Thesis

Adult Osteosarcoma: a retrospective study of single center cohort database

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the medical University's regulations and procedures concerning plagiarism.

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Zusammenfassung

Einführung: Das Osteosarkom, als häufigster hochmaligner Knochentumor, weist eine bimodale Altersverteilung auf. Bei der Diagnosestellung befinden sich die meisten Patient:innen in ihrem zweiten und dritten Lebensjahrzehnt, mit einem zweiten Gipfel nach dem 40. Lebensjahr. Seit der Einführung der Polychemotherapie in den 1970er Jahren, zusätzlich zur radikalen Resektion, gab es in den letzten Jahrzehnten wenig Fortschritte bei den Therapiemöglichkeiten. Die 5-Jahres-Überlebensrate bleibt stabil bei etwa 60%.

Material und Methoden: 79 erwachsene Patient:innen mit Osteosarkom, die zwischen 2000 und 2020 im Universitätsklinik Graz, einem Referenzzentrum für Weichteil- und Knochentumore, diagnostiziert und behandelt wurden, wurden eingeschlossen. Für jeden Patient/jede Patientin wurden das Alter bei der Diagnose, das Geschlecht, die Krankengeschichte, das klinische Erscheinungsbild, radiologische Diagnostik, betroffene Seite, Lokalisation, Anzahl und Größe der Läsion(en), histologischer Typ, Tumor-Grad, Behandlungsstrategie, Entwicklung von Metastasen und Rezidiven, Nachbeobachtungszeit und das „Outcome“ erfasst. Diese Parameter wurden zwischen zwei Gruppen von Patient:innen, unter und über 50 Jahren, verglichen.

Ergebnisse: Von 79 Patient:innen waren 31 männlich und 48 weiblich. Das durchschnittliche Alter unserer Patient:innen betrug 45.8 Jahre (Spanne von 18 bis 93 Jahren). Zum Zeitpunkt der Diagnose waren 48 (60.8%) Patient:innen unter 50 Jahre alt, 31(39.2%) Patient:innen waren über 50 Jahre alt. Die Lokalisation betraf in 72 (91.1%) Fällen die Gliedmaßen und in 7 (8.9%) Fällen den Stamm. 56 (70.9%) Patient:innen hatten ein konventionelles Osteosarkom, elf (13.9%) hatten ein Parosteales Osteosarkom, sechs (7.6%) hatten ein *Low-Grade Central* Osteosarkom, zwei (2.5%) hatten *High-Grade Surface* Osteosarkome, und bei vier (5.1%) Patient:innen wurde ein Sekundäres Osteosarkom diagnostiziert. Die durchschnittliche Zeit von Symptombeginn bis zur Diagnosestellung betrug 5.3

Monate (Spanne von 0.5 bis 60 Monate); bei vier Tumoren handelte es sich um einen Zufallsbefund. 70 (88.6%) Patient:innen wurden operiert, 46 (58.2%) erhielten eine Chemotherapie, 35 (44.3%) erhielten das empfohlene Therapieschema mit neoadjuvanter und adjuvanter Chemotherapie zusätzlich zur radikalen Resektion. 38 (48.1%) Patient:innen entwickelten Metastasen, bei acht (10.1%) Patient:innen kam es zu einem lokalen Rezidiv. Die durchschnittliche Nachbeobachtungszeit betrug 50.7 Monate. 25 (31.6%) Patient:innen verstarben an der Krankheit, die durchschnittliche Zeit bis zum Tod betrug hierbei 32.2 Monate.

Fazit: Das hochgradige konventionelle Osteosarkom ist der häufigste Subtyp beim Erwachsenen und tritt meist in der Knieregion auf. Extremitäten-erhaltende Operationsverfahren mit anschließender Implantation einer Tumorendoprothese sind die Standardbehandlungsoption für Patient:innen jeden Alters, während der Einsatz von Chemotherapie, vor allem bei älteren Patient:innen, niedrig bleibt. Ältere Patient:innen entwickeln häufiger Metastasen, haben eine höhere Rezidivrate und die Zeit bis zum Tod ist kürzer als in der jüngeren Altersgruppe. Angesichts der zunehmenden Lebenserwartung in der allgemeinen Bevölkerung und damit einer wahrscheinlich höheren Inzidenz von Osteosarkomen bei älteren Menschen sind weitere Studien in der geriatrischen Bevölkerung mit Osteosarkomen hinsichtlich möglicher Behandlungsoptionen erforderlich.

Schlüsselwörter: Osteosarkom; Erwachsene; Ältere; Bösartige Knochentumore

Abstract

Introduction: Osteosarcoma, the most prevalent highly malignant tumor of bone, has a bimodal age distribution. At diagnosis, most patients are in their second and third decade of life with a second peak after the age of 40. After the invention of polychemotherapy regimes in addition to radical resection in the 1970s, no improvement regarding therapy modalities occurred in the last decades and the 5-year OS remains stable with around 60%.

Material and methods: 79 adult patients with osteosarcoma diagnosed and treated between 2000 and 2020 at the Medical University Hospital of Graz, a reference center for soft tissue and bone tumors, were included. For each patient we registered age, gender, medical history, clinical appearance, radiological findings (location, affected side, number and size of lesion(s), joint involvement), histological type, tumor grading, treatment, occurrence of metastasis, recurrence, follow-up time and outcomes. These parameters were compared with the published literature. In addition, the comparison between the patients under and above the age of 50 years was performed.

Results: Out of 79 patients, 31 were male and 48 were female. The median age was 45.8 years (range from 18 to 93 years). At time of diagnosis, 48 (60.8%) patients were under the age of 50 and 31 (39.2%) patients were above 50. Localization included appendicular skeleton in 72 cases and axial skeleton in 7 cases. 56 (70.9%) patients had conventional osteosarcoma, eleven (13,9%) parosteal osteosarcoma, 6 (7.6%) low grade-central osteosarcoma, 2 (2.5%) high-grade surface osteosarcoma and secondary osteosarcoma was diagnosed in 4 (5.1%) patients. The mean time from onset of symptoms until diagnosis was 5.3 months (range from 0.5 to 60 months); 4 tumors were an incidental finding. 70 (88.6%) patients underwent surgery, 46 (58.2%) received chemotherapy, 35 (44.3%) were treated with neoadjuvant and adjuvant chemotherapy in addition to radical resection. 38 (48.1%) patients developed metastasis; local recurrence was seen in eight

(10.1%) patients. Mean follow-up time was 50.7 months. 25 (31.6%) patients died of disease (mean time 32.2 months).

Conclusion: High grade conventional osteosarcoma is the most common subtype occurring in the adults, most frequently in the knee region. The limb-salvage surgery with implantation of tumor endoprosthesis is a standard treatment option for all patients, whereas the use of chemotherapy in the elderly patients remains low (especially in the group of patients over the age of 50). The older patients more commonly develop metastasis, have higher recurrence rate and time to death is shorter than in the younger age group. Considering increasing life span in the general population and therefore a probable higher incidence of osteosarcoma in the elderly, further studies in the geriatric population with osteosarcoma regarding possible treatment options are warrant.

Keywords: Osteosarcoma; Adults; Elderly; malignant bone tumor.

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1 Introduction

Osteosarcoma is the most common malignant primary bone tumor in children and adolescents with a second peak after the age of 40. The tumor cells directly form osteoid and mineralized bone tissue. Low- and high-grade intramedullary osteosarcomas, juxtacortical osteosarcomas, and extraskeletal osteosarcomas are distinguished based on their location and histological appearance, with the high-grade intramedullary form being the most common one, comprising 75% of all cases. High-grade central osteosarcomas can be further subdivided into various subtypes based on their histological differentiation pattern. (2-5)

The pathogenesis of osteosarcoma is unclear, but alterations in tumor suppressor genes like *RB* and *p53* have been described. Osteosarcomas can also occur secondarily after radiation or in patients with Paget's disease. (1, 6-8)

High-grade intramedullary osteosarcoma preferably occurs in the metaphysis of long tubular bones, especially the distal femur, proximal tibia, and proximal humerus. Patients are mostly diagnosed between the ages of 15 and 25, with males being more commonly affected. The tumors are characterized by rapid and invasive growth with early development of lung metastases. Before the introduction of aggressive chemotherapy, the 5-year survival rate was below 20%. After the 1970s, chemotherapy in osteosarcoma got introduced. Ever since then, the 5-year survival rate has expired up to 60-70%. Therefore, the most commonly applied treatment strategy consists of neoadjuvant chemotherapy, radical tumor resection, followed by postoperative chemotherapy cycles. (2-5, 9)

The therapeutic success is histologically assessed by determining the extent of vital tumor sections after neoadjuvant chemotherapy. Depending on the percentage of vital/necrotic areas in the tumor, tumors are classified into a six-stage regression grading scheme (“Salzer-Kuntschik regression grading”).

Due to the diverse differentiation possibilities of osteosarcomas, their diagnosis can be challenging. Depending on the expression of different basic substances and growth patterns, similarities exist with Ewing sarcoma, chondrosarcomas, aneurysmal bone cysts, and other lesions. (2-5)

2 Background

The human musculoskeletal system is composed of highly specialized body tissues including muscles, as the active component and bones, cartilages, joints and ligaments as the passive, stabilizing component. For further comprehension of pathological processes, it is important to outline the basic physiological elements of the skeletal system.

2.1 Bone

The adult human skeleton has 206 bones and can be divided into two major bone types - tubular and flat bones - depending on the skeletal sites and type of bone.
Bone is highly specialized and performs the following functions: stability, mechanics, protection and metabolism. Stability and mechanics allow body movements and a functional process flow. The protective feature provides safety to several vital organs such as heart, lungs, central nervous system and of bone marrow, responsible for sufficient immunological response and blood cell production. Metabolically, bone contains and stores several ions, primarily calcium and phosphorus. Bone is characterized as a tissue with high activity and remodeling processes as a response to physical, endocrine, and metabolic alterations while on the other hand its simplicity in terms of structure allows bone to recover/restore itself to its normal function and structure after fractures. Therefore, local cellular events as well as tissue factors, including cytokines, chemokines, ligands, matrix metalloproteinases and signalling transduction cascades, are important. (2, 10, 11)
Bones possess an outer compact cortex and an inner medullar space. The cortex, comprised of dense and solid compact bone, constitutes approximately 90% bone matrix and 10% space. Within compact bone, the spaces include vascular canals, osteocyte lacunae, and osteocyte canaliculi connecting the lacunae. This structural configuration empowers the cortex to withstand bending, torsion, and shear forces effectively. The medulla encompasses cancellous bone and marrow at the proximal and distal ends of long bones, along with fatty marrow within the shafts of the long bones. The spongy cancellous bone at the ends of long bones is composed of around 25% bone and 75% space. The predominant component of the cancellous

bone space is bone marrow (usually adipocytic or hematopoietic), dependent on location of the bone and patients' age. Cancellous bone exhibits an arrangement of highly perforated vertical plates interconnected by thinner horizontal struts, optimizing its ability to resist loading forces. In essence, bone adheres to the principle of maintaining the least mass necessary to provide maximal strength, exemplifying Wolff's law, where form follows function. (2, 9)

2.1.1 Definitions

Cortex: The dense outer section of a bone, also known as compacta.

Medulla: The inner part of a bone located inside the cortex. It may consist of marrow and fat in the midportions of long bones or a combination of marrow, fat, and coarse cancellous bone near the proximal/distal bone ends. Cancellous bone is occasionally referred to as "spongiosa".

Primary spongiosa: Cancellous bone comprising mixed spicules (trabeculae) made up of both bone and calcified cartilage.

Secondary spongiosa: Cancellous bone without residual cartilage.

Mature bone: Bone in which the collagen of the extracellular matrix is organized in layers or lamellae, known as lamellar bone.

Immature bone: Bone in which the collagen of the extracellular matrix is randomly arranged, referred to as woven bone. (9)

2.1.2 Topography and Anatomy of bone

Topographically bone can be divided into five categories: axial, trunk, appendicular, acral and craniofacial. These regions are relevant for diagnosing different bone tumors. Skull, spine, and sacrum are forming the central axial skeleton. All bones forming the extremities of the skeleton, including the scapula and pelvis, are categorized as an appendicular skeleton while all bones forming hands and feet are designated as the acral skeleton. The trunk region is referred to as ribs and sternum.

Further on, bones can be separated into the two main groups of flat and tubular bones. While the bones of the skull, sternum, scapula, clavicle and pelvis are categorized as flat, the bones of the extremities and the ribs are tubular. The tubular bones can be subclassified in long bones (femur, humerus, radius, etc.) and short bones (metatarsals, phalanges, etc.). A special subcategory of tubular bones are the epiphysioid bones, which in general are built like the epiphysis of long bones. Tarsal bones, carpal bones as well as the patella belong to this subcategory. (9, 12, 13)

2.1.2.1 Tubular bones

The epiphysis represents the rounded distal/ proximal ends of tubular bone, contributing to joint formation, and is characterized by articular cartilage. Adjacent to the epiphysis is the growth plate, a cartilaginous region responsible for longitudinal bone growth. The metaphysis is specifically identified as a narrow region directly adjacent to the cartilaginous growth plate (physis) where the primary spongiosa is initially created during the process of enchondral ossification. From a radiological perspective, the metaphysis is widely characterized as the area enclosed by the flaring cortex on the side of the shaft adjacent to the growth plate. This less precisely defined region proves to be diagnostically valuable due to the tendency of certain bone tumors to manifest in this location. The diaphysis comprises the elongated shaft and is a primary site for cortical bone formation. Understanding the topographical distinctions in long tubular bones is essential for comprehending bone development, growth, and pathological conditions. (2, 13-16)

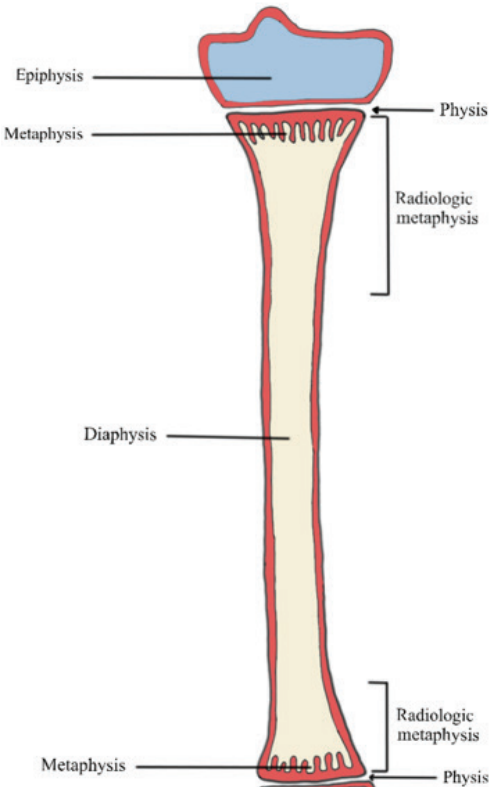


Figure 1: Anatomy of long bone. Image taken fi
Basicmedical Key, 2017 (cited 13.02.2024). (

2.1.2.2 Flat bones

Flat bones represent a distinct category of skeletal elements characterized by their flattened shape and protective function. They serve as shields for vital organs and provide extensive surface areas for muscle attachment. Structurally, flat bones consist of two thin outer layers of compact bone enclosing a layer of spongy or trabecular bone known as the diploe, containing red blood marrow as the vital site of haematopoiesis. Developmentally, flat bones originate from intramembranous ossification, where mesenchymal cells directly differentiate into osteoblasts, forming bone matrix. (2, 17)

2.1.3 Histology

Bone tissue consists of different cell types as well as mineralized extracellular matrix (ECM). Chemically, bone is composed of 45% minerals, 30% organic material and 25% water. (18) The main component are collagen fibrils of Type I and hydroxyapatite crystals, which is composed mainly by Calcium-, Phosphate- and Hydroxide-ions. The ECM is responsible for the bending strength of bone (compressive- as well as tensile strength). Collagen fibrils are arranged differently in immature and mature bone tissue. In immature tissue they are tightly interwoven (woven bone) while in mature bone, they are arranged in histologically visible layers (lamellar bone). Macroscopically bone appears in two different types: spongy bone and compact bone. Histologically both types are categorized as lamellar bone with the difference that in spongious bone, the lamellae are laminarly layered while in compact bone they are arranged in multiple concentrical lamellar systems, so called osteons or Harvers' systems.

Cells of bone tissue are bone lining cells, osteoblasts and their preliminary stages (capable on osteogenesis), osteocytes (built-in, stationary osteoblasts) and osteoclasts ("destructors" of bone). Bone tissue undergoes lifelong remodeling processes induced by all previously mentioned cell types.

All inner bone surfaces are covered by endosteum mostly composed by bone lining cells. On the outer surface, with exception of articular surfaces, bone is covered by periosteum, with an inner layer called stratum osteogenicum which is similarly

composed as the endosteum and an outer layer called stratum fibrosum mostly comprised of connective tissue and elastic fibers as a protectional layer. (12, 15, 18)

2.1.4 Osteogenesis and Longitudinal Growth

Bone tissue develops from consolidation of osteogenic and chondrogenic mesenchymal cells beginning from the 6th week of embryogenesis. Differentiation of bone can go two different ways: desmal osteogenesis and chondral osteogenesis. In desmal osteogenesis, mesenchymal cells differentiate directly from mesenchymal cells to osteoblasts, which are responsible for bone tissue production (ossification). Only a minority of bones such as scull and clavicula result from desmal osteogenesis which starts around the 8th embryonal week. (18)

The majority of ossification develops through chondral osteogenesis, in which mesenchymal cells differentiate into chondroblasts forming a chondrogenic version of the future bone tissue out of hyalin cartilage. The chondrogenic model of bone gets progressively replaced by bone. Depending on the origin of ossification, a distinction is made between perichondral and enchondral ossification. In perichondral ossification, osteoblasts from the perichondrium, which surround the cartilaginous bone model, produce osteoid, leading to thickening of the bone through circular deposition of osteoid. Enchondral ossification, on the other hand, results in lengthening of the bone in the epiphyseal region. Chondroclasts, arising from mesenchymal cells around the 5th-6th week of embryonic development, break down cartilage tissue, while osteoblasts contribute to bone growth. All long tubular bones and flat bones, except for the skull bones and clavicle, develop through endochondral ossification. Only in the epiphyses of long tubular bones enchondral ossification begins partially months to years after birth. The ossification of cartilage progresses slowly over the years, an essential prerequisite for postnatal longitudinal growth into late adolescence. (18, 19)

Longitudinal growth of tubular bones is based on proliferation of chondrocytes, which causes a synchronous move of both growth plates in direction of the epiphysis while they get simultaneously destroyed from within the medullary channel and replaced by bone tissue. The proliferation of chondrocytes reduces towards the epiphysis, while the ossification proliferates. That is why the growth plates do not

consolidate but rather move towards the middle of the diaphysis. Only when the chondrocytes cease proliferation, they get overtaken by ossification, the cartilaginous growth plates get consumed, and entirely replaced by bone, the growth plates close, and longitudinal growth stops. (12, 18)

2.1.5 Modeling/Remodeling

Bone modeling is a dynamic process that adjusts bone size, shape, and strength during development, responding to physiological and mechanical influences. At cellular level, osteoblasts work independently to deposit more matrix than osteoclasts can resorb. Longitudinal and radial growth occur through collagen synthesis, enhancing bone strength by increasing cross-sectional area and placing material farther from the bone centre. Bone formation is happening most rapid during adolescence, influenced by both, non-modifiable (gender, genetics) and modifiable (nutrition, hormones) factors. Although peak bone mass is achieved in the first three decades, bone strength can still increase through remodeling, a continual process replacing old bone with new to maintain structural integrity. Remodeling is a complex, homeostatic process influenced by stochastic and deterministic mechanisms, ensuring a balanced bone structure between excess mass and fragility. Unlike modeling, remodeling involves coordinated cellular responses between osteoclasts and osteoblasts, occurring through basic multicellular units and varying in cortical and trabecular bone. Approximately 3-5% of cortical and 25-28% of trabecular bone is remodeled annually, completely regenerating the adult skeleton every 10 years. (15, 17, 18)

2.2 Bone tumors

Bone tumors can be divided in primary and secondary neoplasms. Primary bone tumors are rare and present with a wide spectrum of morphology. Their biological behaviour range from benign to malignant and they are classified according to their tissue of origin. (4, 5)

Secondary bone tumors, also known as metastatic bone tumors, are a result of the metastasis or spread of cancer cells from their primary location, most frequently including breast, lung, prostate, kidney, and thyroid. (20)

