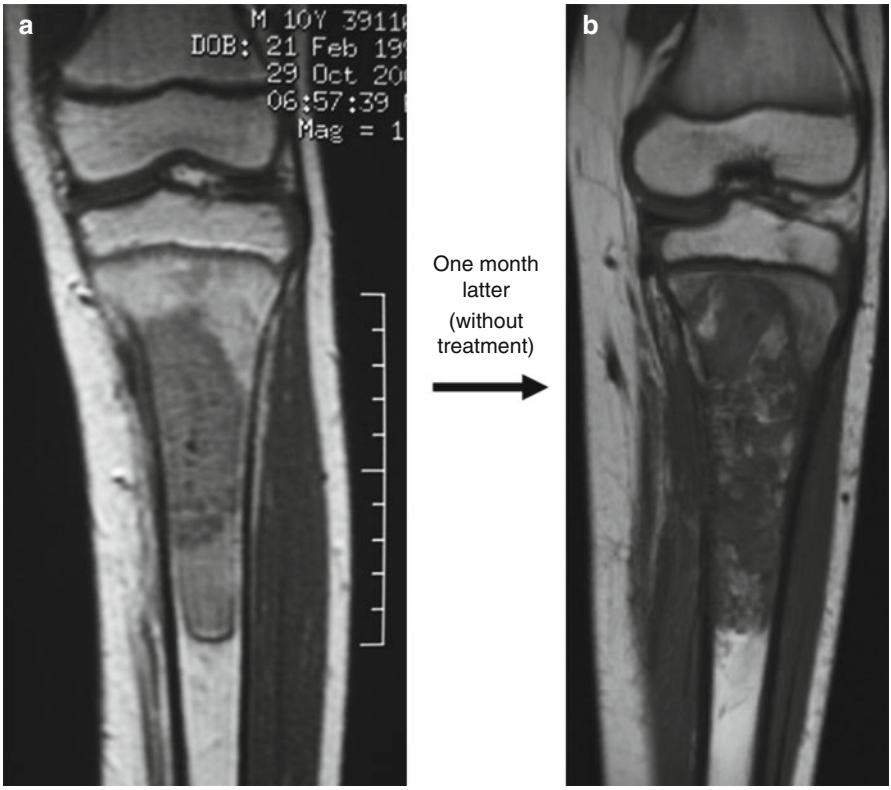

Fig. 6.13 (**a**) The initial MRI scan for this patient with a bone sarcoma was made at a hospital other than our own. Over a month later (**b**), he came to our center, and we took another MRI scan in order to be sure the epiphysis could still be saved. These two consecutive scans and other such sequential scans strongly suggest that invasion of the physis by an osteosarcoma is largely just a matter of time

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