REVIEW ARTICLE

Osteosarcoma: Pathology, staging and management

DJ van der Spuy MBChB (Stell)
Orthopaedic Surgery Registrar, University of Stellenbosch
GJ Vlok MBChB, MMed(Orth), FC(Orth) SA
Professor and Head: Dept of Orthopaedic Surgery, Tygerberg Hospital/University of Stellenbosch

Reprint requests:
Dr DJ van der Spuy
Dept of Orthopaedic Surgery
PO Box 19063
Tygerberg
7505
Tel: +27 21 938-9266

Introduction

Osteosarcoma (OS) is a malignant spindle cell sarcoma in which the malignant cells produce osteoid or bone in the background of a sarcomatous stroma. However, fibrous or cartilaginous tissue may co-exist or even predominate.

The classic or so-called conventional osteosarcoma develops in the medullary cavity of the metaphysis of long bones. It has a predilection for the knee area with 50% of cases in either the distal femur or proximal tibia, with the second most common site being the proximal humerus (10%).[1] However, osteosarcoma has been described in every bone.

Osteosarcoma has a bimodal peak incidence. It is the most common bone tumour in children and adolescents with a peak incidence between ten and 20 years. This correlates with the adolescent growth spurt. It is also the third most common malignancy in childhood following leukaemia and lymphoma.[1] Adults are less affected with a second peak incidence between 50 and 70 years. This later incidence is usually associated with secondary OS that could arise in Paget's disease, bone infarcts and fibrous dysplasia.[2] Surface osteosarcomas tend to affect younger adults in the third and fourth decade.[3,4]

Many variants of the conventional or classic high-grade OS have been described. The two most common of these variants are the surface or juxtacortical group and the telangiectatic osteosarcomas. The surface osteosarcomas arise on the surface of long bones, most prominently the posterior aspect of the femur.[5]

Aetiology

The cause of OS remains uncertain. Many carcinogens and oncogenes have been proposed. Simian virus 40, a contaminant of the polio virus, was implicated but current thinking doubts its contribution in the oncogenesis.[6,7] Irradiation is long known to cause OS in patients receiving this therapy for other malignancies.

There is reason to believe that there might be a genetic component in the aetiology of OS. Osteosarcoma is a component of the familial Li-Fraumeni syndrome,[8] which is known to have mutations in the p53 tumour suppressor gene. This syndrome is associated with OSs, soft tissue sarcomas, breast carcinomas and adrenal cortex tumours. There is an increase in the incidence of OS in hereditary retinoblastoma as well as in the autosomal recessive Rothmund-Thomson syndrome[9] (skin pigmentation, hypogonadism and bone abnormalities).

Clinical presentation

Early diagnosis of a malignancy remains the biggest challenge for general practitioners and orthopaedic surgeons confronted with non-specific pain in a limb. In OS the most common presentation is site specific pain. This is generally worsened by physical exertion. Approximately 20% complain of night pain. Nearly half of the patients associate the pain with a traumatic event (*Table I*).[10]

Table I: Clinical signs of osteosarcoma[10]

Clinical sign	Percentage present at first consultation
Local tenderness	92%
Palpable mass	39%
Painful joint movement	39%
Limp	30%
Limited range of movement	23%
Atrophy of muscle	5%
Fever	3%

Fewer than 5% of those with OS will actually complain of a palpable mass.

In a recent study by Widhe[10] in Sweden it was found that in only 30% of first consultations was a diagnosis of a tumour made. Some incorrect non-specific diagnoses such as tendinitis, osteitis, chondromalacia patella and even Osgood-Schlatter disease were made. Therefore these diagnoses should be made with care in the child and adolescent presenting with pain around the knee. The incidence of pathological fractures either at presentation or during therapy varies between 5% and 10%.[11]

Recognising osteosarcoma on plain radiographs

The X-ray holds the key to confirm a clinical suspicion. The literature makes it clear that OS does not necessarily present with the classic Codman's triangle and the sunburst appearance (that often get portrayed as the mainstay of these diagnoses) and that these signs are non-specific. Conventional osteosarcomas are usually found eccentrically in the metaphysis of long bones with areas of radiodense, radiolucent or mixed patterns (*Figures 1 and 2*). The key to the diagnosis is to recognise the malignant nature of the lesion. This should be evident in recognising cortical destruction, soft tissue infiltration and a wide zone of transition in the medulla (*Figures 3 and 4*). The 'sunburst appearance' results from speckles of bone developing along the newly formed vessels derived from the periosteum, giving the appearance of sunrays. Codman's triangle is thought to be the desperate attempt by the periosteum to contain the tumour by laying down reactive bone and hence lifting up the periosteum.

The key to the diagnosis is to recognise the malignant nature of the lesion - cortical destruction, soft tissue infiltration and a wide zone of transition in the medulla

Surface osteosarcomas usually have a 'patched-on' appearance often leaving an incomplete line between cortex and tumour, sometimes referred to as the 'string-sign'.[4] These tumours do not usually infiltrate the medulla and due to their cartilaginous nature often have radiodense and radiolucent areas creating a lobulated effect on X-ray (*Figures 5 and 6*).