2.2.1 Classification

The WHO classifies bone tumors based on histological and/or genetic types. It includes different groups of tumors: osteogenic, chondrogenic, fibrogenic, vascular, osteoclastic giant cell-rich, notochordal, other mesenchymal and hematopoietic tumors of bone. A comprehensive overview of all bone tumors including morphology codes from the International Classification of Disease for Oncology (ICD-O) is summarized in *Table 1*. In ICD-O the behaviour of the neoplasm is coded /0 for benign tumors, /1 for unspecified, uncertain behaviour or borderline, /2 for carcinoma in situ and grade III intraepithelial neoplasia and /3 for malignant tumors in primary site. (4, 5)

Chondrogenic tumors	ICD-O
Benign	
Subungual exostosis	9213/0
Bizarre parosteal osteochondromatous proliferation	9212/0
Periosteal chondroma	9221/0
Enchondroma	9220/0
Osteochondroma	9210/0
Chondroblastoma NOS	9230/0
Chondromyxoid fibroma	9241/0
Osteochondromyxoma	9211/0
Intermediate (locally aggressive)	
Chondromatosis NOS	9220/1
Atypical cartilaginous tumor	9222/1
Malignant	
Chondrosarcoma, grade 1	9222/3
Chondrosarcoma, grade 2	9220/3
Chondrosarcoma, grade 3	9220/3
Periosteal chondrosarcoma	9221/3
Clear cell chondrosarcoma	9242/3
Mesenchymal chondrosarcoma	9240/3
Dedifferentiated chondrosarcoma	9243/3
Osteogenic tumors	
Benign	
Osteoma NOS	9180/0
Osteoid osteoma NOS	9191/0
Intermediate (locally aggressive)	
Osteoblastoma NOS	9200/1
Malignant	
Low-grade central osteosarcoma	9187/3
Osteosarcoma NOS	9180/3
Conventional osteosarcoma	
Telangiectatic osteosarcoma	
Small cell osteosarcoma	
Parosteal osteosarcoma	9192/3
Periosteal osteosarcoma	9193/3
High-grade surface osteosarcoma	9194/3
Secondary osteosarcoma	9184/3
Fibrogenic tumor	
Intermediate (locally aggressive)	
Desmoplastic fibroma	8823/1
Malignant	
Fibrosarcoma	8810/3
Vascular tumors of bone	
Benign	
Haemangioma NOS	9120/0
Intermediate (locally aggressive)	
Epitheloid haemangioma	9125/0
Malignant	
Epitheloid haemangioendothelioma NOS	9133/3
Angiosarcoma	9120/3
Osteoclastic giant cell-rich tumors	
Benign	
Aneurysmal bone cyst	9260/0
Non-ossifying fibroma	8830/0
Intermediate (locally aggressive, rarely metastasizing)	
Giant cell tumor of bone NOS	9250/1
Malignant	
Giant cell tumor of bone, malignant	9250/3
Notochordal tumors	
	ICD-O
Benign	
Benign notochordal tumor	9370/0
Malignant	
Chordoma NOS	9370/3
Chondroid chordoma	
Poorly differentiated chordoma	9370/3
Dedifferentiated chordoma	9372/3
Other mesenchymal tumors of bone	
Benign	
Chondromesenchymal hamartoma of chest wall	
Simple bone cyst	
Fibrous dysplasia	8818/0
Osteofibrous dysplasia	
Lipoma NOS	8850/0
Hibernoma	8880/0
Intermediate (locally aggressive)	
Osteofibrous dysplasia-like adamantinoma	9261/1
Mesenchymoma NOS	8990/1
Malignant	
Adamantinoma of long bones	9261/3
Dedifferentiated adamantinoma	
Leiomyosarcoma NOS	8890/3
Pleomorphic sarcoma, undifferentiated	8802/3
Bone metastases	
Haematopoietic neoplasms of bone	
Plasmacytoma of bone	9731/3
Malignant lymphoma, non-Hodgkin, NOS	9591/3
Hodgkin disease NOS	9650/3
Diffuse large B-cell lymphoma NOS	9680/3
Follicular lymphoma NOS	9690/3
Marginal zone B- cell lymphoma NOS	9699/3
T- cell lymphoma NOS	9702/3
Anaplastic large cell lymphoma NOS	9714/3
Malignant lymphoma, lymphoblastic, NOS	9727/3
Burkitt lymphoma NOS	9687/3
Langerhans cell histiocytosis	9751/1
Langerhans cell histiocytosis, disseminated	9751/3
Erdheim- Chester disease	9749/3
Rosai-Dorfman disease	

Table 1: WHO classification of bone tumors including morphology codes from International Classification of Diseases for Oncology, third edition, second revision (ICD-O-3.2) (4, 5)

2.2.2 Grading and Staging

Various staging and grading systems have been invented, all aimed at categorizing tumors based on prognostic factors including tumor size, compartmentalization, histologic grade, and the absence or presence of metastasis.

At present, there are two staging systems applied to evaluate the clinicopathologic parameters of bone tumors. The initial system is the well-known tumor (T), node (N), metastasis (M), short TNM, staging system invented by the American Joint Committee on Cancer, which got approved by the International Union Against Cancer in 1987. The second system, invented by Enneking et al. in 1986, was also approved by the American Joint Committee Task Force on Bone Tumors. (2)

Recognizing and evaluating bone tumors can be challenging, even for experienced clinicians, radiologists, or pathologists. Consequently, it is recommended, to refer patients with suspected bone neoplasm to specialized bone sarcoma centres for comprehensive treatment. The utilization of grading and staging is crucial not only for predicting the biological behaviour but also for evaluating prognosis, making them essential pieces of information before treatment. (21, 22)

2.2.2.1 Grading

Malignant bone tumors have a wide variety in their biological behaviour and histological type and grading are still the best parameters for the approximate prediction of prognosis. (4, 5) The grading process relies on histological characteristics and is closely linked to the prognosis. Key criteria for grading include evaluating the relative proportion of cells to matrix, nuclear contours, nuclear enlargement and hyperchromasia. Additionally, features like mitotic figures and necrosis are considered and their presence correlates with the grade.

The clinical behaviour and, consequently, the grade in bone sarcomas are frequently influenced by the histological subtype. As shown in *Table 2*, Ewing sarcoma (ES), mesenchymal chondrosarcoma and dedifferentiated chondrosarcoma are always classified high-grade while parosteal osteosarcoma and clear cell osteosarcoma are considered low-grade neoplasm. (4, 5)

Grade	Sarcoma type
Grade 1 (low-grade)	Low-grade central osteosarcoma Parosteal osteosarcoma Clear cell chondrosarcoma
Grade 2 (intermediate-grade)	Periosteal osteosarcoma
Grade 3 (high-grade)	Osteosarcoma (conventional, telangiectatic, small cell, secondary, high-grade surface) Undifferentiated high-grade pleomorphic sarcoma Ewing sarcoma Dedifferentiated chondrosarcoma Mesenchymal chondrosarcoma Dedifferentiated chordoma Poorly dedifferentiated chordoma Angiosarcoma
Variable grading	Conventional chondrosarcoma (grade 1-3) Leiomyosarcoma of bone (grade 1-3, no established grading system) Malignant giant cell tumor of bone

Table 2: Bone sarcomas in which grade is determined by histological type. (4, 5)

Figure 2 shows a microscopic picture of a conventional osteoblastic high grade (G3) osteosarcoma with typical signs of malignancy such as irregular hyperchromatic and enlarged nuclei and abnormal mitotic activity. High-grade tumors (G2, G3 in a three-grade system) are associated with unfavourable prognoses, requiring differentiation from low-grade tumors (G1 in a three-grade system) with better prognoses. (4, 5, 22, 23) It is important to note that the oncological and surgical grading scale may differ from histopathologic grading, leading to variations in the grading classification.

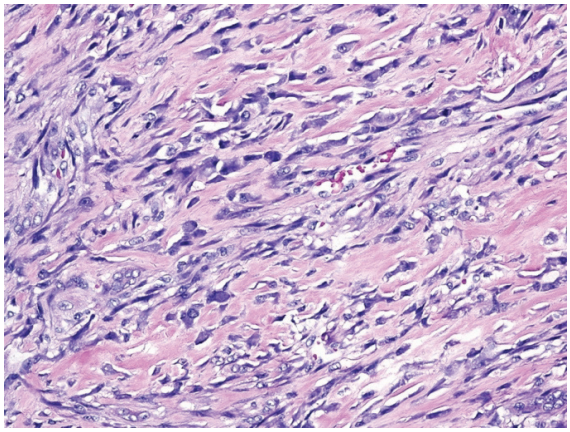


Figure 2: Microscopic pathology of a conventional osteoblastic high grade (G3) osteosarcoma.

2.2.2.2 Staging

The following rules of classification (see *Table 3*) apply to all primary malignant tumors of bone except multiple myeloma, malignant lymphoma, surface/juxtacortical osteosarcoma and juxtacortical chondrosarcoma. The following TNM categories get determined by physical examination and imaging. A histological confirmation and division by histological types and grading should always be given. (4, 5)

A difference can be made between *Clinical TNM* (cTNM) classification and *Pathological TNM* (pTNM) classification.

TNM staging in tumors of bone

cTNM - Clinical Classification

T – Primary Tumor

TX – primary tumor cannot be assessed

T1 – No evidence of primary tumor

Appendicular skeleton

T1 – Tumor 8cm or less in greatest dimension

T2 – Tumor over 8cm in greatest dimension

T3 – Discontinuous tumor in the primary bone site

Spine

T1 – Tumor confined to a single vertebral segment or two adjacent vertebral segments

T2 – Tumor adjacent to three adjacent vertebral segments

T3 - Tumor adjacent to four adjacent vertebral segments

T4a – Tumor invades into the spine canal

T4b – Tumor invades the adjacent vessels or tumor thrombosis within the adjacent vessels

(The five vertebral segments are: Right pedicle, right body, left body, left pedicle, posterior element)

Pelvis

T1a – A tumor 8cm or less in size and confined to a single pelvic segment with no extraosseous extension

T1b - A tumor greater than 8cm in size and confined to a single pelvic segment with no extraosseous extension

T2a - A tumor 8cm or less in size and confined to a single pelvic segment with extraosseous extension or confined to two adjacent pelvic segments without extraosseous extension

T2b - A tumor greater than 8cm in size and confined to a single pelvic segment with extraosseous extension or confined to two adjacent pelvic segments without extraosseous extension.

T3a - A tumor 8cm or less in size and confined to a two pelvic segment with extraosseous extension

T3b - A tumor greater than 8cm in size and confined to a single pelvic segment with extraosseous extension

T4a – Tumor involving three adjacent pelvic segments or crossing the sacroiliac joint to the sacral neuroforamen

T4b – Tumor encasing the external iliac vessels or gross tumor thrombus in major pelvic vessels

(The four pelvic segments are: Sacrum lateral to the sacrum foramen, Iliac wing, Acetabulum/periacetabular, pelvic rami/symphysis/ischium)

N – Regional Lymph Nodes

NX – regional lymph nodes cannot be assessed

N0 – No regional lymph node metastasis

N1 – regional lymph node metastasis

M – Distant Metastasis

M0 – no distant metastasis

M1 – distant metastasis

M1a – Lung

M1b – Other distant sites

pTNM – Pathological Classification

The pT and pN categories correspond to the clinical T and N categories.

pM – Distant metastasis

pM1 – distant metastasis microscopically confirmed

Table 3: TNM classification of malignant tumors, eight edition, 2017. (4, 5)

The regional lymph nodes are regarded according to the site of the primary tumor. Nevertheless, involvement of regional lymph nodes is rare. In cases where nodal status is not assessed either clinically or pathologically, the regional node status should be considered N0 rather than NX. (2, 4, 5)

The actual edition (eight edition, 2020) of the TNM system encloses a differentiated T classification for appendicular skeleton, spine, and pelvis.

Combining the TNM system with the histological grade, malignant bone tumors can be staged. However, the staging in Table 4 can only be applied to lesions of the appendicular skeleton, trunk, facial bones and skull. There are no staging modalities for bone sarcomas of the spine or pelvis. Staging in these tumors is not possible. (4, 5)

Stage	T	N	M	Grade
Stage IA	T1	N0	M0	G1, GX Low Grade
Stage IB	T2/T3	N0	M0	G1, GX Low Grade
Stage IIA	T1	N0	M0	G2, G3 High Grade
Stage IIB	T2	N0	M0	G2, G3 High Grade
Stage III	T3	N0	M0	G2, G3 High Grade
Stage IVA	Any T	N0	M1a	Any G
Stage IVB	Any T	N1	Any M	Any G
	Any T	Any N	M1b	Any G

Table 4: Staging according to TNM classification and grading by histological type.(4, 5)

In 1986, Enneking et al. described a staging system for tumors of the musculoskeletal system (see *Table 5*), which has been adopted by the Musculoskeletal Tumour Society (MSTS) and gained acceptance especially by orthopaedic surgeons because of its ability to make therapeutic decisions. Besides the grade and the presence of metastasis, it provides information about the extent beyond the surgical compartment (intra-/intercompartmental). (4, 5, 24)

Stage	Grade	Site	Metastasis
IA	Low (G1)	Intracompartmental (T1)	None (M0)
IB	Low (G1)	Extracompartmental (T2)	None (M0)
IIA	High (G2/G3)	Intracompartmental (T1)	None (M0)
IIB	High (G2/G3)	Extracompartmental (T2)	None (M0)
III	Any	Any	Regional or distant (M1)

Table 5: Enneking et al. staging for malignant bone tumors in 1986. Modified Version for staging from the MSTS given in brackets. (2, 4, 5, 24)

For better comprehension Musculoskeletal Tumor Society (MSTS) designed a modified version of the Enneking et al. staging for malignant tumors. TNM equivalents given in brackets in *Table 5*. A detailed categorization of intracompartmental (T1) and extracomopartmental (T2), giving information of anatomical extent is shown in *Table 6*.

Intracompartmental (T1)	Extracompartmental (T2)
Intraarticular	Soft tissue extension
Superficial to deep fascia	Deep fascial extension
Paraosseous	Intraosseous or extrafascial extension
Intrafascial compartment	Extrafascial compartment

Table 6: Definition of anatomical extension by the Musculoskeletal Tumor Society (MSTS) according to the Enneking et al. staging system. (2)

2.2.3 Epidemiology

The true incidence of benign bone lesions is unknown. As a result of higher sensitivity in radiological diagnostics the incidence of benign and low-grade cartilaginous tumors has increased. (2, 5) In comparison, malignant bone tumors, with a rate of only 0.2% of all neoplasms are rare. Bone sarcomas exhibit a

significantly lower incidence compared to soft tissue sarcomas, with only one tenth of the incidence rate of soft tissue sarcomas. The annual incidence rate for bone sarcomas remained relatively stable over the last decades with around 0.75 cases per 100.000 population in both sexes. (4, 5)

Osteosarcoma is the most diagnosed primary sarcoma (35.7%), followed by chondrosarcoma (27.3%), ES (13.5%), and chordoma (7.7%).

The age-specific incidence rates for bone sarcomas exhibit a bimodal pattern, peaking in the second decade of life and in patients older than 60 years, unlike the gradual increase seen in soft tissue sarcomas with age. (2, 25) The second age peak is related mostly to risk factors such as st. post. radiation or Paget's disease. ES occurs less frequent than osteosarcoma and most patients get diagnosed under the age of 30. The incidence rates of chordoma and chondrosarcoma increase from adolescence onwards. (4, 5, 26)

2.2.4 Aetiology

Most malignancies of bone seem to occur de novo, although it is progressively markable that some arise in association with diseased bone or benign precursor lesions. The most clearly established precancerous conditions are Paget's disease of bone, chronic osteomyelitis, radiation injury, bone infarction as well as some benign tumors, like osteochondroma. Also cases of bone sarcoma arisen in association with metal implants have been reported. (4, 5)

Genetic variants with high risk on developing bone tumors are rare but nonetheless important to identify as they can have an impact in response to treatment or can predict increased risk for developing other tumor types. Relevant examples therefor are mutations in *TP53*, associated with Li-Fraumeni syndrome, and *RB1*, which both have a predisposition to develop osteosarcoma.

Non-inherited, postcygotic mutations lead to mosaicism and can raise the likelihood of tumor development in specific, affected regions of the human body. For instance, mutations in *IDH1* and *IDH2* contributing to enchondromatosis, while *GNAS* mutations are associated with polyostotic fibrous dysplasia, Mazabraud syndrome and McCune-Albright syndrome. (4, 5)

2.2.5 Clinics

Clinically, bone tumors present with a wide range of symptoms, including swelling, pain, pathological fractures, or neurovascular impact. Sometimes they can be asymptomatic and found as an incidental finding. (4, 5, 21)

In benign lesions, clinical symptoms are often unspecific, swelling and tenderness over the affected bone as well as intermittent aching pain are the most common findings. Examples for benign tumors with specific symptoms are e.g. osteoid osteoma with night pain relieving under anti-inflammatory medication, osteochondroma which presents as a painless lump whereas chondroblastoma causes severe pain. In general, periarticular lesions often affect the joint and present with joint-stiffness and limited range of motion. (4, 5, 27)

Malignant tumors of bone show typical symptoms like non-mechanically influenced worsening pain and night pain interrupting sleep. Swelling usually starts to show as soon as the tumor cells spread from inside the cortex and starts to grow beneath the periosteum. (4, 5, 21) Patients with limb-swelling, especially in the knee-region combined with non-mechanical or night pain should always be considered as malignancy suspect and further investigated.

However, we have to bear in mind, that there is a broad spectrum of differential diagnosis for patients presenting with the symptoms mentioned above such as malignant bone tumors, metastasis of other primary malignant tumors, benign lesions of bone, osseous infections, and haematological disorders. (4, 5, 9)

2.2.6 Diagnostics

Patients with suspect lesions of bone should be transferred to a reference centre for bone and soft tissue tumors and be discussed in a multidisciplinary team of radiologists and pathologists in terms of diagnosis as well as orthopaedic surgeons, radiation therapists and oncologists involved in finding the best possible treatment options. (2-5)

2.2.6.1 Primary diagnostics

In the initial phase of the diagnostic process, a comprehensive evaluation of the patient's medical history is essential, encompassing the onset, nature, and duration of symptoms. Gathering information about previous illnesses, familial

predispositions, unintended weight loss, pallor, diminished performance, and night sweats can contribute to the diagnosis of bone tumors. Subsequently, a thorough clinical examination involving inspection, palpation, and assessment of the relationship to the surrounding area of the suspected tumor, should be conducted. During the inspection, symptoms such as swelling, redness, visible vascular markings, or changes in skin color (e.g., livid or ulcerated) should be carefully observed. The measurable size of the tumor mass needs to be documented in centimeters. Palpation should include an evaluation of potential limitations in movement, intra-articular effusions, or sensory-motor deficiencies. Additionally, a comprehensive examination of the entire body, not just the affected region, is crucial. Certain symptoms, if detected, could contribute to the diagnosis, such as café-au-lait spots indicative of neurofibromatosis. While there are no specific screening tests for musculoskeletal tumors, certain general blood parameters can be valuable in the diagnostic process, including red and white blood cell counts, C-reactive protein (CRP), lactate dehydrogenase (LDH), alkaline phosphatase (AP), calcium levels, among others. (4, 5, 28, 29)

2.2.6.2 Radiology

If there is a suspicion of a bone tumor after clinical examination, first of all, conventional radiographs in two planes (X-rays with anterior-posterior projection and a lateral radiograph) are performed. Some tumors exhibit imaging features so characteristic that, for the experienced Musculo-skeletal radiologist, the diagnosis is nearly certain even in the absence of a biopsy. (2, 9) Parameters to determine the behavior of the tumor are anatomical location, size, composition of matrix, pattern of bone destruction and periosteal reaction. Certain bone tumors exhibit a predilection for specific bones and anatomical regions. For instance, osteosarcomas most commonly manifest in the distal femur, followed by the proximal tibia. The occurrence of bone tumors is often eccentric, primarily affecting the long bones of the extremities. Patient age plays a critical role, with notable age-dependent variations in prevalence. Osteosarcomas (35%) and ES (16%) are prevalent in individuals aged five to fifteen years, while bone tumors in the elderly are frequently metastatic. (4, 5, 9) Radiological assessment of tumor size can offer insights into its nature. Smaller dimensions (<5 cm) are suggestive of benign tumors, while larger

dimensions can be either benign or malignant. A rapid and substantial increase in tumor size over a short duration raises suspicions of malignancy. The matrix type in bone tumors presents radiological distinctions that may indicate specific diagnoses. Chondroblastic osteosarcoma, with mineralized and non-mineralized chondroid matrix, appears white, while osteoblastic osteosarcoma exhibits a more granular structure. Most tumors appear radiolucent, such as lytic or mixed osteosarcomas, while others may appear sclerotic or calcified. (22, 30)

Radiological patterns of bone destruction provide insights into tumor aggressiveness. The geographic pattern (Type 1) involves lytic areas and is further categorized into subtypes from 1A to 1C based on the presence or absence of sclerosis. Type 1 exhibits a rim of sclerosis between lytic areas, Type 1B shows no signs of sclerosis but rather a clear separation from normal bone structure whereas Type 1C is characterized by a less clear separation from normal bone. Type 2, the moth-eaten pattern, features multiple holes in the bone and is more aggressive than Type 1. Type 3, the permeative pattern, is characterized by rapid progression and diffuse lytic areas, representing the most aggressive pattern. These patterns are observed in malignant tumors like ES or osteosarcoma, as well as in some benign conditions (such as osteomyelitis). (22, 31)

The radiological pattern of periosteal formation reflects tumor growth rates. Slowly growing tumors result in a thick layer of bone, while fast and slow growth may lead to the formation of multiple periosteal layers. Features such as extension through the cortex, perpendicular formations, and rapid growth suggest malignancy. The Codman triangle, a disruption of the periosteum during growth, is a commonly described radiological sign of periosteal reaction, observed most frequently in malignant tumors (4, 5, 21, 22, 29).

