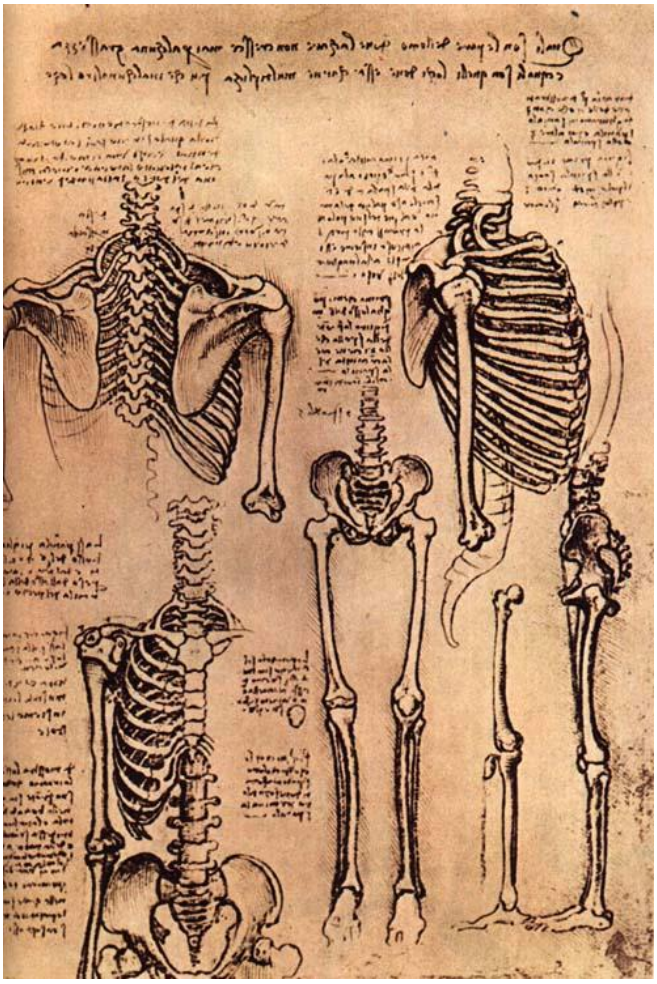
Phenotypic differences and similarities in fibro-osseous tumours of bone: implications for clinical practice and disease management



Esther I. Hauben

Cover: Leonardo da Vinci, The skeleton of the trunk and legs, 1509-1510

Phenotypic differences and similarities in fibro-osseous tumours of bone: implications for clinical practice and disease management

PROEFSCHRIFT

ter verkrijging van
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door

Esther Irène Hauben

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Promotiecommissie

Promotores	: Prof. Dr. P.C.W. Hogendoorn
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Misschien is niets geheel de waarheid, en zelfs dát niet.

[Multatuli, ideeën, eerste bundel, idee 1, 1862]

Aan mijn moeder, Dirk en Moira

Stellingen

behorende bij het proefschrift

Phenotypic differences and similarities in fibro-osseous tumours of bone: implications for clinical practice and disease management

1. Een globaal slechte histologische respons van het chondroblastair ostesarcoom op neo-adjuvante chemotherapie resulteert niet in een slechtere overlevingskans zoals gezien wordt bij osteosarcoom in het algemeen.
(dit proefschrift)

2. Bij jonge patiënten die zich presenteren met een primair osteosarcoom dient men bedacht te zijn op een erfelijk kankersyndroom, en meer nog als het een niet osteoblastair subtype osteosarcoom betreft.
(dit proefschrift)

3. Ewing sarcoom en het adamantinoom van de lange beenderen behoren niet tot éénzelfde tumorfamilie.
(dit proefschrift)

4. De β-catenine route speelt geen essentiële rol in de genese van het desmoplastisch fibroom van het bot, in tegenstelling tot bij desmoid type fibromatose.
(Dit proefschrift)

5. Het vrouwelijke geslacht is een risicofactor voor de ontwikkeling van een tweede primaire tumor.
(Neglia J.P. et al. J Nat Cancer Inst 2001, 93(8):618-29 en dit proefschrift)

6. Osteosarcoom is geen entiteit maar een verzameling van biologisch uiteenlopende tumoren, waarbij de directe afzetting van osteoid en/of bot door de tumorcellen de gemeenschappelijke factor is die hen met elkaar verbindt.
(Raymond A.K. et al. Semin Diagn Pathol 1987, 4(3):212-236)

7. Bij het opzetten van studies met betrekking tot het hooggradig osteosarcoom is het aan te bevelen een onderscheid te maken naargelang het histologisch subtype.

8. Een prospectief opgestelde gegevensbank bevat retrospectief meestal te weinig informatie.

9. Noord- en Zuid-Nederlands, zo gelijkend, maar oh, zo verschillend.
(naar William Wordsworth 1770-1850)

10. De aandrang om nog op het laatste nippertje door te rijden bij vast oranje licht is recht evenredig met het aantal verkeerslichten per 1000m.

Leiden, 1 maart 2006

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1
General Introduction

Primary non-haematogenic tumours of bones are in general rare, accounting for 0.2% of human neoplasms, but these tumours affect mostly children. Based on their histomorphology they are classified in the 2002 WHO classification as chondrogenic, osteogenic, fibrogenic, histiocytic, vascular, neurogenic, lipogenic, notochordal and of unknown histologic type (Table 1) (1). The chondrogenic tumours share the common characteristic of producing chondroid matrix. Osteogenic tumours are defined as any tumour in which osteoid is directly produced by the neoplastic cells. As fibrogenic tumours those lesions are grouped that do not have a mineralising matrix, but generally produce collagen. From a histological point of view, tumours with variable amounts of bone and fibrous tissue or fibrous tissue only can be joined under the heading of osteofibrous tumours. This covers a spectrum from benign to malignant lesions and includes several entities with overlapping histological features suggesting a potential relationship between those entities.
At one end of the spectrum of osteofibrous tumours there is osteosarcoma, the most frequent malignant non-haematogenic bone tumour, at the other end there is the exceedingly rare benign, though potentially locally aggressive desmoplastic fibroma (2).
The phenotypic spectrum of the osteofibrous lesions is mostly a reflection of the spectrum of genetic disturbances involved. This ranges from complex karyotypes in high-grade intramedullar osteosarcoma over numeral changes, translocations as in Ewing sarcoma to point mutations in benign lesions like fibrous dysplasia.
Due to the rarity of these tumours, and for the patients affected by them to benefit from optimal treatment, diagnosis and treatment are reserved to multidisciplinary teams of experts in the field.

1. Osteosarcoma

1.1. Definition, classification and epidemiology

Osteosarcoma is defined as a malignant tumour in which the neoplastic cells produce osteoid, even if only in small amounts (1). It can occur de novo: primary osteosarcoma, or in a pre-existing abnormality, mostly Paget's disease or radiation change in which case these tumours are called secondary osteosarcoma. Osteosarcoma is the most frequent malignant primary bone tumour (2-4). Approximately 20-22% of all primary malignant bone tumours are central high-grade osteosarcoma (3,5). Rarely osteosarcoma has a pure soft tissue localisation. Primary osteosarcoma is predominantly a sarcoma of adolescents: sixty percent of the osteosarcomas occur before the age of 25 years with a peak incidence at 14-20 years. Men are slightly more affected than women. The metaphysis of the distal femur and proximal tibia are most commonly involved. The proximal humerus is the third most frequent localisation. A number of subtypes are recognised depending upon the site of the involved bone and the histo-morphological characteristics (Table 1). Though not included as individual subtypes in the WHO classification, some osteosarcomas present with rather distinct histological features such as pronounced bone formation, the presence of a highly atypical, pleomorphic neoplastic cell population or a mimic

of giant cell tumour. For these subtypes the descriptive terms of sclerotic, anaplastic and giant cell like are used respectively. In this thesis the terms conventional and common are used interchangeably for conventional osteoblastic osteosarcoma.

1.2. Prognosis and role of chemotherapy

Primary intramedullary osteosarcoma of the extremities is a highly malignant tumour. Before the introduction of chemotherapy 5-year survival was approximately 20% with surgical treatment alone. With the introduction of neo-adjuvant (preoperative)

Table 1
WHO Classification of bone tumours [1]

Cartilage tumours
Chondroma
- Enchondroma
- Periosteal chondroma
- Multiple chondromatosis

Chondroblastoma
Chondromyxoid fibroma
Chondrosarcoma
- Central, primary and secondary
- Peripheral
- Dedifferentiated
- Mesenchymal
- Clear cell

Osteogenic tumours
Osteoid osteoma
Osteoblastoma
Osteosarcoma
- Conventional
  - chondroblastic
  - fibroblastic
  - osteoblastic
- Telangiectatic
- Small cell
- Low grade central
- Secondary
- Parosteal
- Periosteal
- High grade surface

Fibrogenic tumours
- Desmoplastic fibroma
- Fibrosarcoma

Fibrohistiocytic tumours
- Benign fibrous histiocytoma
- Malignant fibrous histiocytoma

Ewing sarcoma/PNET
- Ewing sarcoma

Haematopoietic tumours
- Plasma cell myeloma
- Malignant lymphoma NOS

Giant cell tumour
- Giant cell tumour
- Malignant giant cell tumour

Notochordal tumours
- Chordoma

Vascular tumours
- Haemangioma
- Angiosarcoma

Smooth muscle tumours
- Leiomyoma
- Leiomyosarcoma

Lipogenic tumours
- Lipoma
- Liposarcoma

Neural tumours
- Neurilemmoma

Miscellaneous tumours
- Adamantinoma
- Metastatic malignancy

Miscellaneous lesions
- Aneurysmal bone cyst
- Simple cyst
- Fibrous dysplasia
- Osteofibrous dysplasia
- Langerhans cell histiocytosis
- Erdheim-Chester disease
- Chest wall hamartoma

Joint lesions
- Synovial chondromatosis

PNET: primitive neuroectodermal tumour

chemotherapy (6), the prognosis of osteosarcoma has changed dramatically for those patients with good histological response to preoperative chemotherapy. Good histological response is commonly defined as ≥ 90% of necrosis and poor response as < 90% of necrosis. The five-year overall survival for patients with poor histological response is around 50% (7-9). In contrast, the five-year overall survival ranges between 70 to 87% for patients with good histological response (7-11).

1.3. Secondary osteosarcoma

Although the overall incidence of osteosarcoma is low, osteosarcoma is one of the most frequent tumours associated with other malignancies. Aetiology is multifactorial. Osteosarcoma after the age of 40 years (30% of all osteosarcoma patients) is mostly secondary to radiation therapy or Paget's disease (4,12). The occurrence of multiple primary tumours including osteosarcoma can also be the result of a genetic predisposition as is the case in retinoblastoma (RB) (13-21) and the Li-Fraumeni syndrome (22,23). There is also an elevated risk for osteosarcoma in Werner syndrome (24-26) and Rothmund-Thomson syndrome (27). An overview of some of the hereditary syndromes associated with osteosarcoma is too been found in WHO fascicle on Pathology and Genetics of Tumours of Soft Tissue and Bone (1).