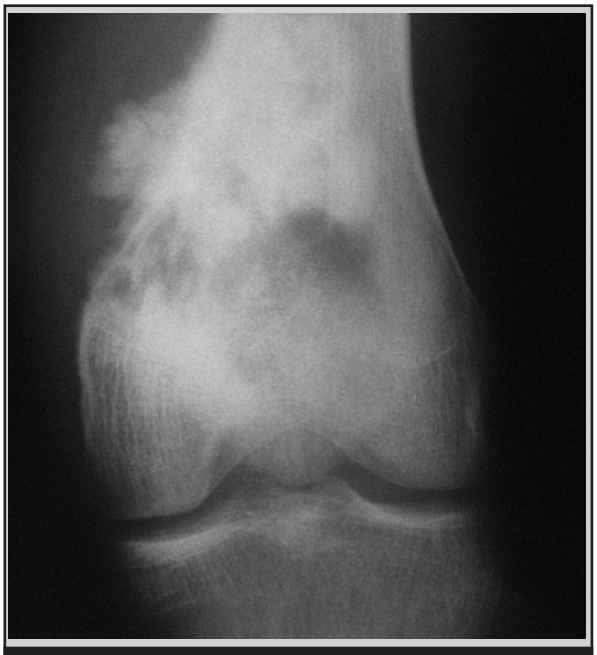

Figure 1: Conventional osteosarcoma: AP distal femur. Combination of sclerotic changes corresponding to new bone formation with lytic changes evident in medulla and cortex

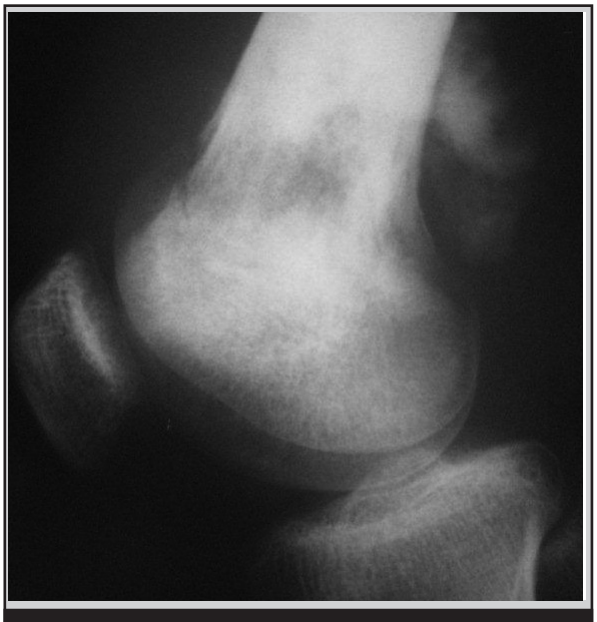

Figure 2: Lateral view of the same tumour

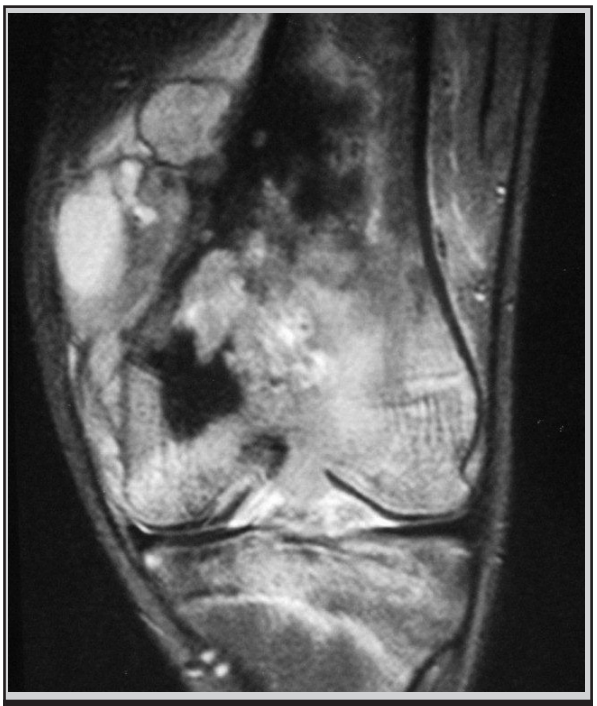

Figure 3: MRI (Gadolinium) of the same tumour. Note the soft tissue element and tumour infiltration into epiphysis

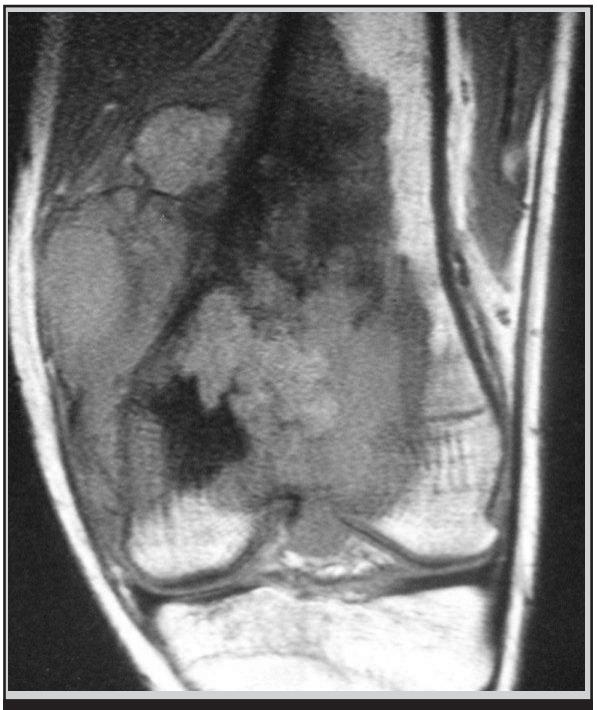

Figure 4: MRI T1 Coronal view clearly defines the borders of the tumour in the bone

Telangiectatic osteosarcomas generally present as radiolucent lesions with aggressive osteolysis and periosteal reactions.[12]

Further staging modalities

Once there is a suspicion of a malignant lesion a complete radiological workup is essential before a diagnosis can be confirmed with a biopsy. The objectives of a good workup are to delineate the local extent of the tumour, to discover any skip lesions in bone and to locate any distant metastases. This facilitates the essential tissue diagnosis with a biopsy.