Basically, two groups of periosteal reactions can be distinguished. On one hand, continuous reactions are observed, typically associated with slow to moderately paced bone processes. On the other hand, interrupted periosteal reactions are usually indicative of accompanying processes in highly aggressive events. Within the continuous reaction category, two subtypes are further differentiated: periosteal reactions with simultaneously obliterated compact bone and periosteal reactions with completely or at least partially preserved compact bone (see *Figure 3*). (32)

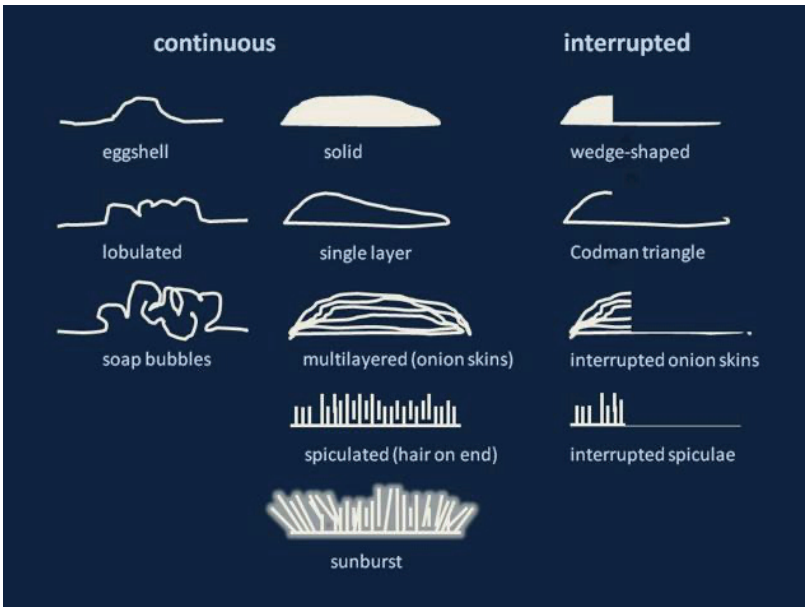


Figure 3: Overview on continuous and interrupted periosteal reaction in courtesy of Dr. Roberto Schubert. Image taken from Radiologe. 1999; 39(10):910-20. (32)

Additionally, attention should be given to the interface between the tumor and its surrounding area. Poorly defined lesions with a broad transition zone are indicative of a likely malignant tumor. A characteristic radiological sign for osteosarcoma is ossification of surrounding tissues, referred to as a "sunburst pattern," although it is non-specific. Conversely, a sclerotic rim often appears in slow-growing, likely benign tumors. (4, 5, 22, 30)

Despite numerous radiological indicators of malignancy, additional imaging techniques are essential to assess the tumor's extent within the bone and surrounding areas. Magnetic Resonance Imaging (MRI) is crucial for evaluating intraosseous extension, extension to muscles, important neurovascular structures, subcutaneous fat, and involved joints. Staging is primarily based on MRI due to its accuracy, high tissue contrast without radiation, and the ability to generate three-dimensional images. MRI can detect skip lesions or local metastases. Therefore, making a contrast-enhanced MRI is mandatory before definitive surgery. (4, 5, 9, 21, 22, 29, 33) Usually, a bright appearance on a T1-weighted image indicates protein-rich fluid like blood, fat, or, in some cases, calcifications. Conversely, if a lesion appears dark on T1-weighted images but bright on T2-weighted images, it suggests higher water content, as seen in edema, hemorrhage, or inflammation. A dark appearance on a T2-weighted image typically indicates the presence of fibrous

tissue, hemosiderin, or melanin. Therefore, in some cases MRI can also contribute to differential diagnosis such as aneurysmal bone cysts or telangiectatic osteosarcoma. (9)

Computerized tomography (CT) of the affected bone is employed to assess size, periosteal reaction, destruction patterns, ossification processes, and soft tissue involvement. However, CT is not the preferred method due to its high radiation impact. CT is valuable for visualizing cortical bone structures and detecting metastases, particularly in the chest, which may appear as calcified nodules. (28, 29) A radionuclide bone scan, using technetium 99m, aids in visualizing the extent of the primary tumor and identifying skip lesions. Local uptake provides information about the tumor's biological activity but not its malignancy. Other imaging techniques, such as positron emission tomography (PET), angiography, and whole-body MRI, are still undergoing evaluation. (4, 5, 21, 22, 30, 33, 34)

Considering the potential serious implications of treatment or occasional misdiagnosis based on imaging alone, a histologic diagnosis becomes necessary, especially in malignant diseases. In many non-neoplastic diseases, imaging studies are often sufficient for clinical diagnosis and their treatment. (9)

2.2.7 Processing of bone specimen

Bone specimen submitted for pathological assessment requires specific procedures. Bone specimens can be categorized into following major groups:

- Intraoperative biopsy
- Diagnostic or therapeutic biopsies and curetting
- Resection specimen. (2)

The definitive diagnosis of a bone tumor relies on a correlation of histological and radiological findings. Biopsies should be taken from the suspected site identified by radiology. The attending surgeon, preferably an orthopaedic oncologist or experienced musculoskeletal radiologist should perform the biopsy. (2, 4, 5, 21, 22, 28, 29, 35) Biopsies should minimize contamination of normal tissue, especially in the context of planned limb salvage surgery. Failure to do so might result in local recurrences, eventually resulting in an amputation of the limb. Thus, precise imaging - including X-rays in two planes, MRI and/or CT, radionuclide bone scan, or PET - and preoperative surgery planning are mandatory before performing a biopsy.

Histological specimens can be obtained through open (surgical, excisional, or incisional) or closed (percutaneous, e.g., core needle) biopsy methods. (21, 22, 28) Closed biopsy techniques, such as core needle biopsy (CNB) or fine needle aspiration biopsy (FNAB) (nowadays extremely rarely used), are performed under sterile conditions using 22-25-gauge needles, generally under local or general anesthesia. CNB is most common, faster, simpler, and more cost-effective than open biopsy (29).

However, the latter can be useful in heterogenous tumors and for acquiring more material for analysis. Proper documentation and marking of the biopsy canal are crucial in closed biopsies, as the biopsy tract is always considered contaminated and must be resected in cases of malignancy. The decision between open and closed techniques should be made individually, with the operability of open biopsy depending on the location and local expertise. In open biopsies, a small longitudinal incision is made to minimize contamination of surrounding tissues. The advantage of open biopsy lies in obtaining a representative amount of tissue, especially in cases where histology and clinical presentation correlation is inconclusive. Key considerations for successful biopsies include avoiding haste, preventing contamination of surrounding structures, conducting operations only after preoperative imaging, anticipating future treatment considerations, obtaining sufficient tissue, avoiding hematoma formation, and employing a drain from the biopsy tract. (28, 29) Excision biopsy is only indicated for clearly benign tumors; otherwise, it is contraindicated due to the inability to adhere to the adequate oncologic-surgical margin.

Following a biopsy, specimens should be fixed in 10% neutral buffered formalin or snap-frozen at -70°C for future studies or, in case of surgery, for determining the resection margin. Intraoperative diagnosis usually used to evaluate the acquired tissue, typically relies on frozen sections stained with hematoxylin-eosin. Gross specimens designated for frozen sections has to be carefully evaluated to identify heavily mineralized tissue, such as cortical bone fragments. It is necessary to exclude these fragments from the specimen since they cannot undergo sectioning without prior decalcification. Conversely, most bone tumors can be sectioned without pre-decalcification, even in the presence of matrix mineralization. When

planning intraoperative frozen sections, it is advisable to select a less mineralized (softer) portion of the lesion for the biopsy specimen. (2)

Decalcification with acid solutions used to be the routine method for fixing specimens; (4, 5, 21, 22) A method used nowadays, in accordance with the guidelines of European Society for Medical Oncology (ESMO), involves decalcifying material in ethylenediaminetetraacetic acid (EDTA) rather than harsher acidic reagents (especially if material should undergo molecular analysis). (4, 5, 21)

Unfixed specimens must be delivered to the laboratory immediately, at least within half an hour. Regardless of fixation status, every specimen must be interpreted by an experienced pathologist, who requires additional clinical information (see *Table 7*) to classify the tumor according to the latest WHO criteria (see *Table 3*). The final classification of every bone tumor should be made according to the current WHO classification and utilizing ICD-O codes. (4, 5, 21, 22)

Clinical features associated with bone tumors	Age, Sex, Racial background of the patient
	Anatomical location
	Nature and duration of symptoms (pain, swelling, previous trauma)
	Pre-existing and concomitant skeletal disease, family history
	Occupational and treatment history, systemic disease, test results

Table 7: Clinical features relevant for pathologists in diagnosis of tumors of bone. (22)

2.2.8 Localisation

Bone tumors usually have predilection sites for certain bones and characteristic locations within individual bones, which can provide information about the nature of the lesion. *Figure 4* illustrates the favored sites of common bone tumors, offering diagnostic clues based on location. Following examples show predilected spots for different tumors: tumors on the posterior surface of the distal femur with ossified tissue are likely for parosteal osteosarcoma. Adamantinoma and osteofibrous dysplasia predominantly affect the fibula or tibia. Chondroblastoma is commonly observed to arise epiphysesal in long bones in children or in epihysioid tarsal or carpal bones. Periosteal chondroma has an inclination for the surface of the proximal humerus in metaphyseal region. In Children, solitary bone cysts are mostly

found in the proximal humeral shaft or the proximal femur. Non-ossifying fibromas in childhood tend to favor the metaphysis of long bones in the lower extremities. (2, 3, 36) Similar observations can be extended to malign bone sarcomas. Malign tumors of bone mainly arise in metaphyseal location. Osteosarcoma typically arises around the knee, more specifically in the distal femur or proximal tibia before the closure of growth plates, with minimal penetration of the cartilaginous physis into the epiphysis. (2, 3) As an exception, ES and admantinoma are usually found in diaphyseal region. ES typically originates from the diaphysis, although metaphyseal involvement is also possible, but rarely the epiphyseal ends of bones are affected. Atypical cartilaginous tumor/chondrosarcomas predominantly affect the femur, pelvis, ribs, and sternum, rarely occurring in the spine or short tubular bones. *Figure 4* illustrates the growth patterns of the most common primary tumors of bone. (2, 3, 36)

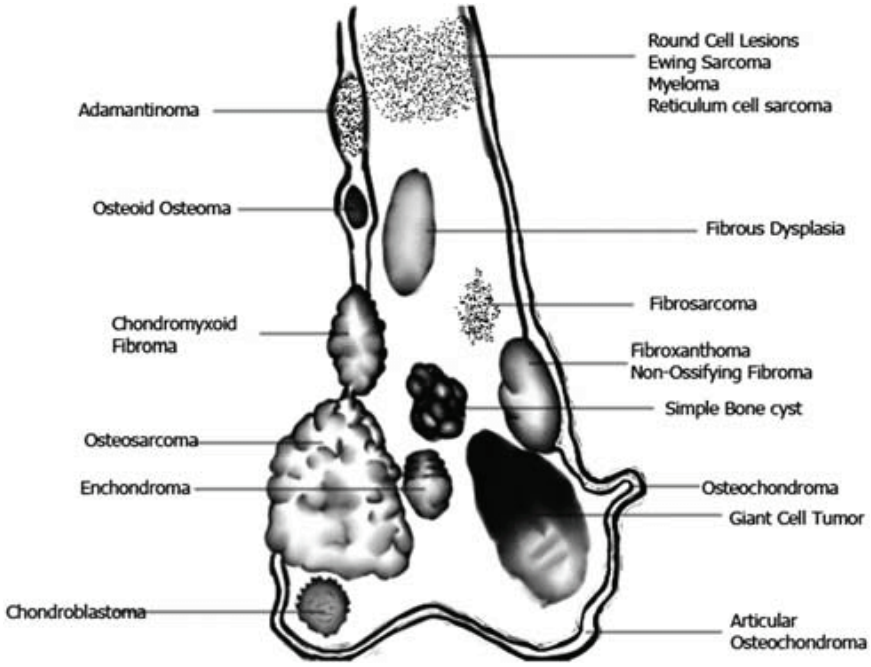


Figure 4: Common locations for frequent bone lesions. Image taken from Int J Surg Oncol (N Y). 2017 Dec; 2(11): e46. (36)

In numbers (see *Table 8*), with 43%, most malign bone tumors arise around the knee, rising to even 56% in patients aged under 20, followed by the pelvis with 31% as the second most common site, especially common in chondrosarcoma and ES. Shoulder girdle, including proximal humerus scapula and clavicula, is the third most

common spot for malign tumors with around 11% in all diagnoses. Lower leg, upper limb, and trunk all together are rarely affected by malignant tumors and only make around 15% of all malignant bone lesions. (2, 4, 5, 27)

Diagnosis	Knee[a]	Hip/Pelvis[b]	Shoulder girdle[c]	Lower leg	Upper limb	Trunk[d]	Risk of pathological fractures
Osteosarcoma	66%	15%	10%	5%	3%	1%	9%
Chonndrosarcoma	17%	48%	15%	4%	9%	7%	12%
Ewing sarcoma	22%	44%	11%	13%	7%	3%	6%
UPS*	41%	29%	9%	5%	14%	2%	16%
All diagnoses	43%	31%	11%	7%	5%	3%	10%

[a]Knee includes distal femur, proximal tibia and proximal fibula.

[b]Hip/Pelvis includes all pelvine bones and the proximal femur.

[c]Shoulder girdle includes proximal humerus, scapula and clavicule.

[d]Trunk includes spine, ribs and sternum.

*UPS = undifferentiated pleomorphic sarcoma

Table 8: Most common locations of primary malignant bone tumors including an overall risk for pathological fractures at time of diagnosis as shown in the WHO Report out of a single centre cohort database out of 3000 patients in the UK. (4, 5)

2.3 Osteogenic tumors

Osteogenic tumors can be rather benign, intermediate or malignant (see *Table 9*) Benign osteogenic tumors include bone islands, osteoma, and osteoid osteoma. Osteoblastoma is an intermediate osteogenic tumor, with a locally aggressive growth pattern. All kind of osteosarcomas are classified as malignant osteogenic tumors. Those can be further differentiated by localization into intramedullary and surface/juxtacortical tumors. Conventional osteosarcoma (osteoblastic, fibroblastic and chondroblastic), Telangiectatic osteosarcoma and small-cell osteosarcoma are intramedullary osteosarcomas while parosteal, periosteal and high-grade surface osteosarcoma have their origin on the surface/juxtacortical part of the bone. However, both intramedullary and surface/juxtacortical can have tumor tissue in one another but are named by their tissue of origin. (4, 5)

Malignant tumors can be further differentiated low-grade (G1), intermediate-grade (G2) and high-grade (G3) sarcomas. Low-grade (G1) includes well-differentiated osteosarcoma and parosteal osteosarcoma. Periosteal osteosarcoma is intermediate-grade (G2). Conventional osteosarcoma can be intermediate (G2) or

high-grade (G3) and high-grade surface osteosarcoma is always high-grade (G3). (4, 5)

Benign	Intermediate	Malignant – Osteosarcoma (OS)	
		Intramedullary	**Surface/Juxtacortical**
Bone island Osteoma Osteoid osteoma	Osteoblastoma	Conventional OS (1) Osteoblastic OS (2) Fibroblastic OS (3) Chondroblastic OS Telangiectatic OS Small cell OS Well differentiated OS	Parosteal OS Periosteal OS High-grade surface OS

Table 9: Osteogenic tumors, WHO classification of bone tumors, 5th edition 2020. (4, 5); OS: Osteosarcoma

Osteosarcomas can be schematically categorized by their location within the bone into intramedullary and surface/juxtacortical subtypes. *Figure 5* shows typical locations of Osteosarcoma.

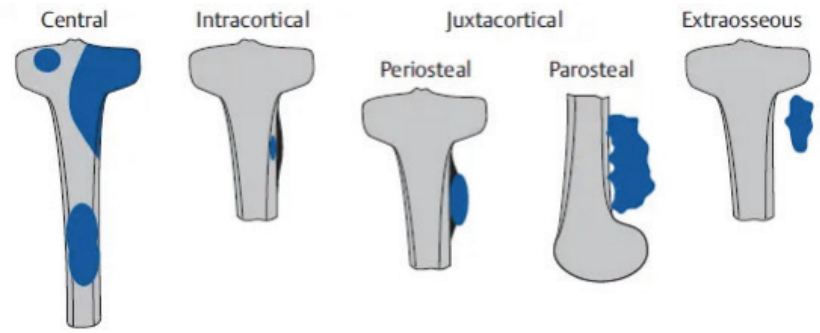


Figure 5: Typical locations of Osteosarcoma. Image taken from Musculoskeletal Key. 2023 Jan. (37)

2.3.1 Intramedullary Osteosarcoma

2.3.1.1 Conventional osteosarcoma

The conventional osteosarcoma as the most common primary high-grade sarcomas affecting the skeletal system will be described further on in chapter *2.4: Osteosarcoma.*

2.3.1.2 Telangiectatic osteosarcoma

Telangiectatic osteosarcoma, a high-grade sarcoma with frequent pleomorphism, typically produces minimal osteoid and features blood-filled spaces separated by

thin septa. Telangiectatic osteosarcoma is a rare subtype, constituting 4% to 10% of all cases, occurring predominantly in the second life-decade. (4, 5, 9)

The tumor, like conventional osteosarcoma, is usually metaphyseal, extending into the epiphysis. Common sites include distal femur, proximal tibia, proximal humerus, and proximal femur. Presentation often involves pain, soft tissue mass, and a higher incidence of pathologic fractures compared to conventional osteosarcoma.

Radiographically, more than 90% exhibit an aggressive lytic appearance with an ill-defined transition zone. Expansile growth and extensive periosteal reaction are common. MRI aids soft tissue assessment, revealing high signal intensity corresponding to hemorrhage and cysts with fluid-fluid levels. Grossly, the tumor is medullary, multicystic, and predominantly composed of blood- and fluid-filled spaces. (2, 4, 5, 9, 38)

On low power, blood-filled spaces are separated by thin fibrous septa, resembling aneurysmal bone cysts. The inclusion of benign osteoblast-like giant cells can contribute to the misleadingly bland appearance when observed at low power. Sparse matrix production and reactive osteoid at the periphery are characteristic. Cytologic atypia is variable, and necrosis may be present. A typical differential diagnosis to telangiectatic osteosarcoma is aneurysmal bone cysts (ABCs). Necrosis is frequently observed in telangiectatic osteosarcoma even in the absence of a substantial fracture, while it is an uncommon occurrence in aneurysmal bone cysts.

Immunohistochemical studies offer limited diagnostic utility. Telangiectatic osteosarcoma has a complex karyotype similar to other osteosarcomas. In distinguishing it from primary aneurysmal bone cysts, fluorescence in situ hybridization can probe the *USP6* locus, which is translocated in ABCs.