2. Ewing sarcoma and Adamantinoma

2.1. Ewing sarcoma

Small cell tumours of the Ewing sarcoma/primitive peripheral neuroectodermal tumour (PNET) family are highly malignant skeletal and extra-skeletal tumours defined as a primitive malignant tumour composed of uniform densely packed small cells, without distinct cytoplasmic borders or prominent nucleoli. This characteristic histology is a near constant feature of Ewing sarcoma with only sporadic cases presenting with atypical larger cells and nuclei (28). They are the second most frequent malignant bone and soft tissue tumour in children and represent 6 to 8% of primary malignant bone tumours. Eighty percent occur before the age of 20 years with a slight male predilection. In the skeleton, the diaphysis and metaphyseal-diaphyseal portion of the long bones are most commonly affected, together with the ribs and pelvic bones (1). In the soft tissue they occur most frequently in the upper thigh and buttock, upper arm and shoulder (29). The Ewing sarcoma/PNET group is characterised in 90 to 95 % of cases by a t(11;22)(q24;q12) (30-32), resulting in the fusion of the EWS gene on chromosome 22 with the FLI-1 gene on chromosome 11 (33).

2.2. Adamantinoma

Adamantinoma of the long bones is a malignant bone tumour with a predilection for the tibial cortex (85-90% of the cases), and is composed of variable amounts of epithelial cells set in a fibrous or osteofibrous stroma. It comprises 0.4% of all primary bone tumours and affects all age groups with a median of 25-35 years (34). Adamantinoma of the long bones is a lesion of epithelial nature (35,36) with expression of basal cell type cytokeratins (37). Histological subtypes of adamantinoma are basaloid, spindle-cell, tubular, squamous, osteofibrous and mixed (34). There is considerable clinical and histological overlap between osteofibrous dysplasia of the long bones and adamantinoma of the long bones, especially the

osteofibrous subtype which suggests that osteofibrous dysplasia and adamantinoma represent the ends of a spectrum of one tumour entity (34).
There have been also descriptions of rare cases with overlapping features between adamantinoma of long bones and Ewing sarcoma suggesting a relationship between these two entities (38-41).

3. Desmoplastic fibroma and desmoid-type fibromatosis

3.1. Desmoplastic fibroma

Desmoplastic fibroma of the bone is a rare benign tumour of bone that can be locally aggressive. It is considered to be the bony equivalent of the desmoid-type fibromatosis (aggressive fibromatosis) of soft tissue (42), since it has the same histology and clinical behaviour as desmoid tumours. It accounts for 0.1% of all primary bone tumours. It occurs in adolescents and young adults with near equal sex distribution. It can involve any bone but is most frequent in the mandible. Due to its rarity, it is not a popular object for research, since meaningful conclusions are hard to be drawn from a handful of cases. Little is thus known about its tumourigenesis and international literature on the subject hardly goes beyond one more case report.

3.2. Desmoid-type fibromatosis

Desmoid tumours of soft tissue occur mostly sporadic but are also seen in Gardner syndrome. This is a hereditary syndrome with familial adenomatous polyposis coli (FAP), osteomas, fibromas, epidermal or sebaceous cysts and a variety of malignant tumours (43). The basic defect in FAP is a mutation in the adenomatous polyposis (APC) gene. Germ line mutations in the APC gene are restricted to patients with FAP or familial hereditary desmoids (44). In sporadic desmoid tumours, somatic mutations in the APC gene have been described (44-46). The APC gene product is involved in the breakdown and regulation of the cellular level of β-catenin. Mutations in the APC gene result in the accumulation of β-catenin. Accumulation of β-catenin in the cell can also be the result of mutations in the gene for β-catenin (47). In sporadic desmoids β-catenin gene mutations occur more frequently than somatic mutations in the APC gene (48).

4. Aims of the study and outline of the thesis

The purpose of this thesis is to reveal the meaning of the phenotypic spectrum of osteofibrous tumours. Osteosarcoma is a single entity with many phenotypes, adamantinoma and Ewing sarcoma are two different entities with histologic overlap in rare cases, and desmoplastic fibroma of bone and desmoid tumour of soft tissue are supposedly one entity because of their identical histologic features.
Validity of subtyping of osteosarcoma on the biopsy specimen, the correlation between osteosarcoma subtype and response to chemotherapy and the relation between subtype and survival are assessed in **chapter 2**. The predictive value of an uncommon osteosarcoma subtype for a possible hereditary cancer syndrome is studied in **chapter 3**. Clinical and histological variables, with a special interest in the histological osteosarcoma subtype, predictive for late relapse are under investigation in **chapter 4**. Ewing sarcoma/PNET is classically described as a small blue cell tumour and adamantinoma of the long bones is an osteofibrous lesion. The result of a study on the putative presence of t(11;22) and t(21;22) in 14 cases of adamantinoma

by RT-PCR is described in **chapter 5**. Desmoplastic fibroma of bone and desmoid-type fibromatosis is histologically identical. Desmoplastic fibroma is considered to be the bony counterpart of desmoid-type fibromatosis (38). This is further investigated by immunohistochemistry and DNA sequencing for activating β-catenin mutations in **chapter 6**. Summary and concluding remarks are presented in **chapter 7**.

References

1. World Health Organization Classification of tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone Lyon: IARC Press, 2002.

2. Unni KK. Dahlin´s bone tumors: general aspects and data on 11,087 cases. Philadelphia: Lippincott-Raven, 1996.

3. Dorfman HD, Czerniak B. Bone tumors. St. Louis: Mosby, 1997.

4. Huvos AG. Bone tumors. Diagnosis, treatment and prognosis. Philadelphia: WB Saunders, 1991.

5. Mirra JM. Bone tumors: clinical, radiologic and pathologic correlations. Philadelphia: Lea & Febiger, 1989.

6. Rosen G, Marcove RC, Caparros B, Nirenberg A, Kosloff C, Huvos AG. Primary osteogenic sarcoma: the rationale for preoperative chemotherapy and delayed surgery. Cancer 1979;43:2163-2177.

7. Bramwell VHC, Burgers M, Sneath RJ *et al.* A comparison of two short intensive adjuvant chemotherapy regimens in operable osteosarcoma of limbs in children and young adults: the first study of the European Osteosarcoma Intergroup. J Clin Oncol 1992;10:1579-1591.

8. Provisor AJ, Ettinger LJ, Nachman JB *et al.* Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children´s Cancer Group. J Clin Oncol 1997;15;76-84.

9. Souhami RL, Craft AW, Van der Eijken JW *et al.* Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. Lancet 1997;350:900-901.

10. Bacci G, Ferrari S, Longhi A *et al.* High-dose ifosfamide in combination with high dose methotrexate, adriamycin and cisplatin in the neoadjuvant treatment of extremity osteosarcoma: preliminary results of an Italian Sarcoma Group/Scandinavian Sarcoma Group pilot study. J Chemother 2002;14:198-206.

11. Bacci G, Ferrari C, Longhi A *et al.* Neoadjuvant chemotherapy for high grade osteosarcoma of the extremities: long-term results for patients treated according to the Rizzoli IOR/OS -3b protocol. J Chemother 2001;13:93-99.

12. Huvos AG. Osteogenic sarcoma of bones and soft tissues in older persons. A clinicopathologic analysis of 117 patients older than 60 years. Cancer 1986; 57:1442-1449.

13. Abramson DH, Ellsworth RM, Zimmerman LE. Nonocular cancer in retinoblastoma survivors. Tr Am Acad Ophth & Otol 1976;81:454-457.

14. Abramson DH, Ellsworth RM, Kitchin FD, Tung G. Second nonocular tumors in retinoblastoma survivors. Are they radiation induced. Ophthalmology 1984;91:1351-1355.

15. Abramson DH, Ronner HJ, Ellsworth RM. Second tumors in nonirradiated bilateral retinoblastoma. Am J Ophthalmol 1979;87:624-627.

16. Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. Br J Cancer 1986;53:661-671.

17. Hawkins MM, Draper GJ, Kingston JE. Incidence of second primary tumours among childhood survivors. Br J Cancer 1987;56:339-347.

18. Meadows AT, Baum E, Fossati-Bellani F *et al.* Second malignant neoplasms in children: an update from the late effects study group. J Clin Oncol 1985;3:532-538.

19. Roarty JD, McLean I, Zimmerman LE. Incidence of second neoplasms in patients with bilateral retinoblastoma. Ophthalmology 1988;95:1583-1587.

20. Schwarz MB, Burgess LPA, Fee WE, Donaldson SS. Postirradiation sarcoma in retinoblastoma. Induction of predisposition? Arch Otolaryngol, Head Neck Surg 1988;114:640-644.

21. Abramson DH, Ellsworth RM, Kitchin FD. Osteogenic sarcoma of the humerus after cobalt plaque treatment for retinoblastoma. Am J Ophthalmol 1980;90:374-376.

22. Li FP, Fraumeni JF, Mulvihill JJ *et al.* A cancer family syndrome in twenty four kindreds. Cancer Res 1988;48:5358-5362.

23. Strong LC, Stine M, Norsted TL. Cancer in survivors of childhood soft tissue sarcomas and their relatives. J Nat Cancer Inst 1987;79:1213-1220.

24. Goto M, Miller RW, Ishikawa Y, Sugano H. Excess of rare cancers in Werner syndrome (adult progeria). Cancer Epidemiol, Biomarkers Prevention 1996;5:239-246.

25. Ishikawa Y, Miller RW, Machinami R, Sugano H, Goto M. Atypical osteosarcomas in Werner syndrome (adult progeria). Jpn J Cancer Res 2000;91:1345-1349.

26. Tsuji Y, Kusuzaki K, Kanemitsu K, Matsumoto T, Ishikawa Y, Hirasawa Y. Calcaneal osteosarcoma associated with Werner syndrome. A case report with mutation analysis. J Bone Joint Surg (Am) 2000;82A:1308-1313.

27. Vennos EM, Collins M, James WD. Rothmund-Thomson syndrome: review of the world literature. J Am Acad Dermatol 1992;27:750-762.

28. Nascimento AG, Unni KK, Pritchard DF, Cooper KL, Dahlin DC. A clinicopatholigcal study of 20 cases of large-cell (atypical) Ewing's sarcoma of bone. Am J Surg Pathol 1980;4:29-36.

29. Enzinger and Weiss's soft tissue tumors St. Louis: Mosby, 2001.

30. Aurias A, Rimbaut C, Buffe D, Dubousset J, Mazabraud A. Chromosomal translocations in Ewing's sarcoma. N Engl J Med 1983;309:496-497.