Magnetic resonance imaging

MRI is the best investigation to define the primary tumour. It provides a good assessment of the degree of medullary infiltration, cortex destruction, soft tissue invasion, neurovascular bundle invasion and it identifies skip lesions[13] (*Figure 3*). Occult skip metastases of 2 mm or more can be picked up on MRI and therefore all MRI series should include coronal T1 sequences to scout the whole affected bone. The T2 sequence shows peri-lesional oedema well and low-mineralised areas have a high signal intensity. Gadolinium has the advantage of defining the border between the tumour and cartilaginous areas very well[12] (*Figure 3*).

Computed tomography

Computed tomography (CT) is of great value to evaluate the lungs for metastases. Pulmonary metastases of 3 mm and greater can be picked up on CT scan.[14] Spiral CT is superior to conventional CT for this purpose.

Scintigraphy (nuclear bone scan)

Bone scans with technetium-99m show an increased uptake in primary tumour corresponding with bone formation and increased vascularity in the tumour area. Nuclear bone scanning is therefore very useful in evaluating skip metastases and metastases in other skeletal sites.[15] Positron emission tomography (PET) is also becoming an important nuclear imaging modality.[16] The most commonly used tracer is fluorine-18 fluorodeoxyglucose (F-18 FDG) which is a glucose analogue and taken up by the cell's glucose transporters. Concentration of this marker in sarcomatous areas is an indicator of increased metabolic activity.[17-18] This 3-D image of metabolic activity is not only useful in staging the tumour but it also allows the evaluation of treatment response with pre- and post-treatment image comparisons.

The objectives of a good workup are to delineate the local extent of the tumour, to discover any skip lesions in bone and to locate any distant metastases

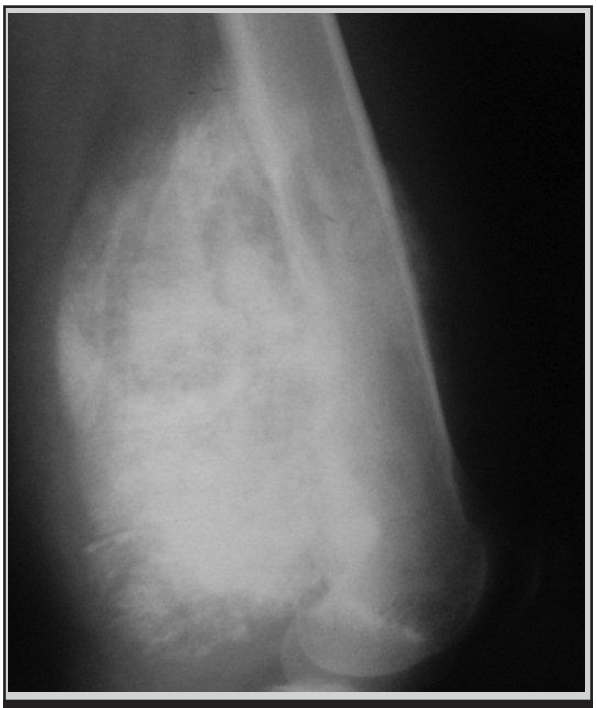

Figure 5: X-ray lateral view of parosteal osteosarcoma – the classic position on the posterior aspect of the femur

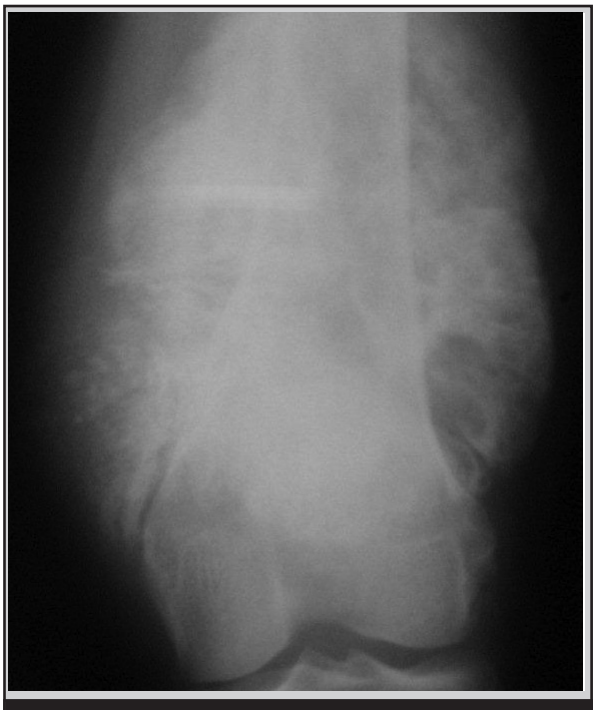

Figure 6: X-ray AP of parosteal osteosarcoma – the lobulated effect is usually created by mixed osseous and cartilage tissue

Angiography

Angiography is useful to define the tumour in relation to adjacent neurovascular bundles and soft tissues. This intervention has been partially replaced by MRI. However in parosteal OS (usually on the posterior distal surface of the femur) angiography plays a vital role defining the femoral artery in relation to the tumour site.[4]

Pathology

This discussion focuses mainly on the classic medullary OS, followed by important characteristics of the variants.

Macroscopic

The tumour is hard and compact, light yellow in colour, localised to the medulla of the metaphysis and tends to penetrate the soft tissue via cortex destruction. Generally the tumour does not cross the physis; however some osteosarcomas in children do cross the physis and extend into the epiphysis.[19] The tissue comprises varying amounts of mineralised bone, with or without foci of cartilage or fibrous tissue. A pseudo-capsule is often observed at the tumour edge in the soft tissue.