Treatment involves neoadjuvant chemotherapy and limb-sparing surgery, mirroring strategies for other osteosarcoma subtypes. In the era of neoadjuvant and limb salvage therapy, the prognosis and histologic response for TAEOS appear comparable to or better than other types of osteosarcomas. (2, 9, 38)

2.3.1.3 Small-cell osteosarcoma

Small cell osteosarcoma, categorized as a high-grade osteosarcoma, is characterized by cells that often resemble those found in Ewing sarcoma or other

small blue cell tumors, producing focal osteoid. Historically, this rare subtype was first identified as a variant of osteosarcoma in the mid-20th century, before it was mostly categorized as ES. Constituting 1% to 2% of osteosarcomas, it shows a slight male predominance, with patients typically presenting in their second and third decades, and an average age of around 25 years. (9)

These tumors mostly occur in long bones, with one-third in the femur, but lack the specific predilection spots observed in conventional osteosarcoma. Common symptoms include pain, swelling, and palpable mass. Radiographically, small cell osteosarcoma exhibits variable appearance, ranging from features akin to conventional osteosarcoma to those resembling ES or other marrow space processes. Grossly, the majority have a substantial soft tissue component with cortical disruption. (9, 39)

Microscopically, small cell osteosarcomas consist of small round cells with relatively uniform nuclei, displaying more pleomorphism than ES. Lacelike osteoid is a consistent feature, though it may be scant in some cases as well as fibrosis and myxoid changes that may be present. The differential diagnosis includes various small blue cell tumors, with immunohistochemistry aiding in differentiation through markers like *CD99* and *FLI1*.

Additional diagnostic studies may involve the expression of osteocalcin and osteonectin, with occasional positivity for various markers. Cytogenetic analysis reveals a complex karyotype similar to conventional osteosarcoma, and in some cases, a translocation involving *EWSR1-CREB3L1* has been identified. (9, 39)

Treatment involves neoadjuvant chemotherapy, akin to conventional osteosarcoma, with the 5-year survival rate slightly inferior to that of conventional high-grade osteosarcoma. Overall, small cell osteosarcoma remains challenging due to its rarity and overlapping features with other small blue cell tumors.(9)

2.3.2 Surface/Juxtacortical osteosarcoma

2.3.2.1 Parosteal Osteosarcoma

Parosteal osteosarcoma is classified as a low- grade malignant bone forming tumor, arising on the cortical surface of bone. (4, 5)

Although constituting only 4% of osteosarcoma cases, parosteal osteosarcoma prevails as the most common juxtacortical malignancy, being three times more prevalent than periosteal osteosarcoma. Typically affecting individuals 10 years older than those with conventional osteosarcoma, the majority of cases emerge between the ages of 20 and 40, with a third occurring in the third decade. A slight female predominance is observed in up to two-thirds of cases. Around 15% to 20% of instances exhibit dedifferentiation, often occurring in older patients.

More than two-thirds of cases manifest in the distal femur – mostly in the posterior part of the distal femur in the metaphyseal region - followed by the proximal tibia and humerus. Uncommonly, involvement extends to the small bones of the hands and feet, with rare occurrences in flat bones. Patients typically present with a painless mass limiting joint flexion or rotation. Pain, if present, tends to be chronic and dull. (2-5, 9)

Characterized by a distinctive appearance, in parosteal osteosarcoma surgery can often be performed based on radiological findings alone. Radiographically, the mostly metaphyseal and periosteal-based tumor typically wraps around the shaft, sometimes almost circumferentially and has characteristic periosteal reaction such as "hair on end”- sign and seldom Codman triangle (see *Figure 8*). The underlying cortical bone exhibits thickening and scalloping. Grossly, the tumor is lobulated, highly ossified, and broadly anchored on the periosteum. Dedifferentiation, when present, introduces a non-ossified intralesional soft tissue component, detectible through CT and MRI.

Histologically, parosteal osteosarcoma is a low to moderately cellular fibroblastic tumor with mild atypia, featuring bony trabeculae of woven and lamellar bone. Approximately 50% of cases may include a cartilaginous component, often at the periphery (see *Figure 7*).

Despite the characteristic radiographic appearance, differentiation from osteochondromas can be challenging but incomplete maturation from cartilage to bone and the presence of cytologic atypia distinguish parosteal osteosarcoma. Distinguishing from high-grade juxtacortical osteosarcoma is aided by the presence of a low-grade fibroblastic component. Parosteal osteosarcoma also overexpresses *MDM2* and *CDK4*, even in high-grade components. (2, 4, 5, 9, 40)

Cytogenetically, parosteal osteosarcoma exhibits a relatively simple karyotype, often with supernumerary ring chromosomes containing amplified segments from the 12q13-15 region. (25) Amplification of *CDK4* and *MDM*2 genes is common, observed in over 85% of cases. A careful search for a low-grade component is advised when high-grade osteosarcoma displays immunoreactivity for *CDK4* and *MDM2.* (9, 40)

Treatment involves complete excision with margins, often requiring limb-sparing excision of the metaphysis and epiphysis. Incomplete excision significantly elevates the risk of local recurrence, reaching up to 88% in some instances. Metastasis is rare in low-grade cases, while dedifferentiation correlates with an increased risk. Chemotherapy is not typically indicated for low-grade parosteal osteosarcoma, but high-grade components may require preoperative chemotherapy if identified by biopsy. (2, 4, 5, 9)

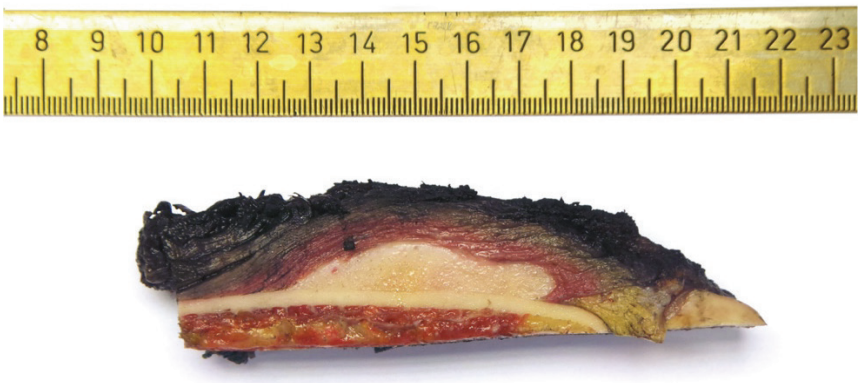


Figure 6: Excision specimen of parosteal osteosarcoma bisected down the long axis with intact cortex and sparing of the medullary space.

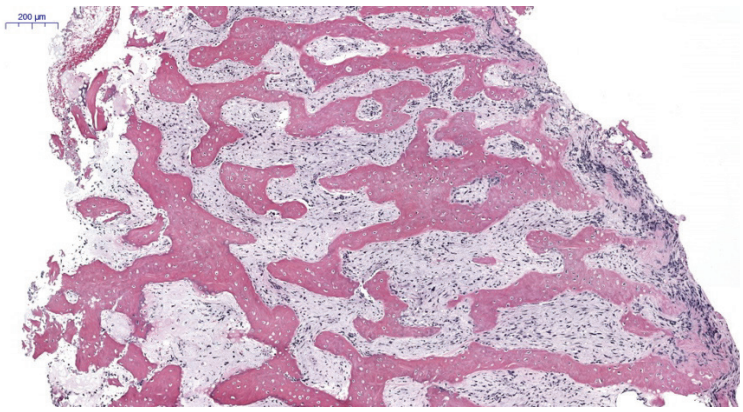


Figure 7: Parosteal OS, low-grade G1.

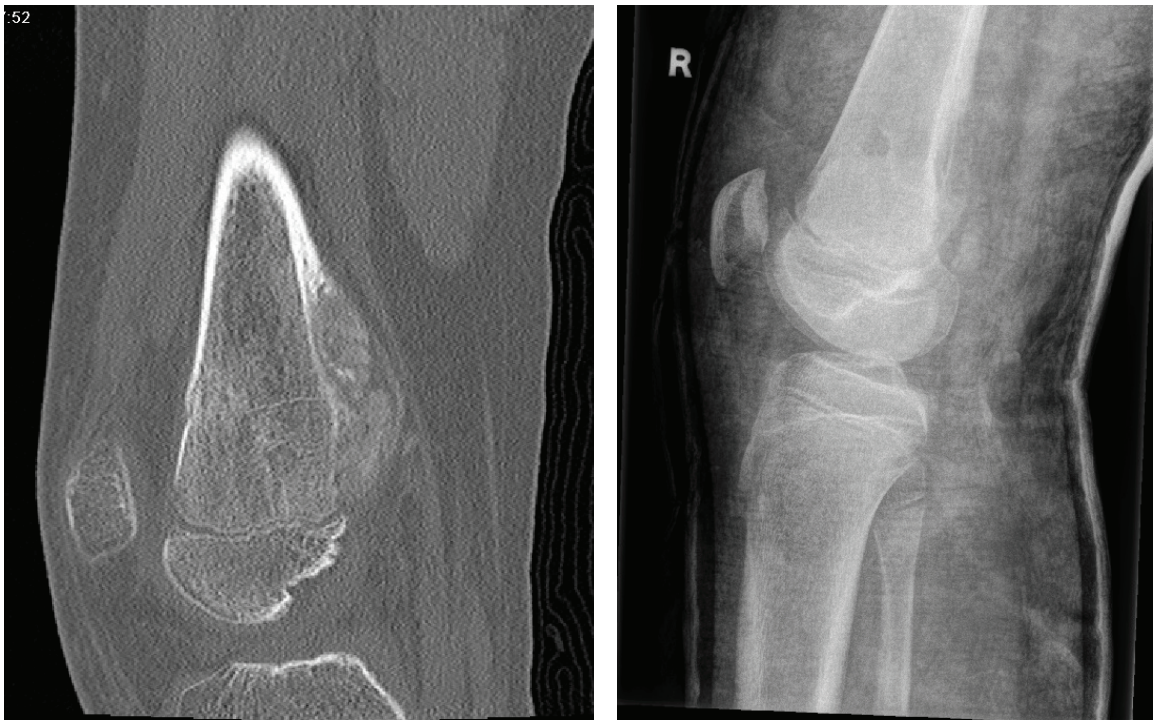


Figure 8: X-Ray of parosteal osteosarcoma in the distal femur (left) and the proximal tibia (right).

2.3.2.2 Periosteal Osteosarcoma

Periosteal osteosarcoma is an intermediate-grade, mainly chondroblastic malignant bone-producing sarcoma, occurring on the bone's surface (juxtacortical) typically underneath the periosteum. (4, 5)

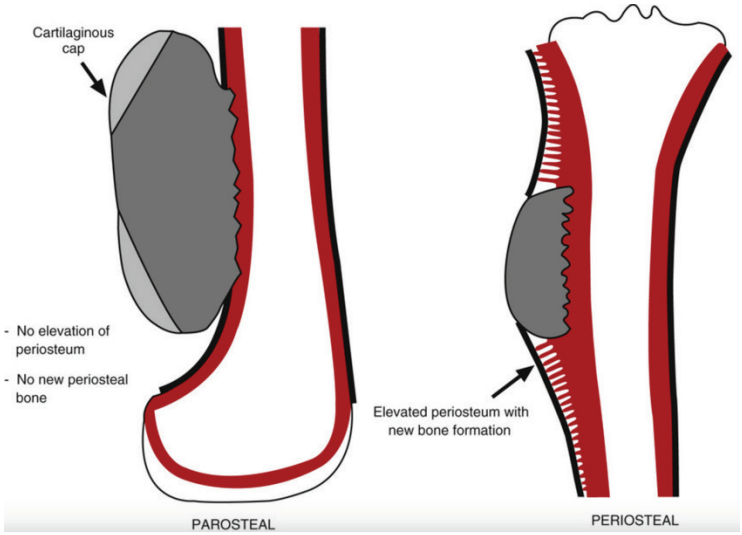


Figure 9: Differences in growth pattern between Parosteal osteosarcoma and Periosteal osteosarcoma. Image taken from Dorfman and Czerniak's Bone tumours, Elsevier, 2015. (2)

Periosteal osteosarcoma constitutes less than 2% of all osteosarcomas and is approximately one-third as prevalent as parosteal osteosarcoma. Its incidence peak occurs in the second and third decade of life, with only 10% of cases found in individuals over 50 years old. While most series show a slight male predominance, a larger interinstitutional series indicates a slight female predominance. Occasional multifocal cases and familial associations have been reported. (2, 4, 5, 9)

In terms of localization, periosteal osteosarcoma displays a distinct diaphyseal or diaphyseal/metaphyseal preference, presenting as a broad, sessile anterior medial lesion that may circumferentially enclose the bone. Predominantly found in the distal femur and proximal tibia, other locations include the humerus, fibula, ulna, pelvis, clavicle, ribs, cranium, and jaw. Common symptoms include limb swelling, mass, and/or pain, typically lasting less than a year, with 50% of cases exhibiting symptoms for less than six months. (9)

Radiographically, the tumor appears as a soft tissue mass firmly based on the cortex, often without intramedullary involvement. Cortical thickening, extrinsic cortical scalloping, and a perpendicular periosteal reaction are typical features, while CT and MRI aid in defining margins and tumor characterizations. Grossly, the tumor emerges from the bone surface with conspicuous cortical thickening and glistening gray cartilaginous matrix. Histopathologically, initial biopsies reveal atypical cartilage, occasionally with myxoid cartilaginous matrix, presenting a challenge in distinguishing from other juxtacortical tumors.

The distinct radiological features usually allow the exclusion of other cartilaginous neoplasms, such as periosteal chondroma and chondrosarcoma. While periosteal chondrosarcoma may share cellular and atypical characteristics with periosteal osteosarcoma, the absence of neoplastic osteoid and the lack of *IDH1* mutations differentiate the two. Immunohistochemistry for *IDH1* proves to be valuable in this differentiation. Parosteal osteosarcoma, despite its juxtacortical and broad-based nature, is metaphyseal and densely sclerotic, contrasting with the diaphyseal location and less osteoid matrix typically seen in periosteal osteosarcoma. Histologically, periosteal osteosarcoma lacks the broad trabeculae and fibroblastic stroma characteristic of parosteal osteosarcoma, though areas of cartilaginous differentiation may be present. Any occurrence of conventional high-grade osteoblastic or fibroblastic osteosarcoma histology suggests the potential for a high-

grade juxtacortical osteosarcoma or dedifferentiation of a parosteal osteosarcoma. (2, 4, 5, 9)

Genetic alterations in periosteal osteosarcoma are inconsistently reported, including cases with *Trisomy 17*, gains of various chromosomal portions, and losses of chromosomes 6, 8p, and 13. Approximately 40% of periosteal osteosarcomas exhibit point mutations in *p53*, a frequency similar to high-grade central osteosarcoma.

Periosteal osteosarcoma generally carries a favorable prognosis, with a 5- and 10-year survival rate of 89% and 83% but metastasis occur in about 15% of cases. Cases without marrow-involvement show better outcome in general. Primary treatment involves surgical excision, often amenable to limb salvage surgery. Marginal or intralesional excisions increase the risk of local recurrence, ranging from 14% to 67%. Chemotherapy has shown no survival benefit, and post-chemotherapy necrosis in the excision specimen is nonpredictive. Age, size, and margin adequacy do not predict long-term survival, but local recurrence significantly elevates the risk of metastasis. (9, 31)

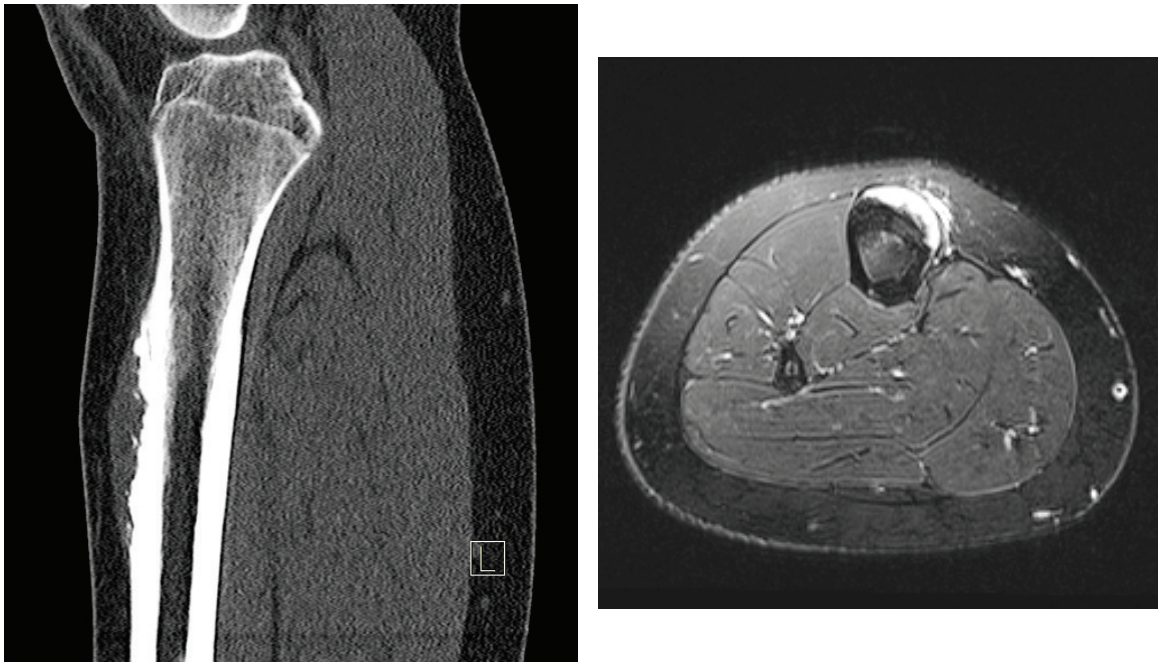


Figure 10: Periosteal osteosarcoma in the proximal tibia.

2.3.2.3 High-grade surface Osteosarcoma

High-grade surface osteosarcoma is a malignant high-grade bone-forming tumor arising on the surface of the bone with histological similarities to high-grade central osteosarcoma with a lacks of low-grade component compared to other subtypes. (4, 5)

High-grade surface osteosarcoma is considered rare, representing less than 5% of juxtacortical osteosarcomas. Predominantly affecting individuals in their second and third decades of life, with a mean age of 21 years, it demonstrates a 2:1 male predominance.

Seventy percent of cases manifest in the long bones of the lower extremity, with the femur accounting for 50% the fibula for 20% and the humerus for less than 10%. Typically diaphyseal, the tumors present as masses, accompanied by pain in two-thirds of cases, with an average duration of 11 months. (4, 5, 9)

Tumors are diaphyseal in 67% of cases, arising from the periosteum and ranging from 4.5 to 22 cm in size. Around 50% are circumferential, displaying frequent disruption of the cortex and marrow space involvement on imaging. Grossly, the tumor is periosteal-based, extending into soft tissue with frequent cortex erosion. Color and texture variations reflect the composition of osteoblastic, fibroblastic or chondroblastic elements. (4, 5, 9, 41)

Eighty percent of cases exhibit osteoblastic characteristics, while the remaining 20% are mostly chondroblastic. Histologically akin to central high-grade osteosarcoma in terms of cellularity and atypia, considerations of dedifferentiation of a parosteal or periosteal osteosarcoma arise in the presence of low-grade fibroblastic elements or well-formed trabeculae.