31. Turc-Carel C, Philip I, Berger MP, Philip T, Lenoir GM. Chromosomal translocations in Ewing's sarcoma. N Engl J Med 1983;309:497-498.

32. Turc-Carel C, Aurias A, Mugneret F *et al.* Chromosomes in Ewing's sarcoma. I. An evaluation of 85 cases and remarkable consistency of t(11;22)(q24;q12). Cancer Genet Cytogenet 1988;32:229-238.

33. Zucman J, Delattre O, Plougastel B *et al.* Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chromosomes Cancer 1992;5:271-277.

34. Hazelbag HM, Taminiau AHM, Fleuren GJ, Hogendoorn PCW. Adamantinoma of the long bones: a clinicopathological study of thirty-two patients with emphasis on histological subtype, precursor lesion, and biological behaviour. J Bone Joint Surg 1994;76A:1482-1500.

35. Rosai J. Adamantinoma of the tibia: Electron microscopic evidence of its epithelial nature. Am J Clin Pathol 1969;51:786-792.

36. Rosai J, Pinkus GS. Immunohistochemical demonstration of epithelial differentiation in adamantinoma of the tibia. Am J Surg Pathol 1982;6:427-434.

37. Hazelbag HM, Fleuren GJ, van den Broek LJCM, Taminiau AHM, Hogendoorn PCW. Adamantinoma of the long bones: keratin subclass immunoreactivity pattern with reference to its histogenesis. Am J Surg Pathol 1993;17:1225-1233.

38. Meister P, Konrad E, Hübner G. Malignant tumor of humerus with features of "adamantinoma" and Ewing's sarcoma. Pathol Res Pract 1979;166:112-122.

39. Van Haelst UJGM, de Haas van Dorsser AH. A perplexing malignant bone tumor. Highly malignant so-called adamantinoma or non-typical Ewing's sarcoma. Virchows Arch A 1975;365:63-74.

40. Ishida T, Kikuchi F, Oka T *et al.* Case report 727: Juxtacortical adamantinoma of humerus (simulating Ewing tumor). Skeletal Radiol 1992;21:205-209.

41. Lipper S, Kahn LB. Case report 235. Ewing-like adamantinoma of the left radial head and neck. Skeletal Radiol 1983;10:61-66.

42. Desmoid tumor of Bone (desmoplastic fibroma or aggressive fibromatosis) Bone tumors. Clinical, radiologic and pathologic correlations. Philadelphia: Lea & Febiger, 1989;735.

43. Gardner EJ, Richards RC. Multiple cutaneous and subcutaneous lesions occurring simultaneously with hereditary polyposis and osteomatosis. Am J Hum Genet 1953;5:139-147.

44. Giarola M, Wells D, Mondini P *et al.* Mutations of adenomatous polyposis coli (APC) gene are uncommon in sporadic desmoid tumours. Br J Cancer 1998;78:582-587.

45. Alman BA, Li C, Pajerski ME, Diaz-Cano S, Wolfe HJ. Increased Beta-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors). Am J Pathol 1997;151:329-334.

46. Bridge JA, Meloni AM, Neff JR *et al.* Deletion 5q in desmoid tumor and fluorescence in situ hybridization for chromosome 8 and/or 20 copy number. Cancer Genet Cytogenet 1996;92:150-151.

47. Miyoshi Y, Iwao K, Nawa G, Yoshikawa H, Ochi T, Nakamura Y. Frequent mutations in the beta-catenin gene in desmoid tumors from patients without familial adenomatous polyposis. Oncology Res 1998;10:591-594.

48. Tejpar, Nollet F, Li C *et al.* Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 1999;18:6615-6620.

2

Does the histological subtype of high-grade central osteosarcoma influence the response to treatment with chemotherapy and does it affect overall survival?

A study on 570 patients of two consecutive trials of the European Osteosarcoma Intergroup.

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On behalf of the European Osteosarcoma Intergroup

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Abstract

Large randomised trials are mandatory when one wants to examine the effect of different aspects (such as the treatment modality) of a pathological condition on the overall outcome. This is especially true when studying a disease in which there is a multi-factorial influence on progression and outcome such as osteosarcoma. Data on 570 patients with biopsy-proven primary central osteosarcoma of an extremity included in two consecutive studies of the European Osteosarcoma Intergroup (EOI) were analysed in order to evaluate if the histological subtype of the biopsy specimen correlated with the subtype of osteosarcoma represented in the resected specimen, if there was a relation between histological subtype and overall survival and if there was a relation between the histological subtype and histological response to chemotherapy. High-grade osteosarcoma, as defined by established criteria, was subtyped as either conventional, chondroblastic, telangiectatic, small cell, fibroblastic, osteoclast rich, anaplastic and sclerotic/osteoblastic well differentiated. A panel of experienced pathologists with a special interest in bone pathology was appointed to review the histological diagnosis and to assess the tumour response to chemotherapy on the resected specimen of each patient entered into the trials. Subtyping on the biopsy specimen proved to be highly representative for the subtype of the whole tumour. In 102 patients for whom subtyping was performed on the biopsy and the resected specimen, there were only 2 discrepancies. Of the 568 patients for whom subtype was available, 404 (71%) were of the common type, 54 (10%) were chondroblastic, 53 (9%) had fibroblastic tumours and the remainder consisted of rare subtypes. A good response to preoperative chemotherapy was defined as 90% or more necrosis. The proportion of patients responding well to chemotherapy differed significantly between subtypes (Chi-square test statistics = 11.44, P = 0,01 on 3 degrees of freedom (df)). In comparison with the conventional subtype, there was a higher proportion of good responders in the fibroblastic group and a lower proportion of good responders in the chondroblastic group. Good responders had a significantly better survival than patients who responded poorly to preoperative chemotherapy (logrank statistic = 7.68, P < 0.01 on 1 df). Survival did not differ significantly according to subtype (logrank statistics = 2.72, P = 0.44 on 3 df), although there was a suggestion that patients with chondroblastic tumours experience a better long-term survival. This large set of prospectively collected data provides important information on the relationship

between pathological subtype, histological response and survival. Histological response has a known prognostic effect on survival, and we have shown that the rates of response differ by subtype. There is some evidence from this study, that the specific histological subtypes, i.e. the chondroblastic subtype, experience better survival. However, despite this large multi-institutional study, we have insufficient numbers of non-conventional tumours to examine this unambiguously for these subsets.

Introduction

Osteosarcoma is the most frequent malignant primary bone tumour (1-3). Approximately 19.9% of all primary sarcomas (2) and 20-22% of all primary malignant bone tumours (3,4) are central high-grade osteosarcoma. A number of subtypes are recognised dependent upon the site of the involved bone and the histo-morphological features (5). As such, among the high-grade central osteosarcomas osteoblastic, chondroblastic, fibroblastic, telangiectatic, giant-cell rich, small cell, and sclerosing types are recognised (6-10). The clinical and biological significance of these subtypes are controversial in literature, because data based upon large enough controlled randomised studies recognising these subtypes as separate entities are lacking. We used the data available from the first two studies of the European Osteosarcoma Intergroup (EOI) (11,12) containing 570 patients with biopsy-proven high-grade central osteosarcoma in order to evaluate the following three questions:
1. Does the histological subtype of the biopsy specimen correlate with the subtype of the resection specimen?
2. Is there a relation between histological subtype and overall survival?
3. Is there a relation between histological subtype and histological response to chemotherapy?

Patients and methods

Data available from the first two studies of the EOI were used. The EOI consists of the European Organization for Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group, the Bone Sarcoma Working Party of the UK Medical Research Council (MRC), the UK Children's Cancer Study Group (UKCCSG), and the Societé Internationale d´Oncologie Paeditrique (SIOP).

Patients
In both studies, patients included were aged 40 years or younger with a diagnosis of primary high-grade intra-osseous osteosarcoma of the extremities, with no evidence of metastasis at the time of diagnosis, with no previous history of malignancy and not yet treated with chemotherapy or radiotherapy. Periosteal and parosteal (13,14) osteosarcomas were excluded because of a different clinical behaviour.
In total, 570 patients were included for the studies running from July 1983 to December 1986 (study BO02/80831, 179 patients) (11) and September 1986 to December 1991 (study BO03/80861, 391 patients) (12). Informed consent was obtained from the patients or their guardians before entering the study. Permission to enter patients into the studies had to be sought from the local ethical committee for each participating centre. The requirements for consent had to follow local practice.

Histological Diagnosis
In order to be included in the study, patients had to have a biopsy-proven high-grade osteosarcoma, which was confirmed after reviewing the slides of the biopsy specimen by a member of the panel of reference pathologists. The initial biopsy to obtain material for histological evaluation and diagnosis was either an open biopsy or a closed (Jamshidi) biopsy and had to contain material from the intra-osseous tumour component, the cortex and the extra-osseous tumour component if this last one was present.

Table 1.
Histological subtypes of high-grade osteosarcoma recognised

Conventional
Chondroblastic
Telangiectatic
Small cell
Fibroblastic
Osteoclast rich
Anaplastic
Sclerotic/osteoblastic well differentiated
Others

When a diagnosis of high-grade central osteosarcoma was confirmed, the subtype was classified according to the categories as given in Table 1, following the criteria of the World Health Organization (WHO) classification for conventional, telangiectatic and small cell osteosarcoma (5). For the others subtypes, criteria commonly in use were applied (1-4,6,7). In short, the common denominator in all subtypes is the presence of osteoid or bone directly formed by the tumour cells. Telangiectatic osteosarcoma is histologically defined by the presence of single or multiple aneurismatic spaces containing blood or degenerated tumour cells. The spaces are separated by septa containing anaplastic sarcoma cells with numerous mitoses. Osteoid formation is scant and has a lace-like filigree pattern. Small-cell osteosarcoma is composed of sheets of small cells comparable to Ewing sarcoma, with small amounts of lacy osteoid. To be classified as chondroblastic, the tumour has to be composed of nodules of cartilage and a malignant cell population directly producing bone or osteoid. In the fibroblastic subtype, the tumour cells are spindle shaped and may take on a herringbone pattern. Only small amounts of osteoid are present. The osteoclast rich variant resembles very much the giant cell tumour in bone, but has definitive cytonuclear criteria of malignancy with osteoid production and is in contrast to the benign giant cell tumour rarely localised in the epiphysis. The sclerotic well-differentiated type shows a bony matrix, filling the marrow spaces and showing maturation and normalisation of the malignant osteoblastic cells. The anaplastic subtype is characterized by highly pleomorphic cells, and can show malignant fibrous histiocytoma (MFH)-like features, but with once more focal direct bone production by the tumour cells. For a tumour to be classified as one or the other subtype, it had to be composed predominantly of tissue characteristic for this subtype. For a tumour to be of the chondroblastic subtype a cut-off of more then 30% of chondroid tissue in the resected specimen was set. Especially in the cases of non-conventional osteosarcoma in which it can be hard to find osteoid or bone formation in the initial biopsy the clinical history, age, localisation and radiographical findings had to be strongly concordant with a diagnosis of osteosarcoma and its subtype.