Microscopic

The principle in diagnosing OS is to identify sarcomatous, spindle-shaped cells producing a calcified tissue, osteoid or bony tissue. Well differentiated sarcomatous osteoblasts are the exception and bizarre undifferentiated spindle cells in masses of osteoid seem to predominate. Bone production is in a rather disorganised woven fashion with sheets of malignant cells pushed against malignant bone. Benign-looking giant cells may be present. Haemorrhage and necrosis are common and predict a poor outcome.

Conventional OS is histologically classified on the tissue type that predominates, as follows:

- osteoblastic
- chondroblastic
- fibroblastic
- dedifferentiated or epitheloid. This subtype is considered when spindle cells are so poorly differentiated that it is impossible to distinguish between the sarcomatous or epitheloid origin of tumour.

Variants of osteosarcoma

Many variants have been described distinct from the classical OS which accounts for 85% of osteosarcomas. Osteosarcoma as such is a rather rare disease and some of these variants are extremely rare and beyond this discussion. However, some of these variants present radiologically very differently from the conventional picture. Therefore, especially in the younger population, OS needs to be considered as part of the differential diagnosis in even radiolucent lesions.

Osteosarcomas can also arise as a secondary phenomenon in a wide range of entities. The most common of these lesions arise secondary to Paget's disease. However fewer than 1% of patients with Paget's disease develop OS.[20] Other malignant tumours such as chondrosarcoma and fibrosarcoma as well as benign tumours can also develop in Paget's disease. Osteosarcoma can also arise in bone infarcts, bone exposed to radiation and in fibrous dysplasia.

Incidence and characteristics of the different primary osteosarcomas

Refer to *Table II.*

Juxtacortical group

The juxtacortical tumours arise on the surface of bone. They tend to present later[4] (third to fourth decade) in comparison with the adolescent presentation of the conventional OS. These tumours arise from cells in the periosteum.

Parosteal OS arises from the outer fibrous layer of the periosteum. Histologically this is a low grade tumour and has a good prognosis.[3] It is classically found on the posterior surface of the femur with a 'patched-on' appearance on X-ray leaving a line between the cortex and tumour.

Dedifferentiated parosteal OS is the high grade variant of parosteal OS. It can arise spontaneously from a low grade parosteal OS or it can develop secondarily from an incompletely resected lesion.[4] Histologically it resembles conventional medullary OS.

Periosteal OS is thought to arise from the inner cambium layer of the periosteum. It is worth mentioning that this layer contains pleuripotent cells as well as osteoblasts. It is a low or medium grade tumour that is predominantly chondroid tissue. It usually arises in the diaphysis of the tibia.[22]

High –grade surface OS is a very rare juxtacortical OS variant.[3] It is a high grade tumour and carries the same metastatic and prognostic potential as conventional OS.

Staging

The purpose of staging is three-fold:

- First, a tissue diagnosis should be established.
- Second, the extent of the local tumour should be defined in terms of medullary extension, soft tissue and neurovascular penetration, joint involvement and skip lesions in the same bone.
- Finally, it is pivotal to identify and quantify metastatic disease.

Biopsies should be planned with caution and should be performed by the surgeon who will perform the definitive procedure. Limb salvage strategies, potential flaps and anatomical considerations (such as the anterior-superior extension of the knee joint) should be considered when planning the biopsy.

There are basically two systems used for staging OS. Enneking's classification (*Table III*)[23] was published in 1980 and contributed significantly to the research of osteosarcomas. His system is simple to use and considers the histological grade of tumour, the local extent and the presence of metastases.

Table II: Greenspan differential diagnosis of tumours and tumour-like lesions of bones and joint[21]

Type of OS	Percentage of OS	Characteristics
Conventional medullary	85%	Arise in the metaphysis of long bones with a predilection for distal femur, proximal tibia and proximal humerus
Juxtacortical group	4–7%	Arise on surface (see discussion below)
Telangiectatic	5%	Very aggressive; resemble aneurysmal bone cyst or bag of blood with fluid levels; radiolucent with aggressive osteolysis
Giant cell rich	3%	Overabundant osteoclast-like cells; benign-looking lesion resembling giant cell tumour histologically
Low grade central	<2%	Slow-growing, benign-looking lesion with well-defined sclerotic rim, usually in older people
Multifocal	1.5%	Lesions develop simultaneously in different bones; existence as separate entity is in doubt
Small cell	1%	Similar to Ewing's sarcoma; radiolucent
Gnathic	1%	Arising in mandible or maxilla; fourth to sixth decade; good prognosis

The other classification system is the American Joint Committee on Cancer (AJCC) staging system[24] for musculo-skeletal tumours (*Table IV*). The AJCC staging score includes tumour size which is now rated as an important prognosticator.[25]

Medical treatment

Before the 1970s the only treatment modality for OS was surgical. Most patients treated with an amputation for local disease eventually developed metastatic disease. This raised the suspicion that micro-metastatic disease was already present (usually in the lungs) at the time of diagnosis. This suspicion was confirmed with studies that showed that within two years 80–90% of patients treated with amputation alone developed metastatic disease.[26-29] Therefore OS is a systemic disease and cannot be treated with surgery alone.

Chemotherapy

The first drug to be proven beneficial over surgery alone was high doses of methotrexate in the early 1970s.[30] Subsequently doxorubicin, cisplatin and in some institutions ifosfamide were added. The Multi-Institution Osteosarcoma Study (MIOS) confirmed the efficacy of multi-drug regimens with up to 66% of patients being relapse-free after two years.[31]

After the great results of these chemotherapeutic drugs neo-adjuvant chemotherapy was introduced hoping to shrink the tumour prior to surgery.