Radiologically distinguishing itself from central high-grade osteosarcoma, high-grade surface osteosarcoma involves the marrow space in 50% of cases, emphasizing its extraosseous predilection. High-grade surface osteosarcoma which arises in (dedifferentiated) parosteal osteosarcoma display *CDK4* and *MDM2* amplification. Chondroblastic high-grade surface osteosarcoma may resemble periosteal osteosarcoma but exhibits a lobulated appearance and less cytologic atypia. (4, 5, 9, 41) Limited data exist on the cytogenetics and biology of high-grade surface osteosarcoma. A reported case displayed amplification of the s*arcoma-*

amplified sequence (SAS) gene from chromosome 12q13-15, also noted in parosteal osteosarcoma. (9)

Treatment mirrors that of high-grade central osteosarcoma, involving neoadjuvant chemotherapy followed by limb-sparing excision and adjuvant chemotherpy. In patients with high-grade surface osteosarcoma the 5-year survival rate is between 62% to 82%. (4, 5, 42)

2.3.3 Secondary Osteosarcoma

Secondary osteosarcoma is defined as osteosarcoma arising in abnormal bone. The bone abnormality is usually caused by Paget disease of bone, radiation-associated, due to chronic osteomyelitis, related to metallic implants or secondary to early postzygotic disorders such as fibrous dysplasia. (4, 5)

Paget's disease serves as the predominant precursor and is the classic nonneoplastic condition preceding for secondary osteosarcoma development. The tumors typically exhibit high-grade features, with the femur (34%), humerus (24%), and pelvis (24%) being the most commonly affected skeletal sites. Radiologically, the pattern often involves a lytic destructive focus within an area affected by Paget's disease. Prognostically, Paget-related sarcomas have a significantly poorer outlook compared to de novo conventional high-grade osteosarcoma. Also, multifocal synchronous tumors may occur. (2, 4, 5)

Radiation-induced osteosarcomas may arise following both external and internal radiation exposure. Cases usually involve a dosage exceeding 3000 Gy, with a latency period extending beyond 3 years, although a shorter latency period has been observed in patients treated in combination with chemotherapy. These tumors are typically high-grade and display an aggressive clinical course, resulting in short survival.

Sarcomas arising from bone infarcts are rare, with most cases identified as malignant fibrous histiocytoma or fibrosarcoma. A limited number of high-grade osteosarcomas have been documented in patients with bone infarcts or metallic prosthetic implants.

Fibrous dysplasia exhibits a very low incidence (0.4%) of secondary malignancy, primarily seen in patients who had previous irradiation. Most occurrences are noted

in individuals in their third and fourth decades of life, with high-grade osteosarcomas being the predominant histologic type.

Chronic osteomyelitis, particularly with sinus track formation and reactive squamous hyperplasia, predisposes individuals to squamous carcinoma development in association with this condition. Sarcomas, including osteosarcoma, rarely develop in connection with chronic osteomyelitis.

Osteosarcoma related to premalignant bone syndromes affect the same regions as non-syndrome-related osteosarcoma. However, in *Rothmund-Thomson syndrome* tumors can be multifocal and occur in unusual sites, like patella and foot as well as the ankle, in *Werner syndrome*. (2, 4, 5)

2.4 Osteosarcoma

The osteosarcoma is the most prevalent primary malignant bone-producing tumor. Morphologically osteosarcoma can be divided in:

- Conventional osteosarcoma (COS)
- Telangiectatic osteosarcoma (TAEOS)
- Small cell osteosarcoma (SCOS) (4, 5)

Depending on localization intramedullary and surface/juxtacortical can be differentiated (see chapter 2.3: Osteogenic tumors). The following chapters mainly describe conventional osteosarcoma.

2.4.1 Epidemiology

Conventional osteosarcomas are the most common primary high-grade sarcomas affecting the skeletal system. They exhibit a bimodal age distribution, with the majority of cases emerging in the second decade of life, more precise between 14 and 18 years, and a second, smaller peak occurring in older adults (30% in individuals over 40 years). The yearly incidence rate is approximately 4.4 cases per 1 million population for individuals aged 0-24 years, 1.7 cases per 1 million population for those aged 25-59 years, and 4.2 cases per 1 million population for those aged ≥60 years. Males are more commonly affected, with a male-to-female ratio of 1.3:1. (2, 4, 5, 30, 43) Jaw tumors typically arise in the third to fourth decades of life. (44) Telangiectatic osteosarcoma represents a rare subtype, constituting 2-12% of all high-grade osteosarcomas. It frequently emerges in the second decade

of life, exhibiting a male predominance akin to conventional osteosarcoma. Small cell osteosarcoma makes only 1.5% of all osteosarcomas and have been documented in patients aged 5-83 years, with a higher incidence during pubertal growth and a slight predominance to females. (M:F ratio: 0.9:1). (4, 5, 43)

2.4.2 Aetiology

Osteosarcomas originate from primitive bone-forming mesenchyme, most commonly observed during periods of rapid growth, occurring not only in children and adolescents but also in fully grown adults. (4-6, 30, 45) This suggests a potential association between osteosarcoma development and progressive, rapid length growth, particularly in younger individuals. Additionally, chronic inflammatory conditions, such as osteomyelitis or bone infarcts, are considered potential etiological factors. Other conditions that may contribute to osteosarcoma development include exposure to metallic implants (e.g., aluminum, chromium, cobalt, methyl-methacrylate, nickel, titanium, and polyethylene) or a history of previous radiotherapy. Studies have reported documented radiation exposure in approximately 3% of patients who developed osteosarcoma. Furthermore, premalignant diseases, multiple exostoses, fibrous dysplasia, and Paget's disease are linked to an increased risk of osteogenic tumors in individuals older than 40 years. (4-6, 30, 43)

While the majority of osteosarcomas occur sporadically, getting symptomatic by persistent load-independent pain and swelling, a few cases are associated with heritable germ line abnormalities. These include Li-Fraumeni syndrome (mutation in *TP53*), Werner syndrome (mutation in *WRN*-Gene 8p11-12), Rothmund-Thomson syndrome (mutation in *RTS1* or *RTS2*), Bloom syndrome (mutation in *BLM* 15q26.1), and hereditary retinoblastoma especially after receiving ionizing radiation therapy. (4, 5, 21, 45) The definitive causative role of osteosarcomas remains inconclusive. Some identified genetic predisposing factors include loss of heterozygosity of the *RB1*, HER2/erbB-2 expression, κB RANK-ligand expression, presence of the *K7M2* cell line, and potential biomarkers such as *MIF* and chronic low *TNF* or *SOX9* up-regulation. However, to this day, the aetiology of osteosarcoma has not been entirely clarified. *Table 10* provides an overview of the predisposing genetic factors. (1, 6-8)

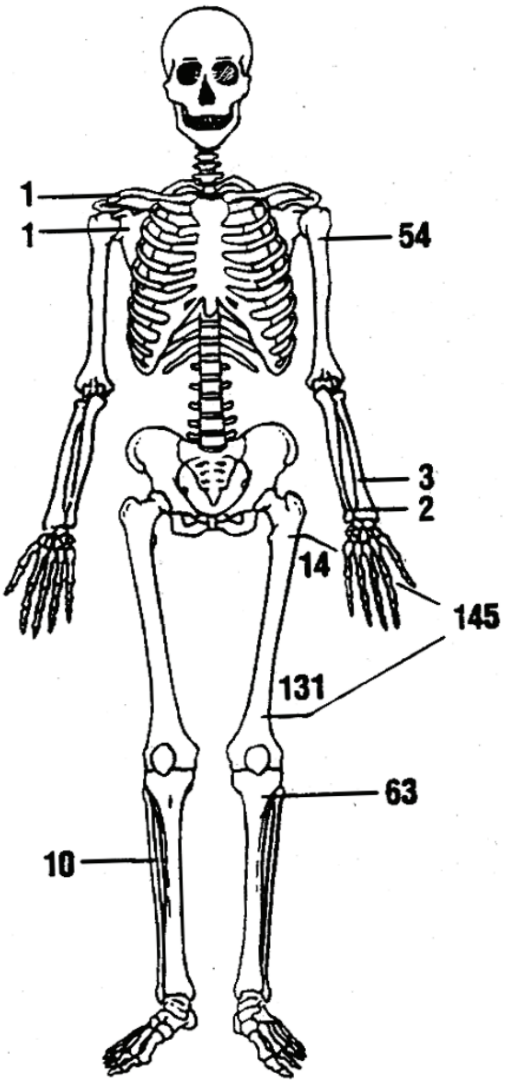
Genetic factors	Explanation
RB gene	Retinoblastoma protein gene, a tumor suppressor gene
HER2/erbB-2	Factor of human epidermal growth factor receptor 2, a proto-oncogene
κB RANK-ligand expression	Receptor activator of nuclear factor κB, a membrane protein of osteoclasts
K7M2	Metalloproteinase, occurs in metastatic osteosarcomas
MIF	Migration inhibitory factor
TNF	Tumor necrosis factor
SOX9	Human protein, involved in initiation and progression of multiple solid tumors

Table 10: Explanation of genetic predisposing factors for genesis of osteosarcoma. (1, 6-8)

2.4.3 Localisation

Osteosarcomas have the potential to manifest in any bone throughout the skeletal system. *Figure 11* gives an overview of the most prevalent locations for all subtypes of osteosarcoma. Regions commonly affected in children and young adults are those undergoing rapid growth, specifically the metaphysis of long bones. (6, 21, 30) Conventional osteosarcomas mostly arises in the distal femur (30%), followed by the proximal tibia (15%) and the proximal humerus (15%) – all three sites with the most active growth plates. In long bones, lesions with around 90% mostly arise metaphyseal and only infrequently develop in the diaphysis (9%) or the epiphyseal region (1%). (2, 4-6, 21, 30) The jaws are the fourth most common site for tumor development. (4, 5, 9)

High-grade central osteosarcomas of the conventional type predominantly originate in the distal femur and proximal tibia, with the proximal humerus following. Low-grade intramedullary osteosarcomas are more frequently observed in the head and neck region. In patients over 50 years, only 15% of the tumors occur in the knee area. The anatomical distribution in elderly patients is diverse and encompasses the axial skeleton as well as nonspecific bone sites such as the pelvis, ribs, or neck. (2, 3, 6, 21, 30)



Involvement of small bones of the extremities as well as multifocal osteosarcoma occurs rather rare. Metastatic spread is more common than multiple independently occurring primary tumors. Telangiectatic osteosarcoma (TAEOS) also primarily arises around the knee (60%) and the proximal humerus (20%), commonly occurring in the metaphyseal region with direct extension to the adjacent epi- and diaphysis. Small cell osteosarcoma (SCOS) has a similar distribution but more commonly arises in the diaphysis of long bones (10-15%) (4, 5)

Figure 11: Localisation of 279 primary osteosarcoma in a study of Glasser et al. (1992). Numbers next to the skeleton showing the frequency of tumours occurring in every anatomical region of the body. Image taken from Eur J Cancer. 2019; 109:36-15. (1)

2.4.4 Clinics

Usually there is only a short period from weeks to months with symptoms like pain, enlarging mass and restricted movement in the adjacent joint. Typical inflammatory characteristics such as hyperthermic or erythematous skin and swelling can occur on the skin around the diseased tissue. 10-15% of patients present first with pathological fractures, mostly seen in long tubular bones such as femur and humerus. The clinical presentation of telangiectatic osteosarcoma and small-cell osteosarcoma is similar to conventional osteosarcoma but with an increase on pathological fractures up to 30% in telangiectatic osteosarcoma. (4, 5) Skin changes and venous dilatation are mostly linked to more vascular lesions. (9) At time of

diagnosis, certain patients may experience weight loss, which is typically linked to disseminating diseases, and in case of osteosarcoma often manifested as lung metastases. (2)

2.4.5 Radiology

The radiologic appearance of conventional osteosarcoma shows a wide variety, depending on the rapidity of growth and the distribution of osteoblastic, chondroblastic, and fibroblastic elements. (9) The tumor tissue can be lytic or sclerotic, but is mostly a combination, which makes a perioperative radiographic osteosarcoma diagnosis possible. Parts of the tumor show mineralization, producing cloudy opacities varying in shape, size, and density. These opacities can be either spread throughout the lesion or accumulate in an area forming a large, irregular sclerotic mass. In rare cases, the tumor may appear uniform with ivory-like density and minimal to no lytic component. (2, 3) The tumor usually shows a destructive growth-pattern with a transition-zone from lytic and/or sclerotic areas to unaffected bone, complicating to assess the lesions margins. The tumor may be solely intramedullary but in most cases the cortex is involved and therefore gets focally destroyed. If the cortex is affected, the tumor mass extends to soft tissue. Cortical penetration can be seen by mineralized shadow overlying the affected area. Cortical destruction is frequent, and more than 80% of patients have extraosseous extension, which in 90% of cases shows mineralization. Typically, the dimensions of extracortical soft tissue span correspond with the size of the intramedullary tumor mass and the dimensions of cortical destruction. (2-5, 9, 43, 46) A larger intramedullary tumor with more extensive cortical disruption generally results in a proportionally larger extracortical soft tissue component. The destructive growth pattern and soft tissue extension often happens asymmetric, with one side of the bone being more affected. One individual spot of cortical destruction and expanse into soft tissue may ultimately develop into an extensive soft tissue mass circumferentially involving the affected bone as the tumor progresses. The outer cortex surface overlying the tumor may display a noticeable periosteal reaction, taking the form of parallelly arranged periosteal new bone, cortical irregularities and fuzziness, or a combination of these features. Another sign is the tumor generating radiating or perpendicular striations, so called "sunburst". This periosteal reaction

can be seen on the bones' surface but does not provide information about soft tissue extension. The tumor growth on the surface of the bone may elevate the periosteum, to the shape of an open triangle known as "Codman's triangle". If the periosteal reaction appears in multiple layers, the so called "onion skin", is commonly seen in undifferentiated small round cell tumors with bone-involvement and in osteosarcomas located in the diaphysis rather than the metaphysis. (2-5, 9, 43, 46)

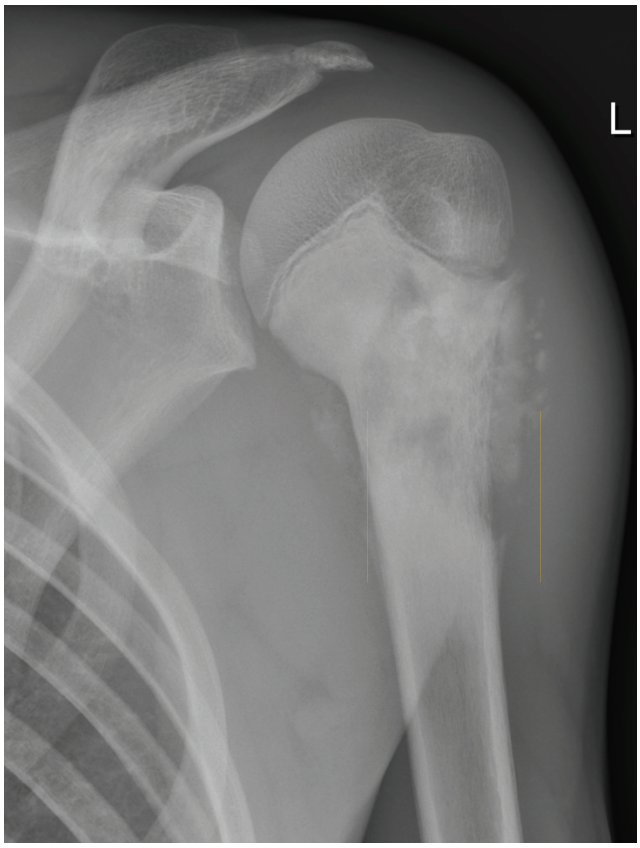


Figure 12: Codman triangle in osteosarcoma of the proximal humerus in X-ray a.p. projection.

Rapidly proliferating tumors can produce lytic tissue with a permeative, bone-destructive growth-pattern and minimal to no periosteal reaction. Diagnosing those types of osteosarcomas requires highly specialized radiologists to differentiate from other bone-destroying lesions like e.g. osteomyelitis. The suspicion of osteosarcoma typically arises from the presence of a destructive lesion in the metaphysis of long bones in adolescents, even in absence of tumor matrix mineralization. From time to time, osteosarcomas of high histologic grade can present with relatively benign-looking radiographic lesions, displaying sclerotic or sharp margins. In extremely rare cases, osteosarcoma originates within the

epiphyseal region, potentially leading to misinterpretation on radiographs as chondroblastoma or clear-cell chondrosarcoma. (2-5, 9, 46)

For evaluating the extent of the disease, CT and MRI are necessary. These imaging modalities play a crucial role in assessing intramedullary involvement and extension into soft tissue. The relation between soft tissue extension and the neurovascular tissue holds particular significance for planning limb-sparing procedures. MRI is especially valuable in delineating the tumor's extent and planning resection planes for surgical excisions. Through variable signal intensity in different tumor tissue areas, MRI reveals the heterogeneous nature of the tumor. Heavily mineralized regions typically exhibit no signal in T2-weighted sections, while sarcomatous highly vascularized areas may display varying degrees of signal enhancement. Overall, MRI coupled with contrast enhancement and fat saturation, elucidates the tumors diverse composition, and helps in evaluating its extent and relationship to surrounding anatomical structures in normal tissue. (2-5, 9, 46)

Although the diagnosis can often be anticipated based on radiographic findings, identifying histological subtypes reliably proves challenging, given the frequent occurrence of atypical appearance. (4, 5)

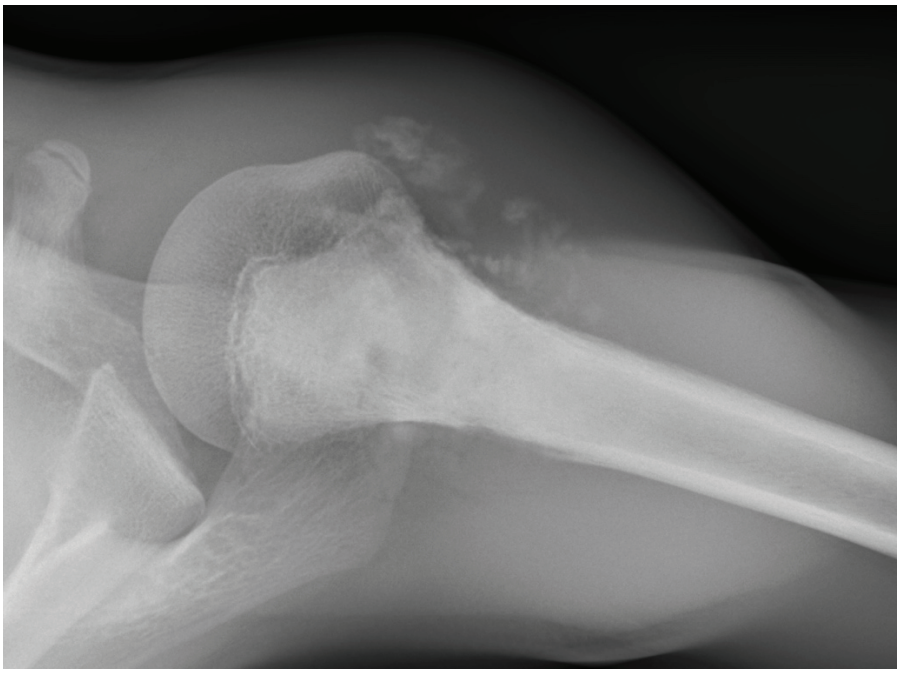


Figure 13: Osteosarcoma of the shoulder as seen in X-ray axial projection.

2.4.6 Gross findings

Besides the typical location of osteosarcoma which is usually in metaphyseal region of long bone there are various other aspects describing the typical gross appearance of osteosarcoma. Conventional osteosarcoma usually presents as a 5-10cm mass, lying intramedullary in the metaphyseal region. (2, 4, 5) It may consist predominantly of ossified or non-ossified tissue, yet the characteristic gross appearance often arises from a combination of bony and soft tissue regions. The cut surface of the tumor is typically highly heterogeneous, featuring areas with varying colors, consistencies, and degrees of ossification. Highly ossified regions resemble ivory-like, exhibiting a yellow-white color and hardness comparable to normal cortical bone, whereas less ossified areas appear softer and yellowish. Regions with minimal to no ossification have a fleshy, tan or chondroid consistency commonly displaying characteristics such as hemorrhagic and necrotic areas. The development of densely ossified areas in osteosarcoma typically results from the interaction between osteogenic tumor cells and preexisting non-tumorous bone. Within the intramedullary region of the tumor, extensive bone-condensation arises as tumor overlays the existing cancellous bone within the medullary cavity. Extramedullary, within the soft tissue, solid areas of bone form through the tumor tissue between spicules of reactive non-tumorous bone originating from the periosteum with the tumor usually demonstrating an invasive and bone-destructive growth pattern. (2, 4, 5, 9)

Cortical destruction is a common occurrence, with over 80% of cases displaying extraosseous extension. In 90% of instances, this extension demonstrates mineralization, frequently creating a distinctive "hair on end" or sunburst pattern perpendicular to the periosteum. (9)

Margins between the tumor tissue and adjacent non-affected structures typically appear irregular, especially in highly ossified tumor tissue fusing the adjacent cortex. Intramedullary small lesions are mostly situated eccentrically but even relatively small lesions frequently extend to the cortex, causing periosteal reaction. As lesions advance, filling the intramedullary space and progressing toward the diaphysis and growth plate, clear signs of cortical destruction, periosteal reaction, soft tissue expanse, or a combination of these characteristics become apparent.