Treatment
The results of patient outcome in both trials incorporated in this analysis have been published previously (11,12). In each trial patients were randomised between two regimens of neo-adjuvant chemotherapy. The first trial (11) compared a two-drug regimen of doxorubicin (DOX) and cisplatin (CDDP) with the same regimen preceded by high-dose methotrexate (MTX). The second trial (12) compared the same two-drug

arm with a multidrug regimen based on the T10 schedule developed by Rosen and colleagues (15). No significant difference in survival was found between the two arms in either trial. In both trials, surgery consisted of limb salvage if complete removal of the tumour with save margins could be achieved. If not, an amputation was performed.

Assessment of histological response to chemotherapy
To assess the histological response to chemotherapy, the local pathologist was requested to make a longitudinal section in the plane of maximum tumour diameter through the resection specimen. After fixation and decalcification, the whole of this slab had to be divided into blocks and all of these blocks had to be embedded in paraffin from which histological slides, haematoxylin and eosin stained, had to be sent in for review together with a diagrammatic map indicating the site of the individual blocks. The reviewer overlaid each slide with a 2-mm squares transparent graph paper on which the respective necrotic and viable areas were drawn. Having done this for each section, a composite reference map was made and percentage of viable and necrotic tumour could be calculated. Necrosis was defined as no detectable tumour or totally necrotic tumour intermingled with non-neoplastic reparative tissue or hyalinised relatively acellular tissue that contained sparse widely scattered pleomorphic cells with degeneration of the nucleus or totally pyknotic cells.

Results

Patients & data forms
Merging the data from the two studies resulted in a total of 570 eligible patients. From all these patients, the biopsy material had been reviewed and a report of this was present. For 369 of these patients, material of the resected specimen was available and reviewed.

General results
As described in the reports of the EOI, there was no survival benefit for patients treated with the three-drug or multidrug regimen when compared with the two-drug arm (11,12). This justifies the merging of the data from the two studies. Good response to chemotherapy was defined as 90% tumour necrosis or more, and poor response as less than 90% necrosis. For the combined data, this resulted in an overall good response to chemotherapy in 102 (28%) patients as illustrated in Table2.

Subtyping and validity of subtyping on the biopsy specimen
Subtyping on the biopsy was done for all of the patients entering the studies (Table 3). In the first study, this was performed for 102 patients on the resected specimen as well. In only 2 cases (2%), there was a discrepancy between the subtype of the biopsy and that of the resected specimen. One case classified on the biopsy as chondroblastic and the other as fibroblastic both proved to be of the conventional subtype in the resected specimen. This was clearly the result of sampling error.

Histological response to chemotherapy by classification of osteosarcoma
The results are summarized in Table 4. Response differs significantly between subtype groups (χ^2 test statistic = 11.44, P = 0.01 on 3 df). Due to the low numbers anaplastic, osteoclast rich, telangiectatic, small cell and other subtypes were combined

Table 2.
Histological response as defined on the resection specimen

Histological Response	BO02/80831 N (%)	BO03/80861 N (%)	Group total N (%)
Good (≥ 90% Necrosis)	22 (22)	80 (30)	102 (28)
Poor (<90% Necrosis)	78 (78)	189 (70)	267 (72)
Total number	100	269	369

Good histological response of the resected specimen to chemotherapy, defined as ≥ 90% necrosis was assessed for 369 patients.

Table 3.
Classification of osteosarcoma according to subtype

Classification	BO02/80831 N (%)	BO03/80861 N (%)	Group total N (%)
Conventional type	144 (82)	260 (66)	404 (71)
Chondroblastic	9 (5)	45 (12)	54 (10)
Fibroblastic	10 (6)	43 (11)	53 (9)
Anaplastic	8 (5)	16 (4)	24 (4)
Telangiectatic	0 (0)	10 (3)	10 (2)
Osteoclast rich	3 (1)	8 (2)	11 (2)
Small Cell	1 (1)	2 (1)	3 (1)
Other	2 (1)	7 (2)	9 (2)
Subtype not specified	2		2
Total	179	391	570

Of the patients recorded as 'Other', 8 were osteoblastic and 1 malignant fibrous histiocytoma (MFH)-like

Table 4.
Histological response by classification of sarcoma

Classification of	Good response N (%)	Poor response N (%)	Total N
Conventional type	76 (29)	183 (71)	259
Fibroblastic	16 (41)	23 (59)	39
Chondroblastic	3 (9)	32 (91)	35
Anaplastic	2 (14)	12 (86)	14
Osteoclast rich	1 (14)	7 (88)	8
Telangiectatic	2 (40)	3 (60)	5
Small cell	0 (0)	3 (100)	3
Other	2 (33)	4 (67)	6
Total	102 (28)	267 (72)	369

Good histological response is defined here as ≥90% necrosis.

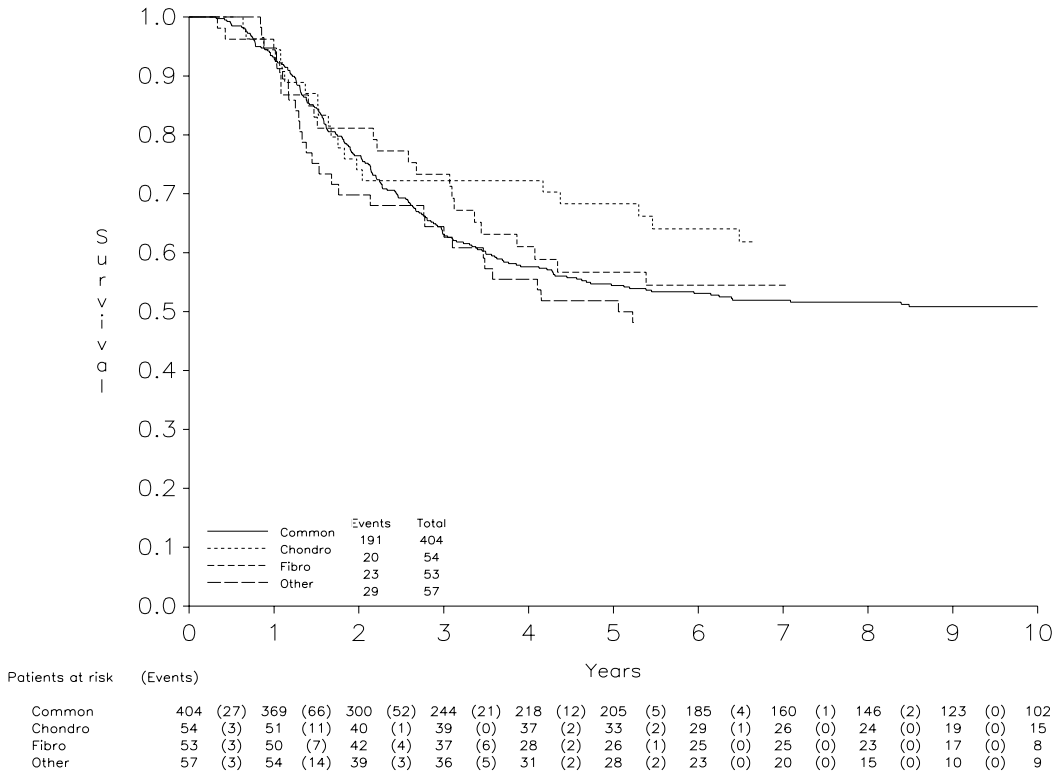
into one group for the test to be performed. The result showed a higher proportion of good responders observed in the fibroblastic group (41%) and a lower proportion in the chondroblastic group (9%) when compared with the conventional subtype (29%).

Histological subtype and survival

Fig.1 shows the overall survival per subtype. Survival was calculated from the date of randomisation, as classification was determined from the biopsy specimens, which were collected before entry. Due to the low patient numbers anaplastic, telangiectatic, osteoclast rich, sclerotic/osteoblastic well-differentiated and small cell subtypes were combined in one group. There was no significant difference in survival between the histological subtypes (logrank statistic = 2.72, P = 0.44 on 3 df). There was a suggestion that patients with chondroblastic tumours experienced a better long-term survival, but there were insufficient patient numbers to confirm this.

Hazard ratios with 95% Confidence Intervals (CIs) for each histological subtype are shown in Table 5. A HR of less than 1 implies a survival benefit for that group. There is a statistically significant difference in the risk of death between the groups at the 5% level if the 95% CI does not cross 1. It can be seen that there appears to be a large

Fig 1.
Survival by classification of sarcoma



Survival curve by histological subtype showing a 15% difference in survival between conventional type and chondroblastic tumours. Logrank statistics = 2.72 (P = 0.44 on 3 degrees of freedom (df)) Chondro, chondroblastic; Fibro, fibroblastic.

reduction (28%) in the risk of death for patients with chondroblastic tumours compared with conventional type tumours. However, this was not a statistically significant difference. In order to prove reliably that a difference of this magnitude in outcome exists between these groups, an excess of 150 patients in each subtype group would be required as calculated by a power analysis.

Histological response was determined from the resected specimen. Thus, for the analyses of survival by response to chemotherapy, survival was calculated from the date of surgery in order to avoid bias.

Survival for patients who respond well to chemotherapy was significantly higher than survival for poor responders (logrank statistic = 7.68, $P < 0.01$ on 1 df). Survival at 5 years for good responders was 75% compared to 45% for poor responders (Fig.2). Due to low numbers of cases in most of the subtypes, survival in relation to the response to chemotherapy per subtype could not be determinated.

The results were not biased by the choice of surgical procedure or patient's age, since there was a rather homogeneous distribution of subtypes over the different age groups and there was a near even distribution in the choice of surgery for each subtype, as can be seen in Table 6.