This proved to be a vital part of management,[32] especially since limb salvage surgery was also introduced. Tumour response to neo-adjuvant therapy became an important prognostic factor as it measured the regression of the tumour in response to these agents. Huvos[33] histological grading system grades the response to chemotherapy looking at the percentage of necrosis (*Table V*). At least 30 different surgical specimens are evaluated for necrosis.

Unfortunately there are relatively few drugs that are effective against OS. Some novel cytotoxic drugs have shown some response against OS and will be considered in the future and reserved for unresectable and chemotherapy-insensitive tumours (*Table VI*).

Gemcitabine is a fluorinated analogue of the nucleoside deoxycytidine and is administered in combination with docetaxel. Some effects have been shown in patients with refractory or relapsed bone metastases.[34]

Pemetrexed inhibits folate-dependent enzymes. It shows limited effects on OS as a single drug but has some promising results in combination with other drugs like platinum and gemcitabine.[35]

Immunotherapy

A recent study by Jeys *et al*[36] showed a survival advantage in patients suffering post-operative infections, suggesting that the induced immune response aids in tumour lysis. The thinking behind immunotherapy (*Table VII*) is that a humoral or cell-mediated attack against the tumour could aid in tumour necrosis.

Table III: Enneking's classification of osteosarcoma[23]

Stage	Grade	Site	Metastasis
IA	Low	Intracompartmental	None
IB	Low	Extracompartmental	None
IIA	High	Intracompartmental	None
IIB	High	Extracompartmental	None
III	Any	Any	Regional or distant

Table IV: The New American Joint Committee on Cancer Staging System[24]

Stage	Grade	Local extent	Metastases
I-A	Low	≤8 cm	None
I-B	Low	>8 cm	None
II-A	High	≤8 cm	None
II-B	High	>8 cm	None
III	Any	Any	Skip metastases
IV-A	Any	Any	Pulmonary metastases
IV-B	Any	Any	Other metastases

Table V: Huvos classification system evaluating osteonecrosis of resected tumour following neo-adjuvant chemotherapy[33]

Grade	Percentage of tumour necrosis
1	<50% of tumour is necrotic
2	Most of the tumour is necrotic <90%
3	Only occasional microscopic tumour viability noted; 90–99% necrosis in each section
4	Tumour is totally necrotic

Table VI: Effective drugs against OS

Conventional drugs
- Methotrexate
- Doxorubicin (Adriamycin)
- Cisplatin
- Ifosfamide

New cytotoxic drugs
- Gemcitabine
- Pemetrexed

Table VII: Immunotherapeutic agents

- MTP-PE
- Inhaled GM-CSF
- Trastuzumab

Muramyl tripeptide phosphatidylethanolamine (MTP-PE) is an analogue of a dipeptide found on the cell wall of the Bacille Calmette-Guerin. This analog dipeptide stimulates a cell-mediated response with the release of multiple cytokines that could be tumouricidal.[37] The Children's Cancer Group and the Pediatric Oncology Group's individual evaluation of MTP-PE showed that MTP-PE as such improved overall survival and improved event-free survival, after it was used in a randomised trial.[38]

GM-CSF is an inhaled agent capable of activating multiple components of the immune system.[36] It has anti-tumour effect in some cancer types.[40] A phase II study is presently being conducted evaluating its efficacy in patients with pulmonary metastatic relapse in OS. This drug could show benefits in patients with metastatic lung disease or as prophylaxis in patients with high risk of developing metastatic disease.[41]

Trastuzumab is a HER-2 monoclonal antibody. The HER-2 gene codes for a transmembrane glycoprotein serving as a receptor for tyrosine kinase. HER-2 expression in OS is often associated with a poor histological response to neo-adjuvant chemotherapy.[42] The role of trastuzumab still needs to be concluded.

Bisphosphonates

Third generation bisphosphonates show promising inhibitory effects on OS cells *in vitro*. It is thought that zoledronic acid enhances specific T-cell major histocompatibility complex mediated lysis that is capable of unrestricted tumour cell destruction.[43] Many tumour cells express tumour-specific, major histocompatibility complexes on their surfaces that could be the target for these T-cells.

Radiation

Generally external beam radiation is not very effective in the treatment of OS.[41] However exceptions to this rule are unresectable pelvic tumours and minimal residual disease post surgery. These two groups could benefit from radiation.[44]

Surgical treatment

Before the 1970s surgery was the only treatment modality and most of the patients were treated with amputations. Since the introduction of chemotherapy, limb salvage therapy has become an option.

The primary goal of surgical management is to limit the local extent of the disease and to prevent metastases. The secondary goal is to restore function.

The question is whether these goals can be achieved successfully with limb salvage therapy instead of amputation. To understand the argument between limb salvage therapy versus amputation, the terms wide margin excision and radical excision should be explained. A **wide margin** is obtained if the reactive zone (the zone of potential infiltration) is not entered and a 'wide' cuff of normal tissue is excised with the whole tumour. The potential downfall of this excision is leaving residual tumour. A **radical margin** is obtained when the whole compartment involved (bone and/or myofascial tissue) is excised. If the tumour is in the distal femur it implies removing the whole femur and all involved muscle compartments. This is therefore only possible if the leg is amputated.

The history evolved very slowly towards limb salvage therapy. In 1980 an article by Campanacci and Laus[45] warned about the danger of recurrence in conservative tumour resection even in the cases of amputation. In the same year Campanacci *et al*[46] confirmed that chemotherapy changed the biological behaviour of most tumours but did not prevent local recurrence rates. Therefore radical (amputation) resections were still advocated.