Radiographically, these characteristics are equivalent with the formation of new periosteal bone. Further growth results in progressing soft tissue expanse overlaying the location of cortical destruction. Indications of periosteal involvement, along with accompanying bone reaction, are frequently observable at the periphery edges of the lesion, particularly in the diaphysis, corresponding to “Codman's triangle” frequently seen in radiographs. Typically, the intramedullary tumor growth is more extensive than predicted on radiographs, and the intramedullary margins show irregularity sharply demarcated dome-shaped structures. The growth plate serves as a natural barrier for tumor progression and is seldomly breached, except in more progressed lesions. In rare instances, articular cartilage destruction may occur with tumor extension into the adjacent joint. Synovial involvement is more common in progressed tumors due to tumor growth along the surface of the bone. (2, 4, 5, 9)

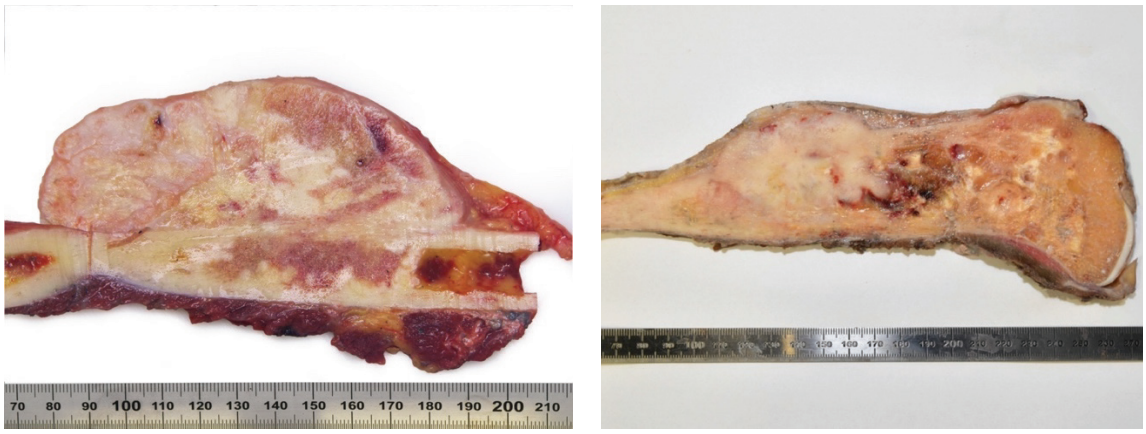


Figure 14: Resection specimen of initially intramedullary osteosarcoma with cortical destruction and soft tissue component.

2.4.7 Histology

Conventional osteosarcoma presents a diverse histomorphological spectrum, with a crucial diagnostic aspect being the identification of neoplastic bone formation. The tumor exhibits a permeative growth-pattern, replacing marrow space, destroying pre-existing trabeculae and later on filling and expanding haversian channels with cortical bone. Neoplastic cells, which may appear fusiform, plasmacytoid, or epithelioid, demonstrate severe anaplasia and pleomorphism. These cells often assume a small, normalized appearance resembling benign osteocytes when

surrounded by bone matrix. Mitotic activity is typically highly active, with abundant atypical mitotic figures as a severe difference to benign osteosarcoma-mimicking lesions. Exhibiting variations in quantity and woven architecture, healthy bone tissue is complexly pervaded by tumor cells. It is composed of primitive, disorganized trabeculae that may form fine or coarse lace-like patterns or large sheets of compact bone through merging trabeculae. (2, 4, 5, 9)

The appearance of the bone matrix on H&E-stained sections, whether unmineralized or mineralized, varies. Unmineralized matrix (osteoid) may appear eosinophilic/red, while mineralized matrix appears basophilic/purple. Collagen in conventional osteosarcoma usually is rather fibrillar than glassy and often cumulated in large clusters or elongated fibrils packed between lesioned cells. The amount of cartilage or/and fibroblastic components in the neoplasm may vary but on this basis the tumor may exhibit different histological patterns, including osteoblastic (76-80%), chondroblastic (10-13%), and fibroblastic (10%) types. (4, 5)

Osteoblastic conventional osteosarcoma, constituting the majority, features neoplastic bone as the primary occurring matrix, ranging from lace-like trabeculae to compact bone tissue.

Chondroblastic conventional osteosarcoma is characterized by a main component of hyaline cartilage with severe cytological atypia, myxoid matrix with single cells or more subtle atypia such as delicate cell-cords. The merging of neoplastic cartilage with areas containing neoplastic bone is a common occurrence, often accompanied by the condensation and spindling of tumor cells at the periphery of chondroid nodules. (2, 4, 5, 9)

Fibroblastic conventional osteosarcoma presents malignant cells that are spindled or epithelioid, often showing severe cytological atypia, with tumor cells associated to an extensive amount of extracellular collagen arranged in a mesh-like, storiform pattern. The spread of osteoclastic giant cells of non-neoplastic type within the tumor is characteristic for the giant cell-rich subtype of osteosarcoma. Some bone sarcomas without associated giant cell tumor of bone histology. Bone tumors with *H3-3A (H3F3A)* or *H3-3B (H3F3B)* p. Gly34 mutation, especially in epiphyseal location in young patients, suggest a link with giant cell tumor of bone.

The epithelioid subtype is characterized by large polyhedral tumor cells. In the osteoblastoma-like subtype, tumor cells typically exhibit less-pronounced atypia and

surround the neoplastic bony trabeculae, while also encasing pre-existing bone trabeculae as a sign of bone-destructive growth. (2, 4, 5, 9)

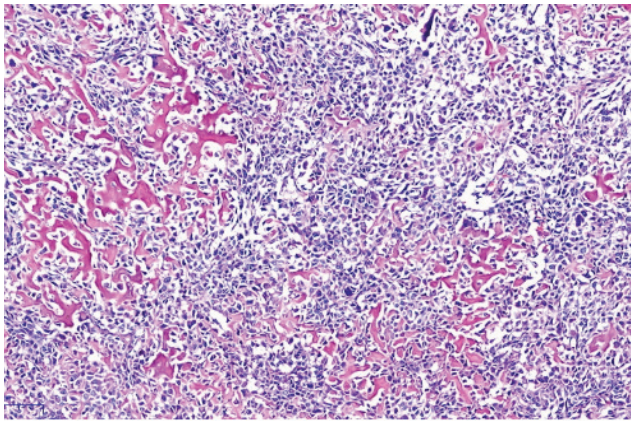


Figure 15: Histology of high-grade osteoblastic osteosarcoma.

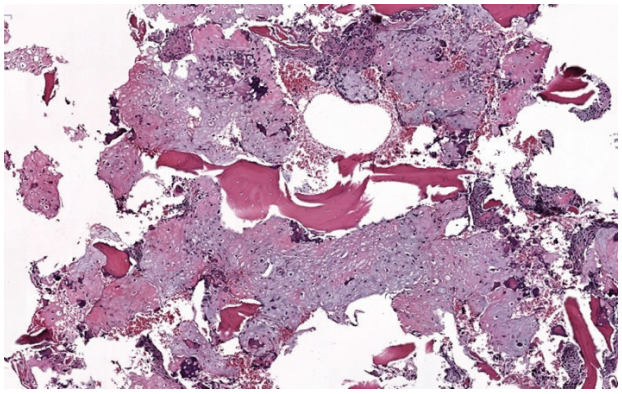


Figure 16: Histology of chondroblastic osteosarcoma.

2.4.8 Differential diagnosis

Osteosarcoma must be differentiated from various bone tumors, as listed above in *Table 1*. Among these, the ES and chondrosarcoma (especially dedifferentiated chondrosarcoma) are the most frequent differential diagnoses.

2.4.8.1 Ewing sarcoma

ES, an undifferentiated small round cell sarcoma is the second most prevalent primary malignancy of bone, affecting primarily children and young adolescents (<20 years) but also occurs in adults and exhibits a slight male predominance (M:F ratio 1.5:1). Unlike osteosarcomas, the etiological factors for ES are identified, including chimeric transcripts of *EWSR* genes with other genomic transcripts such

as *FLI1, ERG2* (human transcription genes), or *DKK2,* a key player in stem cell signaling. (2, 21, 47, 48)

Studies indicate an approximate incidence of 0.3/100,000/year in Caucasians, with minimal occurrence in Blacks and Asians. Long bones of the extremities (50%) are most commonly affected, followed by pelvic bones (25%), and less frequently, ribs or the vertebral column. (2, 21, 47, 48) ES falls within the category of highly aggressive tumors, displaying a significant inclination for both local recurrences and distant metastases, with a predominant occurrence of the latter in the lungs. Prognosis with ES equals that of osteosarcomas, with a 5-year-survival of approximately 70% in localized disease and about 30% in metastatic disease. (2) Factors influencing prognosis include large tumor mass, older age, axial localization, poor response to chemotherapy, and gender, with females having a lower OS (17%-30%) than males (45%-48%). Metastases are present in about 25% of patients at diagnosis, often related to metaphyseal tumors, with bones and lungs being the most common sites. Aggressive multi-agent chemotherapy has improved the prognosis (5-year EFS 30-60%), with a total treatment time of 35 weeks. During this time a combination of Vincristine, Doxorubicin and Ifosfamide or Cisplatin, Doxorubicin and Ifosfamide is administered. Surgery of the primary tumor is scheduled for week 9, with radiotherapy for inoperable cases. (2, 21, 47, 48)

2.4.8.2 Chondrosarcoma

Chondrosarcoma is the third most common primary tumor of bone. It typically occurs between the third and sixth decade of life, equally affecting males and females with an incidence of 0.1/100,000/year. (2, 21, 49)

Chondrosarcomas is commonly localized in the center of diametaphyseal bone, such as the proximal femur, proximal humerus, and distal femur, as well as in the pelvis, ribs, and scapula. Treatment of chondrosarcoma varies based on histological subtypes and grades, with the majority being low-grade (G1) tumors. The 5-year survival is approximately within 75% to 83%. (2, 21, 49, 50)

2.4.9 Treatment

Until the 1970s, osteosarcoma was treated with surgery alone because of a lack in chemotherapeutic agents. Therefore, the event free survival (EFS) was only about

20%. (51) Local therapy alone turned out to be insufficient, since 80-90% of patients developed metastases. (52) Over the years, different strategies in chemotherapy, surgery and radiation developed which improved the percentage of EFS significantly up to 50-60%. Nowadays the standard therapy in osteosarcoma is a combination of neoadjuvant chemotherapy, wide resection, and adjuvant chemotherapy. (52, 53)

2.4.9.1 Chemotherapy

Since the introduction of chemotherapeutics in osteosarcoma in the 1970s, there have been many different attempts in chemotherapy agents and strategies. First approaches in chemotherapy included a complex schedule of cyclophosphamide, dactinomycin, bleomycin, cisplatin, doxorubicin, and high-dose methotrexate (HDMTX). (51) Until now, the use of multi-agent chemotherapy before and after surgery, along with advancements in surgical techniques and radiotherapy, have significantly elevated the five-year DSF. Nowadays, Doxorubicin (DOX; Adriamycin, A), Cisplatin (DDP; Cis–diaminedichloroplatinum II, CDDP; Platinol, P), Methotrexate (MTX; Amethopterin), Ifosfamide (IFX; IFOS, IFO), Etoposide (ETO; VP-16, Vepesid), and Pegylated interferon α-2b (IFNα; Peg-Intron) represent the most potent chemotherapeutic agents utilized across all participating countries of EURAMOS-1. (30) The standard for neoadjuvant chemotherapy is, according to EURAMOS-1, a combination of high-dose methotrexate (M), doxorubicin (A), and cisplatin (P) in two cycles over 10 weeks with following surgery. Within the resection specimen, the histological response on neoadjuvant chemotherapy is being evaluated. The decision on further adjuvant chemotherapy strategies is taken on basis of “good response” or “bad response” (see *Chapter 2.4.9.1.1: Salzer-Kuntschik Regression Grading*). Post-surgery, patients usually get another four cycles (Cycle 3-6) of MAP over a timespan of 18 weeks (week 12-29). According to EURAMOS-1 trail, invented for patients under the age of 40, MAP alone was used for adjuvant standard chemotherapy or MAP got reinforced with weekly Interferon-α (0.5μg/kg/week) for 24 months after MAP in patients with good response to neoadjuvant chemotherapy or MAP with additional Ifosfamide and Etoposide (MAPIE) in patients with bad response to neoadjuvant chemotherapy (see *Figure 17*). However, these additions to conventional MAP chemotherapy have not led to a better outcome but rather to increased toxicity. (30, 54)

The following figure shows the treatment schedule for neoadjuvant and adjuvant chemotherapy according to EURAMOS-1. The postoperative treatment path is randomised and separated by good and bad response to neoadjuvant chemotherapy.

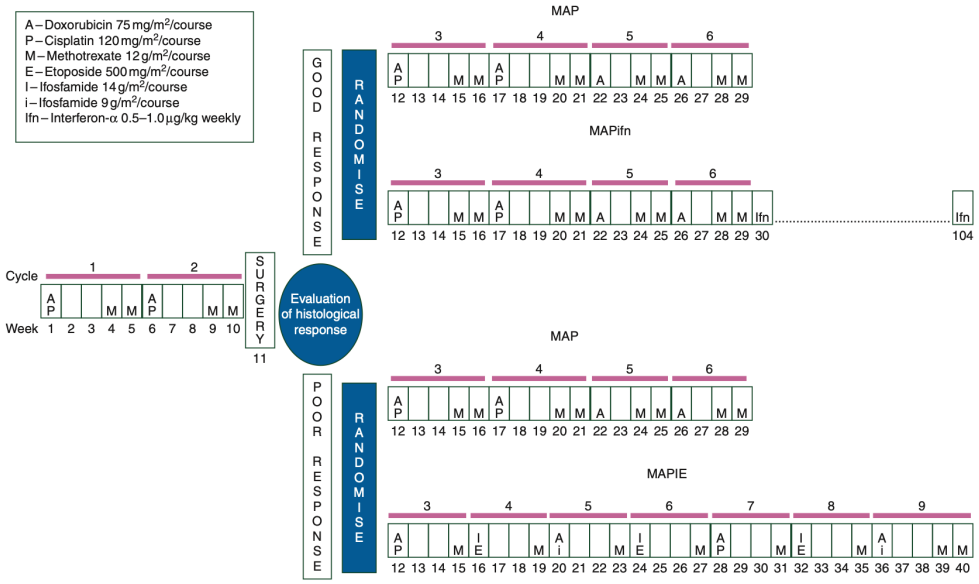


Figure 17: EURAMOS-1 treatment schedule. Image taken from Ann Oncol. 2015;26(2):407-14. (30)

In elderly patients, treatment requires special considerations due to pre-existing comorbidities, but according to the *European Bone over 40 Sarcoma Study* (EURO-B.O.S.S.) a combination of surgical and chemotherapeutical treatment leads to similar outcome as in patients under the age of 40. (54, 55) The main measure for outcome was the time of event-free survival (EFS), defined as the time beginning with adjuvant chemotherapy until the development of metastasis, local recurrence, progression of metastatic disease, or detection of secondary malignancy. (30)

2.4.9.1.1 Salzer-Kuntschik Regression Grading

The effectiveness of neoadjuvant chemotherapy in every patient is analyzed using “Salzer-Kuntschik regression grading” scheme. In the resected tumor tissue, vital tumor cells are quantified. The degree of regression divides the response on chemotherapy administered prior to surgery in correlation to the percentage of vital tumor tissue into six subcategories. While degree of regression 1-3 (<10% of vital tumor cells in tumor tissue/>90% necrotic tissue) corresponds to good response on neoadjuvant chemotherapy, degree of regression 4-6 (>10% of vital tumor cells in

tumor tissue/<90% necrotic tissue) relates with poor or no response on neoadjuvant chemotherapy. (56)

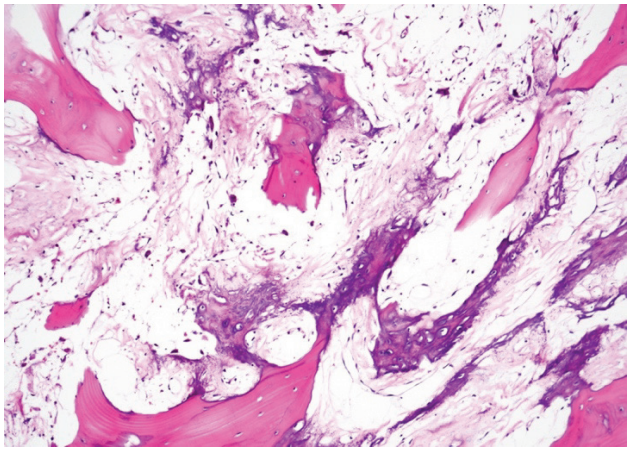


Figure 18: Histology of osteosarcoma after neoadjuvant chemotherapy with "good response" where only scattered vital tumor cells are present.

In terms of survival, patients with good response have a five-year disease-free survival (DFS) of 76-93% while patients with less than 90% of necrosis and therefore bad response have a five-year DFS of only 45-67%. (51)

	Degree of Regression	Description	Alternative description
Good Response	Degree of regression 1	No vital tumor cells	
	Degree of regression 2	Individual vital tumor cells	single vital tumor cells or Cell-clusters smaller than 0,5cm
	Degree of regression 3	Less than 10% of vital tumor tissue	Vital tumor is less than 10% of the whole tumor tissue
Poor Response	Degree of regression 4	10-50% of vital tumor tissue	Vital tumor is less than 10-50% of the whole tumor tissue
	Degree of regression 5	Over 50% of vital tumor tissue	Vital tumor is over 50% of the whole tumor tissue
	Degree of regression 6	Fully vital tumor	No effect caused by neoadjuvant chemotherapy

Table 11: Salzer-Kuntschik Regression Grading. (56)

2.4.9.2 Surgery

Surgical resection is important to obtain local disease control, as the goal is always complete removal of the tumor. (52, 53) According to Enneking et al. margins in resection should be wide (see Table 12). Therefore, the resection tissue should include the tumor, the biopsy scar and a non-contaminated cuff of healthy tissue surrounding the whole tumor.

Margin	Dissection
Intralesional	Within the lesion
Marginal	Through the pseudocapsule or the reactive zone of the lesion
Wide	Lesion including biopsy scar, pseudocapsule, and/or reactive zone, and an unviolated cuff of normal tissue completely surrounding the tumor tissue removed in a single block
Radical	Entire anatomic compartment containing tumor tissue removed in one block

Table 12: Criteria for surgical margins in musculoskeletal tumors by Enneking et al. (24, 52)

While in the past decades, limb amputation was the most prevalent type of surgery for osteosarcoma, being the norm rather than the exception, nowadays, in around 90% of patients a limb-sparing procedure is possible but there are instances where a more radical procedure becomes necessary when a safe tumor removal is not achievable. (24, 52, 53)

The decision for limb salvage surgery is influenced by factors such as the patient's age, tumor size, response to neoadjuvant chemotherapy, and the tumor's location and extent. For small tumors showing a positive response to pre-operative chemotherapy, limb salvage surgery is a suitable option. The pre-surgical planning involves X-rays, MRIs of the affected site to identify the local extent and planning of endoprosthesis, as well as bone scans to identify possible skip lesions. (30) The reconstructive possibilities after limb-sparing surgery are diverse, encompassing endoprosthetic devices, biological reconstruction, or a hybrid approach combining both. Rotationplasty, as a well-established biological reconstruction method for tumors around the knee, have comparable to superior functional and psychological outcomes to endoprosthetic reconstruction, but leads to cosmetical challenges. In cases involving young children with open growth plates, extendable endoprosthesis with lengthening functions are recommended, despite a slightly higher complication-rate. (6, 30, 53) Earliest advancements in reconstruction, achieved due to progress

in bone tissue engineering and materials science, include tumor bone inactivation replantation, allogeneic bone transplantation, autologous bone transplantation, and artificial prosthetic replacement. (46)

In situations with poor response to neoadjuvant chemotherapy, limb salvage surgery should be approached with caution. Several considerations for limb salvage surgery include ensuring that the tumor can be resected as a single block without contaminating surrounding tissues, therefore decisions should be made collaboratively with an experienced oncological and surgical team.