Table 5.
Hazard ratios by histological subtype

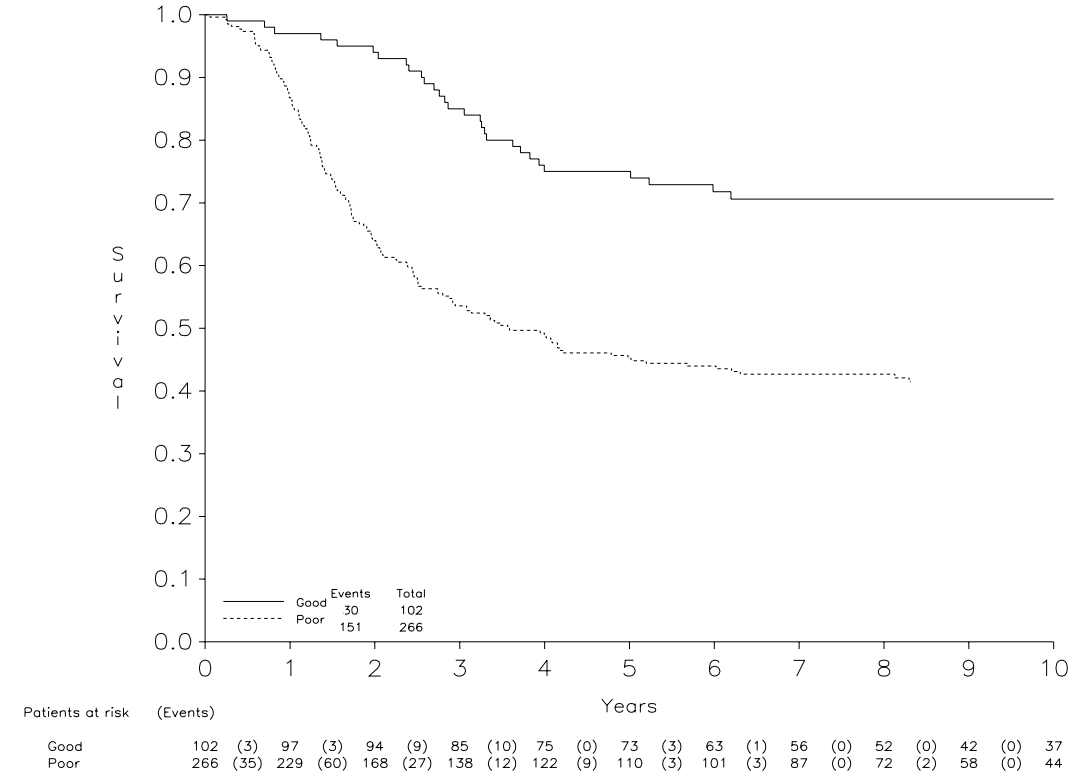
Subtype	N (%)	5-year survival	HR (95% CI)
Conventional	404 (71)	54%	1
Chondroblastic	54 (10)	70%	0.72 (0.48-1.08)
Fibroblastic	53 (9)	57%	0.89 (0.59-1.35)
Other	57 (10)	52%	1.12 (0.74-1.69)

HR, Hazard Ratio; 95% CI, 95% Confidence Interval

Table 6.
Age and type of surgery by histological subtype

Subtype	Conventional		Chondro		Fibro		Other		Total
	N	(%)	N	(%)	N	(%)	N	(%)	N
Age									
<12	70	(72)	11	(11)	9	(9)	7	(7)	97
12-16	168	(73)	21	(9)	22	(10)	18	(8)	229
17+	166	(69)	22	(9)	22	(9)	32	(13)	242
Surgery Received									
Amputation	112	(68)	19	(12)	12	(7)	21	(13)	164
Limb Conservation	263	(71)	34	(9)	40	(11)	34	(9)	371
Rotation Plasty	21	(95)	0	(0)	1	(5)	0	(0)	22
Not Done	8	(72)	1	(9)	0	(0)	2	(18)	11
Total	404	(71)	54	(10)	53	(9)	57	(10)	568

Fig.2.
Survival curve by histological response



Kaplan-Meier survival curve showing a significant better survival for patients with 90% or more of tumour necrosis after preoperative chemotherapy. Logrank statistics = 7.68 (P<0.01 on 1 degree of freedom (df)).

Discussion

Subtyping and validity of subtyping on the biopsy specimen
The data available on 570 patients from two consecutive studies from the EOI were analysed.
In the first years of the study, the subtype was evaluated on the biopsy and the resection specimen. This information was available for a total of 102 patients. In only 2 cases (2%) there was a discrepancy. Since the correlation was so good, the subtype was not reported any more for the resected specimen in these trials. The method of biopsy was not specified and thus the numbers of open biopsies and closed biopsies are not known. However, we do not believe that the method of biopsy influenced the results. The validity of closed biopsy has been proven by a report on the use of the Jamshidi trocar biopsy in 258 patients with 270 biopsies. This revealed an accuracy of diagnosis in 95% of the patients and in 90.7% of the biopsies (16), which is comparable to the open biopsy.
The risk that a chondroblastic osteosarcoma is diagnosed on the biopsy as a chondrosarcoma is rather high as seen in a study by Geirnaerdt and co-workers (17). In this study, 5 of 9 patients with proven chondroblastic osteosarcoma on resection,

had an initial diagnosis on biopsy of chondrosarcoma. However, this was not concordant with the clinical and radiological findings. In 3 patients, a second biopsy revealed the osteosarcomatous nature of the tumour leading to an accuracy of 80%. Whenever a diagnosis of chondrosarcoma is made on a biopsy, this has to be concordant with the clinical information and radiographical findings. The slightest discrepancy should alert the pathologists and the clinicians to the likelihood of a chondroblastic osteosarcoma. From this, it can be concluded that subtyping on the biopsy is reliable in expert hands. This is an important fact since, for patients who respond well to chemotherapy, subtyping on the resection specimen may prove to be impossible. The reliability of the subtyping on the biopsy specimen justifies the inclusion of cases with complete response to chemotherapy in studies looking at the possible prognostic value of the subtype.
By a search of the literature back to the mid- 1960s, only one report was found stating that a given area of chondroid ground substance gives a representative view of the complete tumour (18).

Histological response to chemotherapy by classification of osteosarcoma
For the 369 patients where information from the resected specimen was available, a good response was seen in 102 cases (28%). 75% of these were of the conventional subtype, 16% fibroblastic, 3% were chondroblastic and 7% other subtypes.
The chondroblastic subtype stands out from the other subtypes as these patients show a good histological response to chemotherapy in only 9% of the cases. The proportion of good responders varied significantly between the groups. Comparable results were seen in a study by the group of the Rizzoli Institute analysing 272 patients with primary osteosarcoma of the limb (19). Criteria for including patients in their study were approximately the same as ours, with the exception that they also included 25 patients with resectable lung metastasis at the time of diagnosis. Their preoperative chemotherapy regimen included high-dose methotrexate, doxorubicin and cisplatin. Response was registered as total equalling 100% or incomplete. This was also assessed on complete inclusion of a longitudinal slab through the tumour. 19% of their patients showed complete response. 16.3% of these were osteoblastic, 6.1% chondroblastic, 33.3% fibroblastic and 42.3% telangiectatic. In their study, subtype proved to be an independent predictive factor for histologic response by multivariate analysis. However, subtype was not a predictable factor for local recurrence (20).
The fact that chondroblastic osteosarcomas show a poor histological response to chemotherapy is, however, not so surprising, since it is the chondroblastic component that does not or poorly responds resulting in a higher percentage of remnant viable tumour.

Histological subtype and survival
With regard to subtype being a prognostic parameter for survival, only two studies have been published since the use of neo-adjuvant chemotherapy.
In the study from Pochanugool and colleagues (21), the subtype did not prove to be a prognostic factor. Their study group was composed of only 130 patients with stage IIA and IIB osteosarcoma that could be completely resected with tumour-free margins. The age of the patients was not specified. The study of Petrilli and colleagues (22) was based on 92 patients between the ages of 4 and 28 years with non-metastatic primary osteosarcoma of extremity of which the response to chemotherapy could be evaluated in only 62 cases. The non-osteoblastic subtype was a predictive valuable for recurrence by uni- and multivariate analysis and proved to be

an unfavourable prognostic factor for survival by univariate analysis. The non-osteoblastic subtype, however, was not further specified. Due to the low patient number, the heterogeneous composition of the two patient groups, the lack of data on patient age in one group and the lack of details on the non-osteoblastic subtype in the other, the results are of limited value and are not comparable.

The use of historical controls is also debatable, since with time the natural history of osteosarcoma may have changed (23). Evolving techniques in the field of radiology have resulted in earlier detection of osteosarcoma and have certainly led to an earlier detection of lung metastasis. A solitary lung metastasis is nowadays resected in most centres, some patients having repeated metastasectomies over several years. All these factors, apart from preoperative chemotherapy, have no doubt contributed to the longer survival of osteosarcoma patients (24). In addition, the histological criteria for the diagnosis of osteosarcoma and their subtypes have been refined over time.

However, there are two major studies that can be used as a base-line for the pre-adjuvant chemotherapy period (25,26). In a study of 184 patients operated upon for primary osteosarcoma of the extremity or limb girdles with or without metastasis, a regression analysis revealed a favourable outcome for the fibroblastic subtype (25). Uribe and colleagues included 243 patients with primary high-grade central osteosarcoma. In this group, the fibroblastic subtype had the best prognosis, followed by the chondroblastic subgroup. The osteoblastic subgroup had the worst prognosis (26). This last result is in sharp contrast with a smaller study published in 1966 on only 54 patients in which the chondroblastic subtype has a remarkable bad prognosis when compared with the other subtypes (27).

As previously stated, the chondroid areas are probably the cause of a poor response to chemotherapy. It is therefore initially surprising that the chondroblastic subtype group has a better overall survival than the conventional subtype group, as seen in our study. We would have to presume that a near total necrosis in the non-chondroid areas occurs in all of the osteosarcomas of the chondroblastic subtype. Again, this would be an indication of their different nature.

We conclude that subtyping using the biopsy specimen is highly reliable and predictable for the composition of the whole tumour, chondroblastic osteosarcomas respond poorly to chemotherapy and this subtype can be a prognostic factor for survival.

This last statement finds support from some of the larger studies, both in the period after, as well as before, the introduction of the use of preoperative chemotherapy (19, 25, 26).

Looking at the results of our study, where 28% of patients had a good response to chemotherapy, with only 9% of the patients with a chondroblastic type of osteosarcoma showing good response, raises questions about the current use of chemotherapy in patients with a chondroblastic subtype, especially in a preoperative setting with delayed surgery. The criteria for classifying an osteosarcoma to one or other subtype will also have to be redefined by the biological behaviour of that subtype. In a study of Kersjes and colleagues (18) on 22 osteosarcomas of the lower limb, a mean value of chondroid ground substance of 4,4% was seen in patients with good response (defined as more than 90% necrosis) to preoperative chemotherapy versus 21% in patients with a poor response. Currently, chondroblastic osteosarcoma is defined as an osteosarcoma with more than 30-90% chondrosarcomatous areas.

However, to determine if certain subtypes such as the fibroblastic and chondroblastic osteosarcomas are subtypes with a definitively different behaviour than the classic

osteoblastic osteosarcoma, that could justify a specific therapeutic approach, tailored studies are mandatory.