In 1986 Simon *et al*[47] revolutionised surgical therapy with a multicentre study including 227 patients with OS of the distal femur. They concluded that although radical dissection (amputation) lowered the rate of recurrence, it did not improve survival. They proved that after more than five years limb salvage surgery was as safe as amputation in patients with high grade OS.

In 1988 Springfield *et al*[48] from Florida duplicated these results and also concluded that grade IIB osteosarcoma (extracompartmental high grade with no metastases) could be treated with a wide resection instead of amputation.

In the 90s Rougraff e*t al*[49] showed that, when compared to amputation, limb salvage therapy produced a better functional outcome without decreasing the rate of long-term survival. This finally settled the debate. These results eventually led to better surgical techniques and to the development of hardware to facilitate limb salvage surgery.

Limb salvage surgery

Once the diagnosis of OS is confirmed histologically, neo-adjuvant therapy can be started. The response of the primary tumour is evaluated. Limb salvage (*Table VIII*) can be considered if the follow-up imaging modalities show tumour shrinkage or a reduced inflammatory zone and a wide excision is viable.

Limb salvage can only be considered if there is no progression locally or distally and if blood vessels and nerves are free from tumour. The adjacent joint and growth plates are critically evaluated for involvement. The soft tissue cover is considered in order to allow 3–5 cm margins in bone and approximately 1 cm clean margins in soft tissue in order to achieve a wide resection.

Amputation

Amputation still remains an important surgical modality attaining excellent local control. Every patient is always considered for limb salvage therapy and amputation usually follows when limb salvage therapy is contraindicated.

Indications for amputation:

- The very young, where leg length discrepancy will be a problem. There is a school of thought which suggests that young children cope very well with an amputation.[50]
- Involvement of neuro-vascular bundle.
- Tumour progression on neo-adjuvant chemotherapy.
- Local recurrence or minimal tumour necrosis after neo-adjuvant chemotherapy in limb salvage therapy.

Table VIII: Limb salvage reconstructive options

- Arthrodesis
- Endoprosthesis with or without arthroplasty
- Fibula microvascular graft
- Endoprosthesis with allograft
- Expandable endoprosthesis
- Reconstruction prosthesis like scapular shoulder reconstruction and pelvic reconstruction
- External ring fixator bone transport systems

Pathological fractures do not necessarily warrant amputation and each case should be considered carefully since limb salvage therapy has been shown to be safe.[49]

The kind of amputation should be individualised for the patient. For distal femoral lesions it is unnecessary to do a hip disarticulation and an above-knee amputation is safe.[51]

Rotationplasty

Rotationplasty remains an alternative for an amputation in children with distal femoral lesions. Reconstruction in limb salvage surgery remains very difficult in the very young. Amputation is also problematic in the very young due to the short lever arm for prosthesis fitting. The principle of rotationplasty is to excise most of the distal femur in order to get a clear, tumour-free margin and to utilise the foot as a 'knee-joint' on which a prosthesis can be fitted. Rotationplasty serves as an excellent reconstructive procedure and is generally very well tolerated by children.[52]

Treatment protocol summary

1. Histological diagnosis with a biopsy.
2. Staging: A complete workup to establish the presence of metastases and the local extent of the tumour.
3. Pre-operative chemotherapy (neo-adjuvant). The response is often quantified by repeat MRI scan after completion of the neo-adjuvant chemotherapy course.
4. Surgical ablation of tumour either with limb salvage procedure or amputation. Unresectable tumours will be considered for radiotherapy.
5. Histological analysis of resected tumour for degree of necrosis.
6. If <90% necrosis, postoperative chemotherapy protocol is started. In the case of limb salvage therapy, amputation should be considered.
7. If >90% necrosis, the prognosis seems more favourable and the oncologist will consider chemotherapy.

Please note that pulmonary metastases often get treated primarily with a thoracotomy to enable early resection. Details of this protocol fall outside the extent of this discussion.

Prognostic factors

Many studies with conflicting results have been published on the prognosis. The most important prognosticator is the presence of metastasis at presentation. Survival rates for these patients vary between 10–20% survival after five years.[53] Patients with skip lesions or other bony metastasis do even worse.

The role of the orthopaedic surgeon is to recognise the disease early and to establish a histological diagnosis as soon as possible

The second prognosticator is the response of tumour to neo-adjuvant chemotherapy.[41,54] A good necrotic response usually predicts a long-term survival in up to 90% of patients.

Recent literature[25] suggests that tumour size is a good indicator of histological response to neo-adjuvant chemotherapy, and therefore a good pre-workup prognosticator.

Conclusion

Osteosarcoma is a solid bone tumour usually affecting the adolescent and young adult. Conventional OS is usually found around the knee. Current treatment protocols for high grade conventional OS without metastasis carries a five-year survival for up to 70% of patients. Many variants to the conventional OS can present in atypical ways, and although they are rare, they should be considered in even benign-looking lesions.

In the past five years good progress has been made in the understanding of limb salvage surgery and more functional and durable implants have been developed. However, although many exciting new discoveries are being made on the molecular pathogenesis of OS, not many drugs have been added to current regimens. The role of the orthopaedic surgeon is to recognise the disease early and to establish a histological diagnosis as soon as possible. With better knowledge of the disease, multi-drug chemotherapeutic regimens, proper surgical skills and better surgical hardware it has become possible to cure and salvage limbs, and therefore sustaining a good quality of life.

The content of this article is the sole work of the author. No benefits of any form have been derived from any commercial party related directly or indirectly to the subject of this article.