Amputation is considered when marginal or intralesional resection is not avoidable, as this approach reduces the risk of local recurrence and death and is indicated when complete tumor removal without leaving residual disease is not feasible. Extensive involvement of neurovascular structures may also favor amputation in prospect of a better outcome. The extent of amputation depends on intraosseous tumor involvement and the possibility of sufficient skin closure. (30, 52, 53)

Surgical resection of sarcomas in the axial skeleton remains complex, primarily due to the significant risk of local recurrence and the frequent occurrence of complications following reconstruction. Recent advancements in this field include total en bloc spondylectomy for vertebral sarcomas and hip transposition for pelvic tumors. (52)

2.4.9.3 Radiotherapy

Radiation therapy plays a minor role in the treatment process of osteosarcoma, as it was considered as a radioresistant tumor for a long time. (46, 52) Nowadays, local radiotherapy is used in patients, where surgical resection is impossible, resections is on/within the margins of the tumor or neoadjuvant chemotherapy shows poor response. This applies especially to osteosarcoma in the axial skeleton, where en bloc resection remains difficult. Within the last years, radiosensitizers became the main interest in clinical research, with the goal to increase the sensitivity of tumor cells and kill them without damage of healthy tissue through radiotherapy. (46)

2.4.9.4 Supportive Care

Simultaneously with therapeutic advancements in polychemotherapy in osteosarcoma management, several agents have been developed to mitigate

chemotherapy-related toxicity. The introduction of serotonin antagonists, such as Ondansetron, have significantly reduced emesis induced by chemotherapy. These agents, either alone or combined with Dexamethasone, have become the common method, particularly in the use of highly emetogenic chemotherapy agents such as cisplatin. Other agents employed in the supportive care of osteosarcoma patients are opioids for managing tumor pain and hematopoietic growth factors to decrease the incidence and duration of severe chemotherapy-induced granulocytopenia. (52)

2.4.10 Metastatic disease

At the time of osteosarcoma diagnosis, already 15-20% of all patients have metastasis, with the lungs being affected most commonly. Tumor cells of osteosarcoma typically spread via the hematogenous route. (57) In bone microenvironment, according to Yang et al., mesenchymal stem cells (MSCs) of bone are the most frequent reason for the development of osteosarcoma metastasis (58). In general, MSCs are pluripotent stem cells, highly related to tumor growth and development of metastasis. In case of osteosarcoma, deficiencies in *TP53, RB* and *P16/Cdkn2a* are common reasons for transition of MSCs to osteosarcoma cells. (58) Metastatic disease in osteosarcoma is still linked to a poor prognosis, with only around 20-30% long term survival compared to 60-70% in patients with localised disease (see *chapter 2.4.11: Prognosis*.) (52, 53)

Patients with metastases are approached with a curative intent, as long as the removal of all metastatic deposits through resection is possible. Even in cases where multiple regions and sites are involved, patients can exhibit a prognosis akin to those with localized disease, provided that all deposits can be surgically removed. The curative approach for primary metastatic osteosarcoma mirrors that of localized disease, with the essential inclusion of surgical removal of all identified metastatic foci, typically performed through exploratory thoracotomy, involving the palpation of the entire lung. (52, 57) The role of second-line chemotherapy for recurring localized or metastatic osteosarcoma is less clearly defined than that of surgery, lacking an accepted standard regimen. The choice may consider the prior disease free interval, resectability, and often involves Ifosfamide, Etoposide, and Carboplatin. (52)

2.4.11 Prognosis

Osteosarcoma remains a rare and aggressive disease with a challenging prognosis, reflected by an event-free survival (EFS) of approximately 50-60%. (52, 53) Definitive patient prognosis can only be made during the later stages of the disease, where the outcome of surgical resection quality and chemotherapy response plays a crucial role. Despite therapeutic advancements, around 90% of osteosarcoma patients die from the disease. During therapy, the most common reason for death is pancytopenia, while later on, the deaths in remission are mainly caused by secondary malignant tumors and cardiomyopathy. (1, 6, 8, 59)

The main predictor in primary osteosarcomas' outcome is significantly influenced by the patient's response to pre-operative chemotherapy, as Bielack et al. reported that the timing of surgery does not influence the prognosis. (6) They found no difference in EFS between patients with primary chemotherapy and those with delayed beginning of chemotherapy. Notably, the EURAMOS-1 trial recommends surgery in week 11, after six cycles of neoadjuvant chemotherapy (30).

Adverse prognostic factors include large tumor size, male gender, older age, proximal tumor localization, elevated alkaline phosphatase (AP) and/or lactate dehydrogenase (LDH) values, and the presence of metastases. (6)

The correlation between tumor size and chemotherapy response is lacking, emphasizing the repeated confirmation of chemotherapy response as the most critical prognostic factor. The duration of symptoms does not exhibit a statistically significant correlation with outcome, whereas a longer history confers to a higher risk of poor response. Pre-diagnostic symptoms and previous malignancies show no correlation with prognosis. (6)

Females tend to have a more favorable prognosis, with potential explanations pointing toward hormonal influences, as observed in earlier onset and better chemotherapy response among girls. Female patients exhibit fewer relapses and better overall survival, further establishing being female as a positive prognostic factor. (1, 6) Age above 40 years and a proximal or axial tumor site are associated with a poorer prognosis, with humerus-based osteosarcomas demonstrating the worst prognosis among extremity tumors, while tibia-based tumors exhibit a more favorable outlook. (1, 6)

Elevated AP and/or LDH levels are identified as negative prognostic factors. (21) Additionally, various molecules and biomarkers, such as low TNF-level, epidermal growth factor, hepatocyte growth factor, and prolactin have been implicated in predicting metastatic spread and increased mortality risk. (60) While the presence of primary metastases generally leads to a poor prognosis, the occurrence of lung metastases alone is comparatively better than combined metastatic occurrences. (1, 6, 61)

3 Material and methods

3.1 Patients

We performed a retrospective study of a single center cohort database analyzing 144 patients with histologically confirmed osteosarcoma, which have been diagnosed and treated at the Medical University Hospital of Graz, a reference center for soft tissue and bone tumors, between 2000 and 2020. Out of 144 Patients, 65 got excluded and 79 were analyzed. Reasons for exclusion were age under 18 years as we only included adult patients (46 patients, 31.9%), patients, that only underwent surgery at the Department of Orthopedic Surgery at the Medical University Hospital of Graz and received chemotherapy and follow-up in their home-hospitals (9 patients, 6.3 %) and patients with missing data (10 patients, 6.9%). To ensure data protection, all patients data got anonymized and received ongoing numbers for further statistical analyzation.

3.2 Material

Literature research using medical reference books, scientific database including PubMed, was conducted. Patients' data collection happened via documents stored in our hospital information system MEDOCS.

Regarding chemotherapy, the Medical University Hospital of Graz generally applies *EURAMOS-1* to all patients under the age of 40 and *EURO-B.O.S.S.* scheme for patients over 40 years. Exact information on individually used chemotherapeutic agents was not collected, a difference is only made whether or not polychemotherapy (PCT) was applied.

3.3 Methods

Basic characteristics included age at time of diagnosis, sex, and relevant past medical history. Data including clinical appearance7symptoms , radiological findings (location, affected side, number and size of the lesion(s), joint involvement), histological diagnosis (classification) and grading. Further on, therapy process, such as neoadjuvant chemotherapy, Salzer-Kuntschik Regression Grading after neoadjuvant therapy, limb-salvage surgery vs. amputation, adjuvant chemotherapy and palliative setting was analyzed. Attention was also given to the further process

of the disease including the occurrence of metastasis, their location, recurrence of the disease, follow-up time and outcome. Patients' information was recorded with Microsoft Excel for Mac Version 16.8. that was protected with password.

4 Results

4.1 Basic characteristics

From January 2000 to December 2020, 79 adult patients with histologically confirmed osteosarcoma were included in our patients' data analyzation. The age-range was between 18-93 years with a mean age of 45.8 years, with 48 patients (60.8%) being under 50 years and 31 patients (39.2%) being over 50 years. The exact age distribution in decades is shown in *Table 13*.

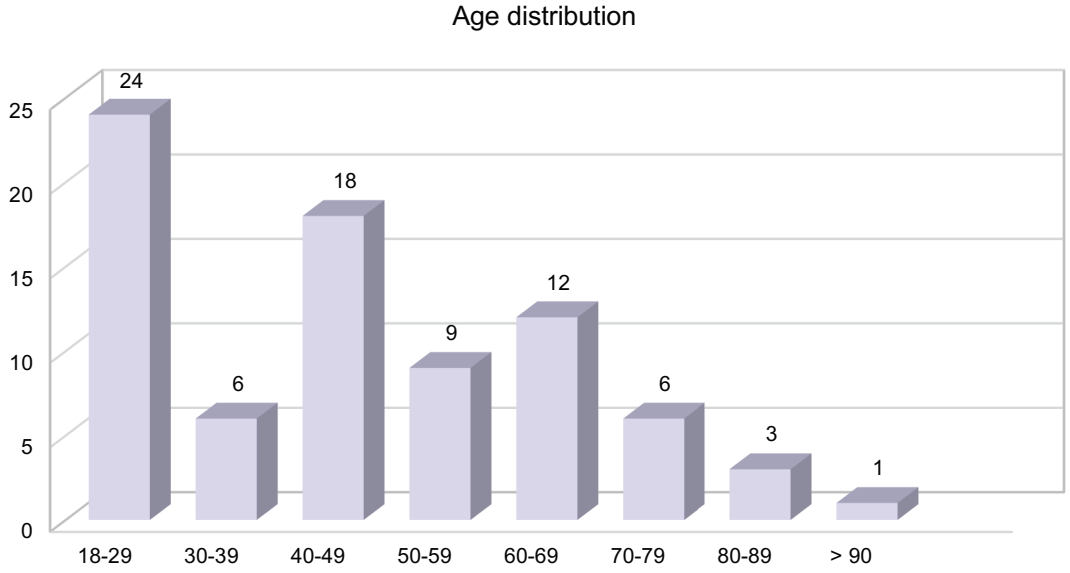


Figure 19: Age distribution.

The gender distribution is almost equal with 40 females (50.6%) and 39 males (49.4%). 18 patients (22.8%) had relevant events in their past medical history: Five patients (6.3%) underwent resection of benign or undefined bone lesions, eight patients (10.1%) suffered from malignant tumors of other organs, two patients had osteosarcoma in another location before (2.5%), two patients had fractures in the same anatomical region (2.5%) and one patient (1.3%) recently received endoprosthesis in the location of the osteosarcoma.

		79 patients	%
Age	Mean age (min - max) in years	45.8 (18 - 93)	
	< 50 years	48	60.8
	> 50 years	31	39.4
Gender	Male	39	49.4
	Female	40	50.6
Relevant past medical history	Yes	18	22.8
	No	61	77.2

Table 13: Basic characteristics of the patients.

4.2 Clinical, radiological and pathological findings

Clinics. The time between first symptoms and diagnosis was recorded in 53 patients with a mean delay of 5.3 months (range from 0.5 to 36 months) except for one patient with recurring pain-episodes for 60 months. Osteosarcoma was an incidental finding in 4 patients (5.1%).

Radiology. Radiologically, 58 patients (73.4%) received MRI for diagnosis, eleven patients (13.9%) received CT whereas in 10 patients (12.7%) MRI and CT were applied to enable more precise radiologic diagnosing.

Localization. Most cases (35 cases, 44.3%) were seen around the knee (including the distal femur and proximal tibia and fibula). The second most common location, with 18 cases (22.8%), was the pelvis including all pelvine bones and the proximal femur. The upper limb was affected eight times (10.1%), followed by trunk in seven cases (8.9%) and shoulder girdle in six cases (7.6%). Three osteosarcomas (3.8%) were uncommonly found in the diaphyseal femur, two (2.5%) in the lower leg, exactly in the calcaneus.

Affected side. Equally**,** 36 osteosarcomas (45.6%) could be found of the right side and another 36 on the left side. Seven osteosarcomas (8.9%) had central location within the trunk.

Number and size of the lesion(s). In 91.1% (72 cases) a single lesion could be found, in six cases (7.6%) there were two lesions. Three lesions could only be found in one patient (1.3%). Lesion varied greatly in size (range 1.9cm in smallest dimension and 20.3cm in biggest dimension). The smallest lesion at time of

diagnosis was 2.8x1.9x1.9cm (in vertebra) whereas the biggest lesion (in proximal femur + pelvis) was 20.3x12.5x12.3cm in size.

Joint involvement. Involvement of the adjacent joint was seen in 48 patients (60.8%) of which two recently received endoprosthesis of the knee. In 31 patients (39.2%) the adjacent joints showed no involvement in the malignant process.

Type of specimen taken for the diagnosis. Specimens taken for diagnosis included biopsy in 62 cases (78.5%) and curettage in 15 cases (19.0%). In two patients (2.5%) histologically confirmed diagnosis was made postoperative through analysis of the resection specimen.

		79 patients	%
Clinics	Mean delay until diagnosis (min. – max.) in months	5.3 (0.5 – 36)	
	Symptomatic patients	53	67.1
	Incidental finding	4	5.1
	Unknown	22	27.8
Radiology	MRI	58	73.4
	CT	11	13.9
	MRI + CT	10	12.7
Specimen type	Biopsy	62	78.5
	Curettage	15	19.0
	Resection specimen	2	2.5
Number of lesions	1	72	91.1
	2	6	7.6
	3	1	1.3
Joint involvement	Yes	48	60.8
	No	31	39.2

Table 14: Clinical and radiological findings including type of the specimen taken for the diagnosis.

Osteosarcoma histological type. Five different types of osteosarcomas were found in our patients: conventional osteosarcoma (56 cases, 70.9%), parosteal osteosarcoma (11 cases, 13.9%), low-grade central osteosarcoma (6 cases, 7.6%), high-grade surface osteosarcoma (2 cases, 2.5%) and secondary osteosarcoma (4 cases, 5.1%). In the patients with conventional osteosarcoma, predominant histological type was osteoblastic osteosarcoma (27 cases, 34.2%) followed by

fibroblastic (10 cases, 12.7%), chondroblastic (7 cases, 8.9%) and small cell (2 cases, 2.5%). 10 cases (12.7%) were categorized “mixed” with almost equal amounts of at least two of the mentioned above.

Grading. Low grade central osteosarcoma was always categorized as low-grade (G1). Parosteal osteosarcoma in our patients was graded G1 in six cases, G2 (intermediate-grade) in four cases with long growth-duration and intramedullary expanse and one case as dedifferentiated parosteal osteosarcoma, G3. Conventional osteosarcoma, high-grade surface osteosarcoma and secondary osteosarcoma were always graded high-grade (G3) tumors.

		79 patients	%
Osteosarcoma type	Conventional osteosarcoma	56	70.9
	Osteoblastic	27	34.2
	Fibroblastic	10	12.7
	Chondroblastic	7	8.9
	Small-cell	2	2.5
	Mixed	10	12.7
	Parosteal Osteosarcoma	11	13.9
	Low-grade central osteosarcoma	6	7.6
	High-grade surface osteosarcoma	2	2.5
	Secondary osteosarcoma	4	5.1
Localization	Knee [a]	35	44.3
	Hip and pelvis [b]	18	22.8
	Upper limb	8	10.1
	Trunk [c]	7	8.9
	Shoulder girdle [d]	6	7.6
	Diaphyseal femur	3	3.8
	Lower leg	2	2.5
Affected side	Right	36	45.6
	Left	36	45.6
	Central/Trunk	7	8.9
Grading	G1 (low-grade)	11	13.9
	G2 (intermediate-grade)	5	6.3
	G3 (high-grade)	63	79.7

[a] Knee includes distal femur, proximal tibia and proximal fibula.
[b] Hip and pelvis includes all pelvine bones and proximal femur.
[c] Trunk includes spine, ribs and sternum.
[d] Shoulder girdle includes proximal humerus, scapula and clavicula.

Table 15: Distribution by osteosarcoma type, localization, side and grading.

4.3 Treatment

35 patients (44.3%) fulfilled the requirements for treatment following the recommended scheme including neoadjuvant chemotherapy, surgery and adjuvant chemotherapy according to *EURAMOS-1* or *EURO B.O.S.S.* scheme.

Amongst the 35 patients that received neoadjuvant chemotherapy, there were 14 patients (17.7%) with “good response” (vital tumor cells <10%; Salzer-Kuntschik regression grading I – III) and 21 patients (26.5%) with “poor response” (vital tumor cells >10%, Salzer-Kuntschik regression grading IV-VI).

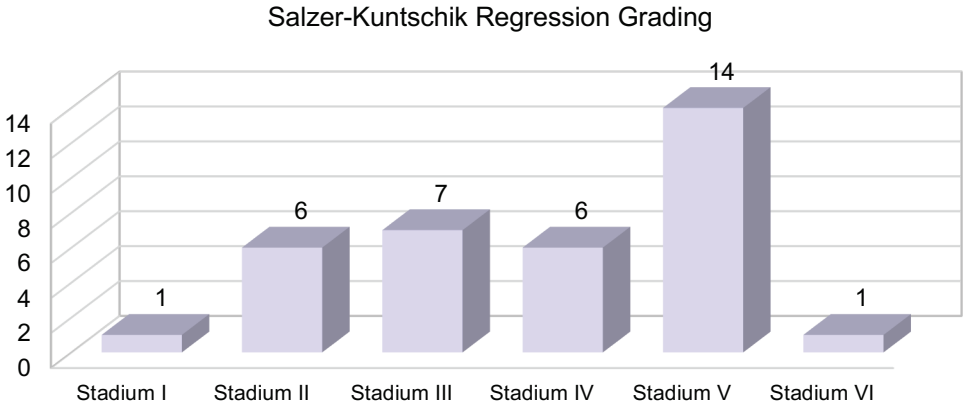


Figure 20: Salzer-Kuntschik regression grading in 35 patients with neoadjuvant chemotherapy.

70 patients (88.6%) underwent surgery: 55 (69.6%) of them received limb-salvage surgery while 15 (19.0%) underwent amputation. From 55 limb-salvage surgery patients, 39 patients (70.9%) received resection with following implantation of tumor endoprosthesis, 4 patients (5.1%) had resection without reconstruction. In four cases (5.1%) reconstruction was performed via allograft and osteosynthesis, in one case (1.3%) allograft and additional prosthesis were implanted. Autograft in combination with osteosynthesis was used in two patients (2.5%). In all five patients with vertebral osteosarcoma, the affected bone was resected, and the vertebra was stabilized by osteosynthesis.

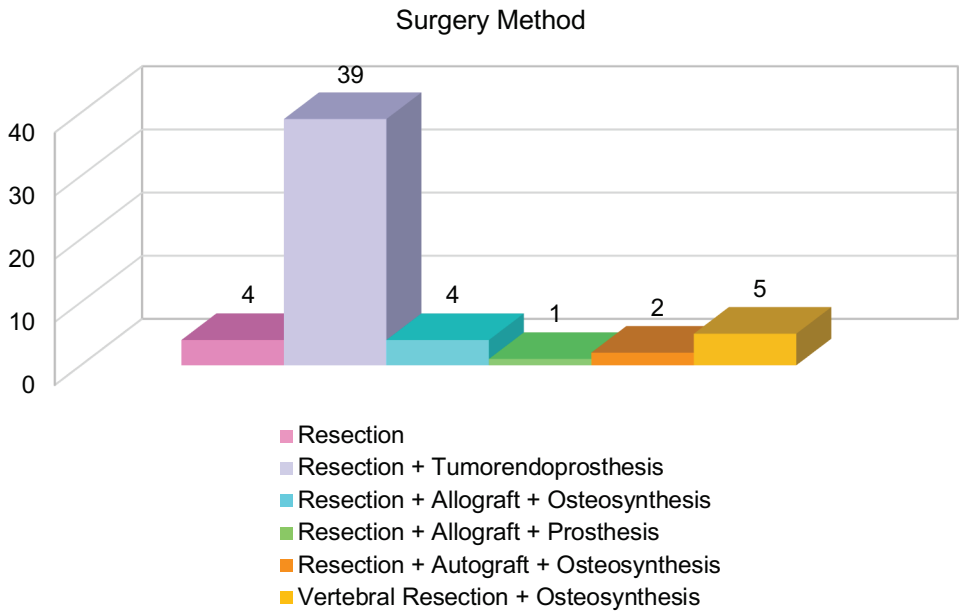


Figure 21: Distribution of surgery methods.

24 patients (30.8%) had surgery without additional application of chemotherapy. Six patients (7.6%) underwent surgery and only received chemotherapy afterwards. Of note, one of these patients was diagnosed with osteosarcoma after resection with histological verification on resection material.