References

1. Huvos AG. Bone tumors. Diagnosis, treatment and prognosis. 2nd ed. Philadelphia: WB Saunders, 1991: 85-155.

2. Unni KK. Dahlin´s bone tumors: general aspects and data on 11,087 cases. 5th ed. Philadelphia: Lippincott-Raven, 1996: 143-183.

3. Dorfman HD, Czerniak B. Bone tumors. 1th ed. St.Louis: Mosby, 1997:128-252.

4. Mirra JM. Bone tumors: clinical, radiologic and pathologic correlations. 2nd ed. Philadelphia: Lea & Febiger, 1989: 248-344

5. Schajowicz F, Sobin LH. World health organization. International histological classification of tumours. Histological typing of bone tumours. Berlin: Springer-Verlag, 1993

6. Simmons CC. Bone sarcoma: factors influencing prognosis. Surg Gynec Obstet 1939;68:67-75.

7. MacDonald I, Budd JW. Osteogenic sarcoma: I. A modified nomenclature and a review of 118 five-year cures. Surg Gynecol Obstet 1943;76:413-21.

8. Ewing J. A review and classification of bone sarcomas. Arch Surg 1922;4:483-533.

9. Ewing J. A review of the classification of bone tumors. Bull Am Coll Surg 1939;24:290-5.

10. Sim FH, Unni KK, Beabout JW, Dahlin DC. Osteosarcoma with small cells simulating Ewing's tumor. J Bone Joint Surg (Am) 1979;61:207-15.

11. Bramwell VHC, Burgers M, Sneath R, Souhami R, van Oosterom AT, Voûte PA et al. A comparison of two short intensive adjuvant chemotherapy regimens in operable osteosarcoma of limbs in children and young adults: The first study of the European Osteosarcoma Intergroup. J Clin Oncol. 1992;10:1579-91.

12. Souhami RL, Craft AW, Eijken JWvd, Nooij M, Spooner D, Bramwell VHC et al. Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. Lancet 1997;350:911-7.

13. Unni KK, Dahlin DC, Beabout JW, Ivins JC. Parosteal osteogenic sarcoma. Cancer 1976;37:2466-75

14. Unni KK, Dahlin DC, Beabout JW. Periosteal osteogenic sarcoma. Cancer 1976; 37:2476-85.

15. Rosen G, Caparros B, Huvos AG, Kosloff A, Nirenberg A, Cacavio R et al. Preoperative chemotherapy for osteogenic sarcoma. Selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer 1982;49:1221-30.

16. van der Bijl AE, Taminiau AHM, Hermans J, Hogendoorn PCW. Accuracy of the Jamshidi trocar biopsy in the diagnosis of bone tumors. Clin Orthop 1997; 334:233-43.

17. Geirnaerdt MJA, Bloem JL, van der Woude HJ, Taminiau AHM, Nooy MA, Hogendoorn PCW. Chondroblastic osteosarcoma; characterization by gadolinium enhanced MR imaging correlated with histopathology. Skelet Radiol 1998;27:145-53.

18. Kersjes W, Heise U, Winkler K, Delling G. Comparison of quantitative ground substance analysis in biopsy and resected tumor in osteosarcomas. Virchows Arch A 1987;412:155-60.

19. Bacci G, Delepine N, Bertoni F, Picci P, Mercuri, M, Bacchini A et al. Predictive factors of histological response to primary chemotherapy in osteosarcoma of the extremity: study of 272 patients preoperatively treated with high dose methotrexate, doxorubicin and cisplatin. J Clin Oncol 1998;16:658-63.

20. Bacci G, Ferrari S, Mercuri M, Bertoni F, Picci P, Manfrini M, et al. Predictive factors of local recurrence in osteosarcoma: 540 patients with extremity tumors followed for minimum 2.5 years after neoadjuvant chemotherapy. Acta Orthop Scand 1998;69:230-6.

21. Pochanugool L, Subhadharaphandou T, Dhanachai M, Hathirat P, Sangthawan D, Pirabul R, et al. Prognostic factors among 130 patients with osteosarcoma. Clinical Orthop 1997;345:206-14.

22. Petrilli AS, Gentil FC, Epelman S, Lopes LM, Bianchi A, Lopes A, et al. Increased survival, limb preservation, and prognostic factors for osteosarcoma. Cancer 1991; 68:733-7.

23. Taylor WF, Ivins JC, Pritchard DJ, Dahlin DC, Gilchrist GS, Edmonson JH. Trends and variability in survival among patients with osteosarcoma: a 7 years update. Mayo Clin Proc 1985;60:91-104.

24. Simon MA. Causes of increased survival of patients with osteosarcoma: current controversies. J Bone Joint Surg (A) 1984;66-A:306-10.

25. Bentzen SM, Poulsen HS, Kaae S, Myhre Jensen O, Johansen H, Mouridsen HT et al. Prognostic factors in osteosarcomas. A regression analysis. Cancer 1988;62:194-202.

26. Uribe-Botero G, Russell WO, Sutow WW, Martin RG. Primary osteosarcoma of bone. Clinicopathologic investigation of 243 cases, with necropsy studies in 54. Am J Clin Pathol 1977;67:427-35.

27. Price CHG. The prognosis of osteosarcoma: an analytical study. Br J Radiol 1966;39:180-8.

3

Multiple primary malignancies in osteosarcoma patients. Incidence and predictive value of osteosarcoma subtype for cancer syndromes related with osteosarcoma.

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Abstract

The overall incidence of osteosarcoma is low. However, the occurrence of osteosarcoma in a setting of multiple primary tumours is not infrequent, although population-based incidence numbers are unknown. The occurrence of osteosarcoma and other malignancies is frequently related to treatment, and can also be the result of genetic predisposition as in patients with retinoblastoma, Li-Fraumeni syndrome, Werner syndrome and Rothmund-Thomson syndrome. The aim of our study is to establish the incidence of osteosarcoma associated with other malignancies in a population wide study and to find out if these osteosarcomas have a specific subtype, that could draw attention to a genetic predisposition to malignancy. A list of all patients registered in the Dutch National Pathology Register, named PALGA, with a diagnosis of osteosarcoma between 1975 and May 2000 was retrieved. All patients with another malignancy besides osteosarcoma were selected. All patients registered in the same period with a tonsillectomy served as a control for the occurrence of malignancy in a normal population. In a second step, only osteosarcoma patients with a history of retinoblastoma or a malignancy before the age of 46 years, since these are most probable to have a hereditary cancer syndrome, were retained for further analysis. The osteosarcomas were subtyped as conventional, chondroblastic, fibroblastic, telangiectatic, anaplastic, osteoclast-rich or small cell. As a control for osteosarcoma subtypes the data of 570 patients entered in two studies from the European Osteosarcoma Intergroup (EORTC/MRC) were used. Of all 938 patients registered with the diagnosis of osteosarcoma, 66 had a history of multiple primary tumours. Four patients had a surface osteosarcoma, three an extra-skeletal osteosarcoma and 59 had intramedullary high-grade osteosarcoma. Of this last group, one patient was known with Rothmund-Thomson syndrome, one had retinoblastoma and 30 had their malignancy before the age of 46. Of these 32 patients, 17 had osteosarcoma of the long bones. Especially women seem to be more susceptible for the development of multiple primaries. In 9 patients, the histological subtype could be assessed by revision of available histological slides. All of these patients had an osteosarcoma subtype other than conventional as opposed to 29% in the control group of the European Osteosarcoma Intergroup. It is concluded that although the incidence of osteosarcoma is low, the occurrence of another malignancy in osteosarcoma patients is higher than in the normal population. Specifically, osteosarcoma patients have a relative risk of 2.4 (95% confidence interval 1.88 - 3.07) to develop another malignancy. A non-conventional subtype of osteosarcoma should draw attention to a possible genetic predisposition of the patient involved.

Introduction

Osteosarcoma is the most frequent non-haematogenic malignant bone tumour (1-3). Central high-grade osteosarcoma accounts for approximately 20-22% of all primary malignant bone tumours. Although the overall incidence of osteosarcoma is low, osteosarcoma is one of the most frequent tumours associated with other malignancies. Aetiology is multifactorial. Case reports on second malignancies including osteosarcoma after radiation or chemotherapy treatment in cohorts of patients with cancer in childhood and adolescence are many. These studies have been substantially reviewed by Cullen and Meadows (4,5). The occurrence of multiple primary tumours including osteosarcoma can also be the result of genetic predisposition as is the case in retinoblastoma (6-13) and the Li-Fraumeni syndrome (14,15). There is also an elevated risk for osteosarcoma in Werner syndrome (16-18) and Rothmund-Thomson syndrome (19). Data on syndrome-related osteosarcoma patients are mostly calculated from clusters and families of patients already diagnosed with a specific genetic syndrome and thus give no indication of the incidence of hereditary cancer syndromes including osteosarcoma in a population. Based on histological criteria, osteosarcomas can be subtyped as conventional, chondroblastic, telangiectatic, fibroblastic, small cell, giant cell rich, and sclerotic/osteoblastic. The histological subtype of osteosarcoma is a predictive factor for response to chemotherapy (20,21), is related with disease free survival (22) and tends to be related with overall survival (21).
In order to have an estimate of the number of osteosarcoma patients with a possible hereditary cancer syndrome in a population, we studied all patients with high-grade intramedullary osteosarcoma, who either had a retinoblastoma in the antecedents or any other primary tumour at a relative young age.
In addition, we were interested to see if osteosarcoma subtypes reflect a possible hereditary background for malignancy.

Material and methods

Database
Listings of all patients between 1975 and the 20th of May 2000 diagnosed with osteosarcoma were obtained through the national computerised pathology records system of Foundation PALGA (The Dutch National Pathology Registry). The PALGA registration is obligatory for all pathology labs in the Netherlands. It contains the full conclusions of the pathology report and includes as such all histological and cytological and autopsy data for every patient in the Netherlands. This registration started in 1975 and reached 100% participation of all pathology labs since 1990. This database serves for several population-based questions and forms among others the database source for the Dutch Cancer Registry.
All patients with a reliable diagnosis of osteosarcoma and any other primary tumour, not carcinoma *in situ*, were selected for further investigation.

Selection of syndrome-suspected study group
The syndrome-suspected study group was composed of all osteosarcoma patients with one of the following criteria: (1) a medical history of retinoblastoma; (2) a primary tumour diagnosed before the age of 46 years, since 70% of the Li-Fraumeni patients are at risk to develop a second primary malignancy after a first primary before that age (23); (3) patients with carcinoma of the skin before the age of 44 years. This age

was chosen as a cut off, since only 4% of all squamous cell carcinomas of the skin and only 10% of all basal cell carcinomas of the skin occur before the age of 44 as has been shown by a study covering the region of south-east Netherlands (24). Of these patients all available patient data and histological material was reviewed.