References

1. Gibbs CP, Weber K, Scarborough T. Malignant Bone Tumours; *J Bone Joint Surg* 2001;**83A**:1728-44.
2. Dahlin DC, Coventry MB. Osteosarcoma, a study of 600 cases. *J Bone Joint Surg* 1967;**49A**:101-10.
3. World LE, Unni KK, Beaubout JW, Sim FH, Dahlin DC. Dedifferentiated parosteal osteosarcoma. *J Bone Joint Surg* 1984;**66A**:53-9.
4. Sheth DS, Yasko AW, Raymond AK, Ayala AG, Carrasco CH, Benjamin RS, Jaffe N, Murray JA. Conventional and dedifferentiated parosteal osteosarcoma: Diagnosis, treatment and outcome. *Cancer* 1996;**78**(10):2136-45.
5. Okada K, Frassica FJ, Sim FH, Beabout JW, Bond JR, Unni KK. Parosteal osteosarcoma. A clinicopathological study. *J Bone Joint Surg [Am]* 1994;**76**:366-78.
6. Malkin D, Chilton-MacNeill S, Meister LA, *et al*. Tissue specific expression of SV40 in tumors associated with the Li-Fraumeni syndrome. *Oncogene* 2001;**20**:4441.
7. Carbone M, Rizzo Procopio A, *et al*. SV40-like sequences in human bone tumours. *Oncogene* 1996;**13**:527.
8. Porter DE, Holder ST, Steel CM, *et al*. A significant proportion of the patients with osteosarcoma may belong to the Li-Fraumeni cancer families. *J Bone Joint Surg [Br]* **74-B**:883-6.
9. Cumin I, Cohen JY, David A, *et al*. Rothmund-Thomson syndrome and osteosarcoma. *Med PediatrOncol* 1996;**26**:414.
10. Widhe B, Widhe T. Initial symptoms and clinical features in osteosarcoma and Ewing sarcoma. *J Bone Joint Surg [Am]* 200l;**82**:667-74.
11. Jaffe N, Spears R, Efthekari F, Robertson R, Congir A, Takaue Y, Carrasco H, Wallace S, Agala A, Raymond K, *et al*. Pathological fractures in osteosarcoma. Impact of chemotherapy on primary tumor and survival. *Cancer* 1987;**59**:701-9.
12. Bloem JL, Kroon HM. Osseous lesions. *Radiol Clin North Am* 1993;**31**:261-78.
13. Hudson TM, Enneking WF, *et al*. Magnetic resonance imaging of bone and soft tissue tumors: Early experience in 31 patients compared with computed tomography. *Skeletal Radiol* 1983;**10**:137-46.
14. Pass HI, Dwyer A, Makuch R, *et al*. Detection of pulmonary metastasis in patients with osteogenic osteosarcomas: The superiority of CT scans in comparison to conventional tomograms using dynamic analysis. *J Clin Oncol* 1985;**3**:1261-5.
15. Chew FS, Hudson TM. Radionuclide bone scanning of osteosarcoma: Falsely extended uptake patterns. *Am J Roentgenol* 1982;**139**:49-54.
16. Kole AC, Nieweg OE, van Ginkel RJ, *et al*. Detection of local recurrence of soft-tissue sarcoma with positron emission tomography using [18F]fluorodeoxyglucose. *Ann Surg Oncol* 1997;**4**:57-63.
17. Early JF, Mankoff DA. Tumor metabolic rates in sarcoma using FDG PET. *J Nucl Med* 1998;**39**:250-4.
18. Yamamoto T, Seino Y, Fukumoto H, *et al*. Over-expression of facilitative glucose transporter genes in human cancer. *Biochem Biophys Res Commun* 1990;**170**:223-30.
19. Panuel M, Gentet JC, Scheiner C, *et al*. Physeal and epiphyseal extent of primary malignant bone tumour in childhood: Correlation of preoperative MRI and the pathological examination. *Pediatr Radiol* 1993;**23**:421-4.
20. Dickson GR, Shirodria PV, Kanis JA, *et al*. Familial expansile osteolysis: a morphological, histomorphometric and serological study. *Bone* 1991;**12**:331-8.
21. Greenspan A, Remagen W. Differential diagnosis of tumors *and* tumor-like lesions of bones and joints. Lippincott-Raven & Williams 1998;59-117. Philadelphia
22. Unni KK, Dahlin DG, Beaubout JW. Periosteal osteogenic osteosarcoma. *Cancer* 1976;**37**:2476-85.
23. Enneking WF, Spanier SS, Goodman MA. Current concept review: Surgical staging of musculoskeletal sarcoma. *J Bone Joint Surg* 1982;**62A**:1027-30.