			79 patients	%
Surgery	Yes		70	88.6
		Limb salvage	55	69.6
		Amputation	15	19.0
	No		9	11.4
Chemotherapy	Yes		48	60.8
		Neoadjuvant + Adjuvant	35	44.3
		Adjuvant	6	7.6
		Palliative	7	8.9
	No		31	39.2
Palliative treatment	Yes		16	20.2
		Primary palliative setting	6	7.6
		After primary curative approach	10	12.7
	No		63	79.8

Table 16: Distribution in treatment.

Palliative treatment was applied to 16 patients (20.3%), six of them went straight into palliative care because of advanced disease at diagnosis or inoperability while ten patients received it following unsuccessful primarily curative approach. Radiotherapy was applied only in three patients (3.8%), all of them in palliative setting. One patient (1.3%) refused all kind of therapy.

4.4 Progression and follow-up

Metastasis. Metastasis were seen in 37 patients (46.8%) whereas in 42 patients (53.2%) no metastasis were detected. 17 patients (45.9%) had monolocular metastasis while 20 patients (54.1%) had multilocular metastasis in more than one region displayed. Notably, 34 (91.9%) of 37 patients with metastasis had metastasis in their lungs. Bone constitutes the second most common area for metastasis (27.0%), followed by intraabdominal metastasis (24.3%), lymphnode metastasis (6.3%), cerebral metastasis (3.8%) and skin metastasis (2.5%). Hematogenous metastasis, mediastinal metastasis as well as breast and ovary metastasis individually occurred once.

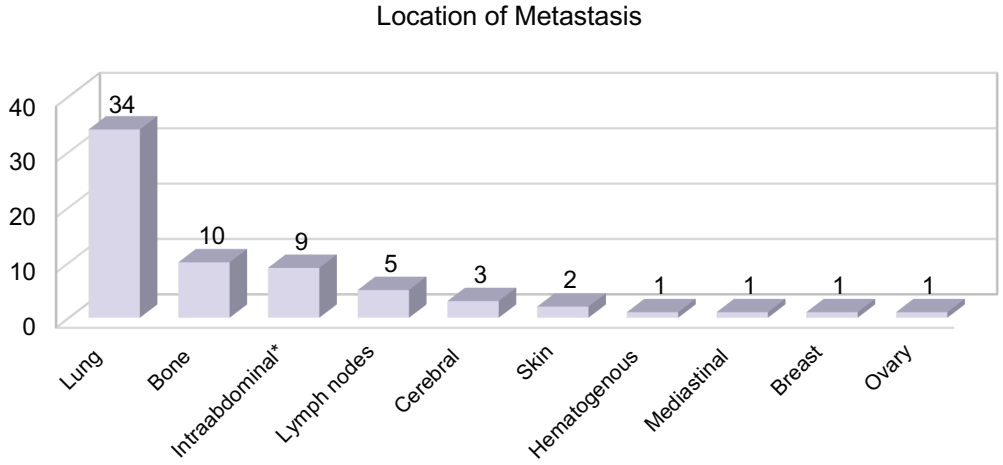


Figure 22: Distribution of location of metastasis.

24/37 (64.9%) patients with metastasis died from disease (DOD) while another 13 patients (35.1%) with metastatic disease were alive with metastasis at last follow-up. 31/56 (55.6%) patients with conventional osteosarcoma had metastasis. In

secondary osteosarcoma metastasis were seen in 3/4 (75%) patients and 2/2 (100%) patients with high-grade surface osteosarcoma developed metastasis. In parosteal osteosarcoma metastasis got detected in 2/11 (18.2%) patients. Patients with low-grade central osteosarcoma developed no metastasis during follow-up time.

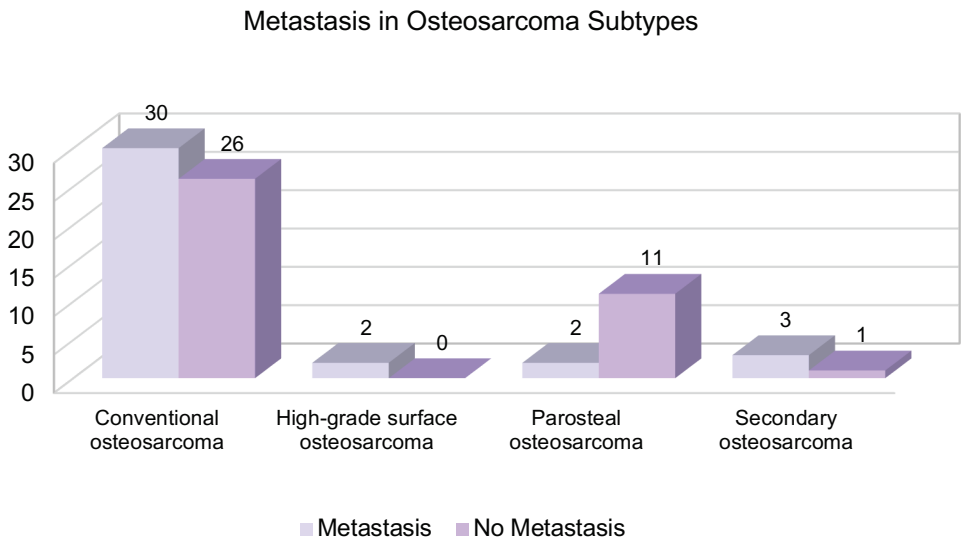


Figure 23: Distribution of metastasis according to osteosarcoma subtype.

Recurrence. Eight patients (10.1%) developed local recurrence of the tumor. Six of them DOD and two were alive at last follow up.

Follow-up. Mean follow-up time was 50.7 months (range from 1 to 256 months) in all patients. In detail, 53 patients (67.1%) were alive with mean follow-up time of 56.2 months (range from 1 to 210 months). 25 patients (31.6%) DOD and their mean follow-up time was 33.3 months (range from 2 to 256 months). Most of these patients died due to respiratory insufficiency in consequence of pulmonary metastasis. In 27 patients (34.2%) no evident information was given about whether they died from disease or not. These patients were assumed as “no DOD” in all other context where DOD was relevant.

		79 patients	%
Metastasis	Yes	37	46.8
	No	42	53.2
Recurrence	Yes	8	10.1
	No	71	89.9
Follow-up time	All patients	50.7 (1 - 256)	
(Mean, min.- max. in months)	Patients without DOD	56.2 (1 - 210)	
	Patients with DOD	33.3 (2 - 256)	
Dead of disease (DOD)	Yes	25	31.6
	No	53	68.4

Table 17: Development and follow-up in our osteosarcoma patients.

4.5 Comparison in patients under/over 50 years

To outline differences between younger and older patients, we divided our cohort in two groups: patients diagnosed under 50 years (48 patients, 60.8%) and over 50 years (31 patients, 39.2%). The mean age in the younger group was 32.4 years (range from 18 to 49 years) and 66.5 years (range from 50 to 93 years) in the older patients group. The mean time from onset of symptoms to verified diagnosis was 6.6 months (range from 0.5 to 30 months) in the younger group and 6.9 months (range from 0.75 to 60 months) in the older group. In 4/31 (12.9%) cases of patients over 50 years, osteosarcoma was an incidental finding. There were no incidental findings in the younger patients.

In both groups there were more female than male patients, 58.3% in the younger and 64.5% in the older group. The rate of axial bone involvement was slightly higher in the younger group (10.4%) than in the older one (6.5%). In the younger group, patients without metastasis were more frequent (56.2% vs 43.8% with metastasis) while in the older group, with 54.8%, more than half of the patients developed metastasis. In both groups, approximately 90% of patients (85.4% in younger group, 93.5% in older group) underwent surgery.

Grading (G1 to G3) showed approximately similar distribution in both groups. In patients under 50 years, we found six (12.5%) low-grade (G1), three (6.5%) intermediate-grade (G2) and 39 (81.3%) high grade (G3) tumors. The distribution in

the older group was as follows: five (16.1%) low-grade (G1), two (4.4%) intermediate-grade (G2) and 24 (77.4%) high grade (G3) tumors.

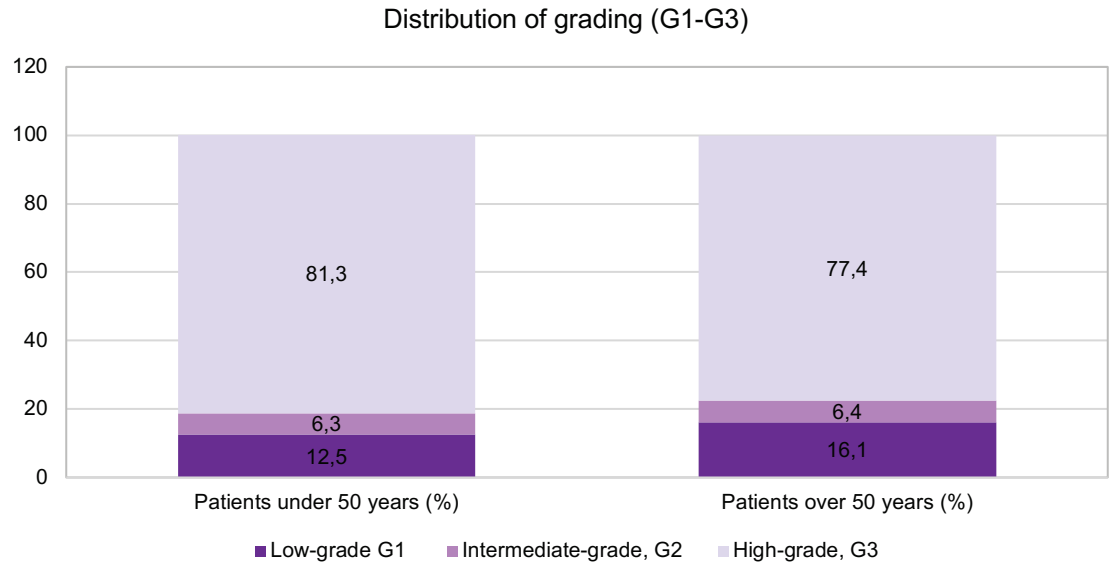


Table 18: Distribution of tumor grading (G1-G3) compared in patients under and over 50 years.

In the younger group, surgery of the primary tumor was performed in 85.4% (41/48 patients) while in the older group with 93.5% (29/31 patients) the percentage was slightly higher. Overall, the amount of limb-sparing surgery was significantly higher in patients under 50 years (82.9% in the younger group, 72.4% in the older group) than the amputation-rate (17.1% in the younger group, 27.6% in the older group).

The application-rate of chemotherapy outlines a main difference between the younger and the older group. While in the younger group 81.3% (39/48 patients) received chemotherapy, in the older group chemotherapy was only applied in 7/31 (22.6%) cases. Neoadjuvant chemotherapy, following the nowadays recommended scheme in osteosarcoma therapy, was applied in 30/48 (62.5%) of the younger group, while only 5/26 (16.1%) patients over 50 years received neoadjuvant chemotherapy.

The effectiveness of neoadjuvant chemotherapy in every patient was analyzed using “Salzer-Kuntschik regression grading” scheme, divided in “good response” (vital tumor cells <10%; Salzer-Kuntschik regression grading I – III) and “poor response” (vital tumor cells >10%, Salzer-Kuntschik regression grading IV-VI). In

the younger group, 13 (43.3%) patients showed “good response” and 17 (56.7%) showed bad response. In the older patients only one patient (20%) showed “good response” while four (80%) patients showed “bad response”.

	Under 50 years	%	Over 50 years	%
Patients	48 (60.8)		31 (39.2)	
Mean Age, years (min.-max.)	32.4 (18-49)		66.5 (50-93)	
Mean time from onset of symptoms to diagnosis in months (min.-max.)	6.6 (0.5-36)		6.9 (0.75-60)	
Incidental finding				
Present/none	0/48	0/100	4/27	12.9/87.1
Male/Female ratio	20/28	41.7/58.3	11/20	35.5/64.5
Extremity/axial ratio	43/5	89.6/10.4	29/2	93.5/6.5
Metastasis				
Present/none	21/27	43.8/56.2	17/14	54.8/45.2
Grading				
G1/G2/G3	6/3/39	12.5/6.3/81.3	5/2/24	16.1/6.4/77.4
Surgery				
Present/none	41/7	85.4/14.6	29/2	93.5/6.5
Chemotherapy, all				
Present/none	39/9	81.3/18.7	7/24	22.6/77.4
Chemotherapy, neoadjuvant				
Present/none	30/18	62.5/37.5	5/26	16.1/83.9
Salzer-Kuntschik regression grading				
I-III/IV-VI	13/17	43.3/56.7	1/4	20/80
Palliative treatment				
Present/none	7/41	14.6/85.4	8/23	25.8/74.2
Local recurrence				
Present/none	3/45	6.2/93.8	5/26	16.1/83.9
DOD				
Present/none	15/33	31.3/68.7	10/21	32.3/67.7
Mean time until DOD in months (min.-max.)	37.9 (2-256)		26.5 (7-92)	
Mean Follow-up time in months (min.-max.)	53.4 (2-256)		46.5 (1-133)	

Table 19: Comparison between patients under and over the age of 50

Local recurrence occurred in 3 (6.2%) patients from the younger group and 5 (16.1%) patients from the older group. In both groups almost equally, approximately

32% of patients DOD (31.3% in the younger group, 32.3% in the older group). While in the younger group mean time until death was 37.9 months (range from 2 to 256 months), mean time in the older group was 26.5 (range from 7 to 92 months). Mean follow-up time for all was 53.4 months (range from 2 to 256 months) in the younger group and 46.5 months (range from 1 to 133 months) in the older group.

5 Discussion

Osteosarcoma is an aggressive disease with EFS of 50-60% and poor prognosis. Since the invention of chemotherapy in the treatment of osteosarcoma in the 1970s, there has been no breakthrough in the treatment of bone tumors. (1, 6, 8, 59, 62, 63). Most osteosarcoma patients are in their second and third decade of life at diagnosis. However, due to longer life expectancy, the rate of osteosarcoma in elderly patients is increasing. (64, 65) In this retrospective study of a single center cohort, we confirmed previously published data and observed some relevant clinical findings despite the relatively small number of patients.

A bimodal age distribution is common in osteosarcoma. (2, 4, 5, 8, 43, 63) The distribution in our patients also shows the common first peak in the second/third decade of life and a second one after the age of 40 (see *Table 13*).

A slight male dominance (M:F ratio: 1.3:1) for osteosarcoma is described in the literature. (2, 4, 5, 43) Nevertheless, in our study there were more female than male patients, 58.3% (1:1.4) in the younger group and 64.5% (1:1.8) in the older group.

In our cohort, the osteosarcoma arose most commonly around the knee (including the distal femur and proximal tibia and fibula) with 44.3% (35/79 cases), followed by the hip and pelvis with 22.8% (18/79 cases). This distribution in localisation of osteosarcoma equals the characteristic site distribution described in literature (2, 4, 5, 8, 43). In our patients, with 8.8%, axial bone involvement was below the rate of axial occurrence (between 15 to 48%) reported in other studies. (63, 65-68) Even though a higher axial bone involvement in older patients is described in several articles (64-67, 69, 70), in our older patients group it was even lower than in the younger group (6.5% vs. 10.4%).

In our cohort, high-grade conventional osteosarcoma including osteoblastic, fibroblastic and chondroblastic subtype was the most frequent type. Concerning the distribution of histological subtypes of conventional osteosarcoma, osteoblastic osteosarcoma is described to be most frequently seen (76-80%), followed by chondroblastic osteosarcoma (10-13%), and fibroblastic osteosarcoma (10%) as the third most common subtype. (4, 5) Other studies also describe osteoblastic osteosarcoma to be the most frequent one, followed by varying amounts of

fibroblastic and chondroblastic osteosarcoma as the second most common subtype. (63, 64, 67) The frequencies of different. Subtyped in our cohort were similar: 48.2% (27/56 cases) were osteoblastic osteosarcomas, 17.9% (10/56 cases) were fibroblastic, 12.5% (7/56 cases) were chondroblastic and the remaining 21.4% (12/56 cases) were rather mixed or small-cell osteosarcomas.

Secondary osteosarcoma is frequently described to mainly arise in elderly patients. (69) In our patients we found four cases of secondary osteosarcoma of which three were seen in patients under the age of 50 (one in second decade, two in fifth decade) and only one over the age of 50, at 69 years.

Therapy options for osteosarcoma are limited. Most commonly, patients undergo resection and, if applicable, receive chemotherapy (depending on the age of the patient, tumor grade and stage). Many studies report prolonged disease-free survival after application of neoadjuvant and adjuvant chemotherapy combined with radical surgery. (63, 64, 66, 67, 69, 70) In addition, various studies outline the need of additional approaches to identify more aetiological and prognostic factors to maximize treatment options such as growth receptor modulators, biologic response modifiers and angiogenesis factors and make them accessible and adaptable to all patients' groups. (1, 6, 8, 59, 62, 63) Majority of our patients (88.6%) (underwent resection of the tumour (81.3% patients in the younger and 93.5% patients in the older group). The limb-salvage surgery with following implantation of tumour endoprosthesis was the most frequently used therapy option. As also described in one study (64), we could see a higher amputation rate in the older patients' group (27.6% vs. 17.1%). Notably, all of the eight patients that did not undergo surgery (seven because of advanced disease or inoperability and one that refused all kind of therapy) DOD.

Due to comorbidities, chemotherapy is often contraindicated in older patients. (67) The application rate of neoadjuvant chemotherapy in the published studies was low. (63-65) This was also observed in our study - the rate of chemotherapy applied in the older group, with 22.6% (7/31 cases), was significantly lower than in in the younger group with a rate of 81.3% (39/48 cases). In all patients that receive neoadjuvant chemotherapy, the effectiveness is evaluated by determining "Salzer-Kuntschik regression grading".

Effectiveness of neoadjuvant chemotherapy described in the literature shows different results. Bacci et al. reported good response following neoadjuvant chemotherapy in both, younger and older patients. In contrast, other studies described a higher number of patients with “poor response” rather than “good response”. (63, 65, 69, 71), a finding that was seen in our patients. Out of 35 patients that received chemotherapy prior to resection, 60% showed “poor response” (Salzer-Kuntschik regression grading stadium IV-VI) while 40% showed “good response” (Salzer-Kuntschik regression grading stadium I-III). With 4/5 (80%) cases in the older group, the rate of “poor response” in patients that received neoadjuvant treatment was even higher than in the younger group with 17/30 (56.7%) cases.

Radiotherapy plays a minor role in osteosarcoma treatment and is mainly applied to patients in palliative setting or patients with intralesional excision. (2, 46, 52, 63, 64) In our patients, only four patients (5.1%) received radiotherapy, all of them in palliative setting.

Osteosarcoma patients frequently develop metastasis, most commonly in the lungs (2, 9, 64) In our study, metastasis occurred in 38/79 (48.1%) cases and notably, the lungs were most frequently affected (in 91.2% of cases). Out of 25 patients that DOD, 23 (92%) had metastasis and only two (8%) died without diagnosed metastasis. This finding shows that the presence of metastasis is a prognostic factor for poorer outcome. 13/38 (34.2%) patients that developed metastasis did not DOD during follow-up time.

There are some limitations of this study. First difficulty in this retrospective study of a single center cohort database was the use of our hospital information system MEDOCS. We noticed the major improvement in documentation within the last years. As the study encompassed cases within the timespan of 20 years, in patients diagnosed in the beginning of the 2000s, the data was scars. Second limitation was the differences in treatment as various chemotherapy regimens were used (even though all patients that fulfilled the requirements were treated according to *EURAMOS-1* scheme (for patients under the age of 40) and *EURO-B.O.S.S.* scheme (for patients over the age of 40 years)). Finally, as we only have follow-up data for 5 years in 28 (35.4%) patients, it is not possible to make a valid statement about 5-year overall survival (OS). Additional follow-up data for 10 years was only

available in nine (11.4%) patients. Therefore, this study does not reveal novel findings regarding the long-term prognosis in adult patients with osteosarcoma.

Our results show that high-grade conventional osteosarcoma is the most frequent subtype occurring in the adults, most commonly arising in the knee region. The limb-salvage surgery with implantation of tumor endoprosthesis is a standard treatment option for all patients, whereas the use of chemotherapy in the elderly patients remains low. Considering increasing life span in the general population and therefore a probable higher incidence of osteosarcoma in the elderly, further studies in the geriatric population with osteosarcoma regarding possible treatment options are warrant.

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