Review and classification
Subtyping of the osteosarcomas was performed by two review pathologists of the European Osteosarcoma Intergroup (PCWH and EH). Osteosarcomas were classified as conventional, chondroblastic, telangiectatic, small cell, fibroblastic, osteoclast rich, anaplastic and sclerotic/osteoblastic well differentiated, as defined by established criteria (25-30).

Control groups
As a control for the occurrence of malignancy served 23252 patients with tonsillectomy diagnosed in the same study period (database interval) as the osteosarcoma patients. Using this control group from the same database, instead of computing an expected number of carcinomas in the general population, has several important advantages. The selection of patients with osteosarcoma is the same as the selection of the control group, coming from the same group of pathology labs, so that potential regional differences in completeness of adherence of pathology laboratories to the database are levelled out. Moreover, it is not clear how "person-years" should be calculated, since the survival of the patients with the primary tumours in the database is not known. Patients with lymphoma or malignancy in the head and neck region, in which tonsillectomy is part of the diagnostic work up or treatment, were excluded in order to avoid selection bias. We adjusted the relative risk calculation for age and sex by use of a Mantel-Haenszel procedure.
As control for subtype the histological data from the first two studies of the EORTC, including 570 patients were used (21). The distribution of the subtypes in this group is comparable to that of a similar study group of the Rizzoli Institute (20).

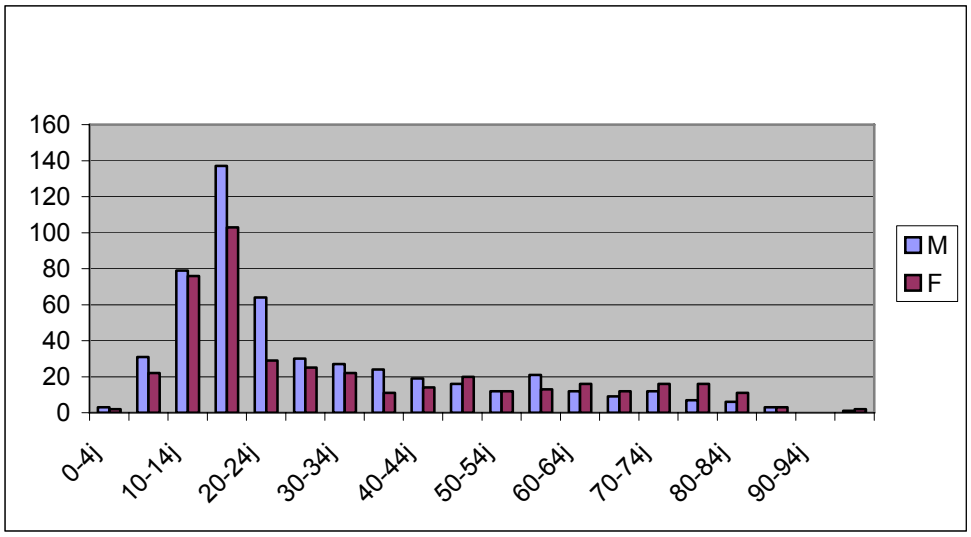
Results

Osteosarcoma in the Netherlands
According to the data obtained from PALGA, 938 patients, 513 male and 425 female, were diagnosed with osteosarcoma between 1975 and May 2000. Age varied from less than 1 year to 99 years with a mean of 20 years. Distribution per age group is given in Fig.1 and distribution by localisation in Table 1.

Patients with multiple primary malignancies including osteosarcoma
A total of 66 osteosarcoma patients (7%) had another malignancy, 24 male (5% of the male osteosarcoma patients) and 42 female (10% of the female osteosarcoma patients) (Table 2). These 66 patients had a total of 73 other malignancies. Multiple basal cell carcinomas in a given patient were counted as one. The nature of these other malignancies is summarised in Table 3. Thirty-five patients had their malignancy prior to osteosarcoma, 31 after. The interval ranged from 0 to 264 months with an average of 92 months prior to the diagnosis of osteosarcoma and 0-231 months with an average of 79 months after the diagnosis of osteosarcoma. In patients with osteosarcoma and another malignancy, the osteosarcomas were more frequently localised in flat bones, bones of the skull and axial skeleton (Table 4).

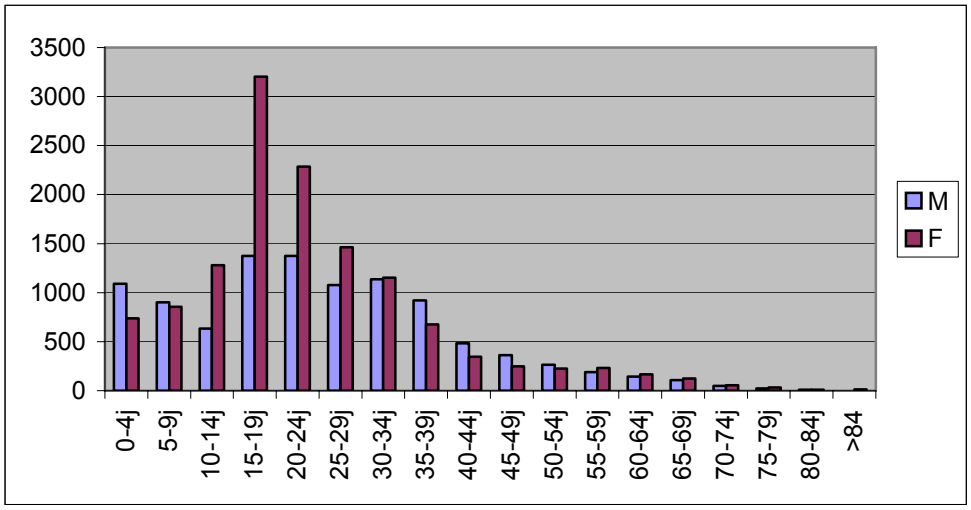
Fig 1.
Number of osteosarcoma and tonsillectomy patients.

A



A. Number of osteosarcoma patients by age as found in the nation wide survey (n=938)

B.



B. Number of tonsillectomy patients by age (n=23,252)

In all, 21% of the patients with intramedullary osteosarcoma of the skull and axial skeleton had other malignancies in contrast to 4% of the patients with osteosarcoma of the long bones of the extremity.
In the control group of 23 252 tonsillectomy patients, 10 142 men and 13 110 women (age distribution in Fig 1), 383 (1.6%) had a malignancy in the observed interval of 120 months before or after tonsillectomy. The crude relative risk of developing a malignancy in osteosarcoma patients is 4.4. After adjustment for age and sex the relative risk is 2.4 (95% confidence interval 1.88-3.07).

Table 1.
Localisation of osteosarcoma by gender

	M	F	
Intramedullary osteosarcoma extremity	374	281	
Intramedullary osteosarcoma axial skeleton	87	97	
Surface osteosarcoma	25	26	
Extra skeletal osteosarcoma	27	21	
Total	513	425	938

Table 2.
Localisation of osteosarcoma in patients with other malignancies

	M	F	
Intramedullary osteosarcoma extremity	9	17	
Intramedullary osteosarcoma axial skeleton	13	20	
Surface osteosarcoma	2	2	
Extra skeletal osteosarcoma	0	3	
Total	24	42	66

Syndrome-suspected study group
After selection of all patients with intramedullary osteosarcoma and a history of bilateral retinoblastoma, carcinoma of the skin before 44 years of age or any primary tumour till the age of 45 years, a group of 31 patients remained. One patient with known Rothmund-Thomson syndrome was also included. The data of these 32 patients are summarised in Table 5. In the syndrome-suspected study group without relation to treatment patients with other malignancies represented 2% of the patients with osteosarcoma of the long bones and 2% of the patients with osteosarcoma of the bones of the skull and axial skeleton. Thus, the observed over-representation of osteosarcoma of flat bones and bones of the axial skeleton in patients with other malignancies was not seen anymore in the syndrome-suspected study group.
The patient data and histological material from contributory departments of pathology all over the Netherlands were reviewed. From 20 patients histological material was available for review and analysis (Table 6). Ethics and privacy guidelines concerning the use of patient files made it impossible to obtain additional data on patients and treatment modalities outside the pathology protocol that was sent in with the histological material.
In the total group of 32 of patients, eight patients developed an osteosarcoma at the site of previous radiation therapy, one patient had lymphoma and one myelodysplastic syndrome after chemotherapy for osteosarcoma. In 14 patients (case numbers in bold), we could make sure that there was no relation with therapy between primary and secondary tumour. One of these patients was known with Rothmund-Thomson, one had a basal cell carcinoma and the remaining 12 patients potentially belonged to a Li-Fraumeni (-like), or other not yet defined cancer syndrome family.

Table 3.
Nature of the second neoplasm besides osteosarcoma

Other malignancy	M < 45 Y	M ≥ 45 Y	F < 45 Y	F ≥ 45 Y
Hodgkin's disease	2			1
Haematogenic diseases	1		2	
Non-Hodgkin's lymphoma	1		1	2
Basal cell carcinoma skin	1	5		6
Squamous carcinoma skin				1
Malignant melanoma skin			1	
Squamous cell carcinoma nose			1	
Squamous carcinoma pharynx/larynx		2	1	
Adenocystic carcinoma palate			1	
Muco-epidermoid carcinoma parotid			1	1
Hepatocellular carcinoma				1
Adenocarcinoma lung		1		
Squamous carcinoma lung		1		
Signet ring cell carcinoma stomach		2		
Adenocarcinoma large intestine				3
Renal cell carcinoma		2		
Urothelial carcinoma bladder		1		
Prostatic adenocarcinoma		1		
Ductal carcinoma breast			2	5
Squamous carcinoma cervix uteri				1
Endometrioid carcinoma				1
Ovarian carcinoma			1	
Embryonal cell carcinoma ovary			1	
Teratoma testis	1			
Oligodendroglioma	1			
Papillary carcinoma thyroid gland			1	
Fibrosarcoma		1	1	
Leiomyosarcoma			1	1
Rhabdomyosarcoma	1			
Ewing sarcoma	1		2	
Liposarcoma				1
Nephroblastoma			1	
Retinoblastoma			2	
Unknown			3	1

Osteosarcoma subtypes in syndrome-suspected osteosarcoma patients
All of the patients in our final selection of osteosarcoma patients with high-grade intramedullary osteosarcoma suspected of a genetic cancer syndrome, and from which material was present for review (Table 7), had an osteosarcoma subtype other than conventional as opposed to 29% in the control group (Table 8).

Discussion

Data on osteosarcoma in occurrence with other malignancies come to us by large follow-up studies on late effects in patients treated for cancer in childhood. With the exception of the study by Hawkins et al (10), all these studies are multi-centre studies.