24. Greene FL, Page DL, Flemming FD: Editors. American Joint Committee on cancer: Cancer staging manual. 6th edition New York: Springer; 2002.
25. Kim MS, Lee S, Cho WH, Song WS, Koh J, Lee JA, Yoo JY, Jeon D. Initial tumor size predicts histological response and survival in localised osteosarcoma patients. *J Surg Oncol* 2008;**97**:456-61.
26. Campanacci M, Cervellati G. Osteosarcoma: A review of 345 cases. *Ital J Orthop Traumatol* 1975;**1**:5-22.
27. Dahlin DC. Osteosarcoma of bone and a consideration of prognostic variables. *Cancer Treat Rep* 1978;**62**:189.
28. Link MP, Goorin AM, Horowitz M, *et al*. Adjuvant chemotherapy of high grade osteosarcoma of the extremity: Updated results of the multi-institutional osteosarcoma study. *Clin Orthop Relat Res* 1991:**8**.
29. McKenna RJ, Schwinn CP, Soong KY, *et al*. Sarcomata of the osteogenic series (osteosarcoma, fibrosarcoma, chondrosarcoma, parosteal osteogenic sarcoma, and sarcoma arising in abnormal bone): An analysis of 522 cases. *J Bone Joint Surg [Am]* 1966;**48**:1-26.
30. Jaffe N, Frei E III, Traggis D, Bishop Y. Adjuvant methotrexate and citrovorum-factor treatment of osteogenic sarcoma. *Eng J Med* 1997;**291**(19):994-7.
31. Link MP, Goorin AM, Miser AW, *et al*. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N Engl J Med* 1986;**314**(25):1600-6.
32. Winkler K, Beron G, Delling G, Heise U, kabisch H, Purfurts C, Berger J, Ritter J, Jurgens H, Gerein V, *et al*. Neoadjuvant chemotherapy of osteosarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy on histological tumor response. *J Clin Oncol* 1988;**6**:329-37.
33. Huvos AG, Rosen G, Marcove RC. Primary osteogenic sarcoma: pathological aspects in 20 patients after treatment with chemotherapy, *en bloc* resection, and prosthetic bone replacement. *Arch Pathol Lab Med* 1977;**101**:14-8.
34. Anderson PM, Wiseman GA, Erlandson L. Gemcitabine radiosensitization after high dose samarium for osteoblastic osteosarcoma. *Clin Cancer Res* 2005;**11**(19 Pt1):6895-900.
35. Kindler HL. The pemetrexed/gemcitabine combination in pancreatic cancer. *Cancer* 2002;**95**(4 Suppl.):928-32.
36. Jeys LM, Grimer RJ, Carter SR, Tillman RM, Abudu A. Postoperative infection and increased survival in osteosarcoma patients: are they associated? *Ann Surg Oncol* 2007;**14**(10):2887-95.
37. Kleinerman ES, Snyder JS, Jaffe N. Influence of chemotherapy administration on monocyte activation by liposomal muramyl tripeptide phosphatidylethanolamine in children with osteosarcoma. *J Clin Oncol* 1991;**9**(2):259-67.
38. Meyer PA, Schwartz CL, Krailo MD, *et al*. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival – report from the Children's Oncology Group. *J Cancer* 2008;**26**(4):633-8.
39. Armitage JO. Emerging applications of recombinant human-granulocyte-macrophage colony-stimulating factor. *Blood* 1988;**92**(12):4491-508.
40. Kumar R, Yoneda J, Fidler IJ, Dong Z. GM-CSF-transduced B16 melanoma cells are highly susceptible to lysis by normal murine macrophages and poorly tumorigenic in immune-compromised mice. *J Leukoc Biol* 1999;**65**(1):102-8.
41. Siegel J, Pressey JG. Current concepts on the surgical and medical management of osteosarcoma. *Expert Rev Anticancer Ther* 2008;**8**(8):1257-69.
42. Gorlick R, Huvos AG, Heller G, *et al*. Expression of HER2/erB-2 correlates with survival in osteosarcoma. *J Clin Oncol* 1988;**17**(9):2781-8.
43. Muraro M, Mereuta OM, Carraro F, Madon E, Fagioli F. Osteosarcoma cell line growth inhibition by zoledronate-stimulated effector cells. *Cell Immuno* 2007;**249**(2):63-72.
44. Ozaki T, Flege S, Kevric M, *et al*. Osteosarcoma of the pelvis: experience of the Cooperative Osteosarcoma Study Group. *J Clin Oncol* 2003;**21**(2):334-41.
45. Campanacci M, Laus M. Local recurrence after amputation for osteosarcoma. *J Bone Joint Surg [Br]* 1980;**2-B**:201-7.
46. Campanacci M, Bacci G, Payani P, Giunti A. Multiple drug chemotherapy for the primary treatment of osteosarcoma of the extremities. *J Bone Joint Surg [Br]* 1980;**62-B**:93-101.
47. Simon MA, Aschliman MA, Thomas N, Mankin HJ. Limb-salvage treatment versus amputation for osteosarcoma of the distal end of the femur. *J Bone Joint Surg [Am]* 1986;**68**:1331-7.
48. Springfield DS, Schmidt R, Graham-Pole J, Marcus RB Jr, Spanier SS, Enneking WF. Surgical treatment of osteosarcoma. *J Bone Surg [Am]* 1980;**70**:1124-30.
49. Rougraff BT, Simon MA, Kreisl JS, Greenberg DB, Mankin HJ. Limb salvage compared with amputation for osteosarcoma of the distal end of the femur. A long term oncological, functional, and quality-of-life study. *J Bone Joint Surg [Am]* 1994;**76**:649-56.
50. Kagen LB. Use of denial in adolescents with bone cancer. *Health Soc Work* 1976;**1**:70-87.
51. Scully SP, Ghert MA, Zurakowski D, Thompson RC, Gebhardt MC. Pathological fractures in osteosarcoma: Prognostic importance and treatment implications. *J Bone Joint Surg [Am]* 2002;**84-A**:49-57.
52. Cammisa FP Jr, Glasser DB, Otis JC, *et al*. The van Nes tibial rotationplasty. A functionally viable reconstructive procedure in children who have a tumor of the distal end of the femur. *J Bone Joint Surg [Am]* 1990;**72**:1541-7.
53. Meyers PA, Heller G, Healey JH, Huvos A, Appelwhite A, Sun M, La Quaglia M. Osteogenic sarcoma with clinically detectable metastasis at initial presentation. *J Clin Oncol* 1993;**11**:449-53.
54. Davis AM, Bell RS, Goodwin PJ. Prognostic factors in osteosarcoma: A critical review. *Clin Oncol* 1994;**12**:423-31.