Table 4.
Proportion of osteosarcomas by localisation with and without other malignancy

	Osteosarcoma only		Osteosarcoma + other	
Intramedullary osteosarcoma extremity	624	71%	26	39%
Intramedullary osteosarcoma axial skeleton/skull/flat bones	155	18%	33	50%
Surface OS	48	6%	4	6%
Extra skeletal OS	45	5%	3	5%
Total	872	100%	66	100%

We present here the incidence of osteosarcoma patients with other malignancies in a population-based study in the time frame of 1975 to May 2000. Of all patients with a reliable diagnosis of osteosarcoma, 7% had other malignancies. The relative risk is 2.4 for developing a second malignancy other than osteosarcoma. 3.3% developed their second tumour after treatment for osteosarcoma. This equals more or less the finding of 2.6% in the studies of Pratt (31) and Longhi (32). In two other studies this number is respectively 1.5 and 5% (33,34). In all, 3.7% of the osteosarcoma patients presented with osteosarcoma after a previous malignancy. In large studies on late effects of cancer treatment in childhood this is 0.07 to 0.34% (10,35-37). Especially women seem to be more susceptible for multiple primary tumours. Also in a recent report from the Childhood Cancer Survivor Study (38), the female sex proved to be an independent risk factor for the development of second primary tumour.
Most of the follow-up studies on childhood cancer deal with the effects of treatment. For al these studies together the most frequent malignancy after osteosarcoma is leukaemia (36%). Radiation therapy is the most important causative factor for the development of osteosarcoma (10,11). However, alkylating agents and radiation therapy are not the sole causes for malignancies associated with osteosarcoma. Abramson (8) described an elevated risk for developing osteosarcoma in patients with retinoblastoma not only in the radiation field, but also outside the radiation field and in patients not treated with radiation therapy. This genetic predilection for retinoblastoma patients to develop osteosarcoma was elucidated by the detection of 13q losses (Rb gene location) in osteosarcoma developing after hereditary as well as sporadic retinoblastoma (39), and in some osteosarcomas in non-retinoblastoma patients (40).
In our 938 osteosarcoma patients, two had a history of retinoblastoma, one developed osteosarcoma of the nose in the field of previous radiation, and at the age of 21 years one had a Ewing sarcoma of the head of the fibula treated with chemotherapy and radiation therapy 13 years after bilateral retinoblastoma. This patient subsequently had a mucoepidermoid carcinoma of the parotid gland at 30 years, an extra-skeletal osteosarcoma at 31 years in the field of previous radiation on the fibula and a squamous cell carcinoma of the concha medialis of the nose at 34 years.
A second hereditary cancer syndrome in which osteosarcoma is seen is the Li-Fraumeni syndrome. Based on our selection criteria, 12 patients of all osteosarcoma patients were suspect for having a Li-Fraumeni syndrome, Li-Fraumeni-like

syndrome or another yet undefined hereditary cancer syndrome. The way we conducted our research, all data were anonymous. Therefore no family histories could be obtained. This makes the application of the criteria we used to compose our study group, in a sense, arbitrary. Still, any Li-Fraumeni syndrome patient has a 70% chance of developing any primary invasive cancer (excluding cancer of the skin) before the age of 45 years (23). By selecting all patients with the development of a primary tumour before the age of 46 years after selecting all patients with a known history of retinoblastoma or a malignancy of the skin before the age of 44 we believe, we extracted those individuals highly suspected for membership of a Li-Fraumeni family.

Table 5.
Syndrome-suspected study group

Nr	Primary tumour	Localisation	Therapy	Secondary tumour	Localisation
118	Osteosarcoma	Femur	NA	Squamous cell ca	Larynx
120	Osteosarcoma	Cervical vertebra	S, RT	Adenocarcinoma	Kidney
121	Osteosarcoma	Cervical vertebra	S, RT	Adenocarcinoma	Kidney
153	Morbus Hodgkin	Neck	RT	Osteosarcoma	Clavicle
162	Osteosarcoma	Femur	S, RT	Basal cell ca	Back
173	Teratoma	Testis	S, RT	Osteosarcoma	Vertebra
196	Osteosarcoma	Fibula	NA	Plasmocytoma	Ileum
217	Oligodendroglioma	Brain	S, RT	Osteosarcoma	Skull
247	Unknown	Unknown,	RT	Osteosarcoma	Tibia
299	Rhabdomyosarcoma	Maxilla	RT, ChT	Osteosarcoma	Palatum
307	Ewing sarcoma	Humerus	NA	Osteosarcoma	Humerus
529	Lymphoma	Abdomen	ChT	Osteosarcoma	Maxilla
732	Osteosarcoma	Femur	NA	Ductal carcinoma	Breast
734	Osteosarcoma	Sternum	NA	Ductal carcinoma	Breast
776	Osteosarcoma	Jaw	NA	Morbus Hodgkin	Axilla
790	Adenocystic Ca	Palatum	S, RT	Osteosarcoma	Jaw
791	Fibrosarcoma	Breast	NA	Osteosarcoma	Rib
803	Unknown	Unknown	RT	Osteosarcoma	Rib
808	Unknown	Unknown	RT	Osteosarcoma	Clavicle
809	Osteosarcoma	Tibia	NA	Mucinous ca	Ovary
883	Osteosarcoma	Tibia	S, RT	Ductal carcinoma	Breast
897	Osteosarcoma	Femur	NA	Melanoma	Arm
906	Leiomyosarcoma	Cervix	NA	Osteosarcoma	Femur
911	Nephroblastoma	Kidney	NA	Osteosarcoma	Rib
912	Ewing sarcoma	Humerus	NA	Osteosarcoma	Humerus
971	Osteosarcoma	Femur	S, ChT	Ductal carcinoma	Breast
1035	Osteosarcoma	Femur	S, ChT	Lymphoma	
1039	Osteosarcoma	Femur	S, ChT	MDS	
1058	Osteosarcoma	Fibula	S, ChT	Squamous cell ca	Tongue
1107*	Osteosarcoma	Tibia	NA		
1125	Osteosarcoma	Femur	NA	Leukaemia	
1148	Retinoblastoma	Eye	S, RT	Osteosarcoma	Nose

* Patient known with Rothmund-Thomson
NA: information not available; S: Surgery; RT: Radiation therapy; Cht: Chemotherapy

Table 6.
Osteosarcoma subtypes in the syndrome suspected group

Subtype	Nr	Primary tumour	Second tumour
Telangiectatic	**120**	Osteosarcoma	Adenocarcinoma
Telangiectatic	**121**	Osteosarcoma	Adenocarcinoma
Telangiectatic	808	Unknown	Osteosarcoma
Chondroblastic	153	Hodgkin's disease	Osteosarcoma
Chondroblastic	776	Osteosarcoma	Morbus Hodgkin
Chondroblastic	**911**	Nephroblastoma	Osteosarcoma
Fibroblastic	**162**	Osteosarcoma	Basal cell carcinoma
Fibroblastic	247	Unknown	Osteosarcoma
Fibroblastic	**897**	Osteosarcoma	Melanoma
Fibroblastic	1039	MDS	Osteosarcoma
Fibroblastic	**1107**	Osteosarcoma	
Fibro/chondroblastic	**971**	Osteosarcoma	Ductal carcinoma
Osteoclast rich	**1058**	Squamous cell carcinoma	Osteosarcoma
Osteoma-like	**529**	Lymphoma	Osteosarcoma
Conventional	217	Oligodendroglioma	Osteosarcoma
Conventional	299	Rhabdomyosarcoma	Osteosarcoma
Conventional	790	Adenocystic carcinoma	Osteosarcoma
Conventional	1035	Lymphoma	Osteosarcoma
Conventional	1125	Leukaemia	Osteosarcoma
Conventional	1148	Retinoblastoma	Osteosarcoma

Table 7.
Osteosarcoma subtypes in the syndrome suspected group without any clear relation with therapy

Osteosarcoma subtype	Case Nr	N	%
Telangiectatic	120,121	2	22%
Fibroblastic	162,897,1107	3	33%
Fibro/chondroblastic	971	1	11%
Chondroblastic	911	1	11%
Osteoma-like	529	1	11%
Osteoclast rich	1058	1	11%
Total		9	

As for osteosarcoma subtypes in patients in our syndrome-suspected study group, there appears to be a large fraction of rare osteosarcoma subtypes in this group. In all, 70% of all reviewed osteosarcomas in the syndrome-suspected study group were other than conventional-type osteosarcoma. When cases without a relation to treatment were left out, all remaining patients had a non-conventional subtype. Of the 81 patients who developed an osteosarcoma after treatment for cancer in a study of the late effects study group by Newton (41), 57% of the patients treated with radiation therapy had a conventional type of osteosarcoma. In contrast, in the patients not treated with radiotherapy the conventional type was most frequent (90%).

Table 8.
Osteosarcoma subtypes in the European Osteosarcoma Intergroup patients

Osteosarcoma subtype	N	%
Conventional	404	71%
Chondroblastic	54	10%
Fibroblastic	53	9%
Telangiectatic	10	2%
Osteoclast rich	11	2%
Other	36	6%
Not sub typed	2	
Total	570	

In this study, there were also five patients with a family history of cancer. Half of these patients had an osteosarcoma of conventional type and half of another subtype. Thus, the histological subtyping of osteosarcomas might be a predictive diagnostic tool in the process of detecting patients with a yet undiagnosed familial predisposition for the development of cancer, more specific, yet undiagnosed cases of Li-Fraumeni syndrome. Osteosarcoma can be the first or even the only primary tumour to occur, not only in the individual patient, but also in a family with no indication for Li-Fraumeni syndrome at the moment of osteosarcoma diagnosis in one of their offspring (42). This has been further proven by the detection of the Li-Fraumeni syndrome specific p53 germ cell mutation (43) in a number of cases of multiple malignant neoplasms in children and young adults without a family history of Li-Fraumeni syndrome (44).
The absolute numbers we presented here are most likely underestimated. Registration in PALGA covers all the departments of pathology in the Netherlands only since 1990. The overall survival for osteosarcoma patients is around 55% and patients diagnosed with osteosarcoma in the later years have not yet developed their possible second tumour. This might mean that the initial number of patients suspected for Li-Fraumeni syndrome in our study is smaller than the actual amount of Li-Fraumeni syndrome patients in the total osteosarcoma patient population.
We conclude that although osteosarcoma is a rare tumour, an osteosarcoma patient has a relative risk of 2.4 (95% confidence interval 1.88 - 3.07) to develop another malignancy. Women are more susceptible than men to multiple primary malignancies including osteosarcoma. At a minimum, 1.2% of the osteosarcoma patients are suspected for a hereditary cancer syndrome, and a non-conventional subtype of osteosarcoma in a young patient raises the possibility of an individual to belong to a family with hereditary cancer syndrome.

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References

1. Dorfman HD, Czerniak B. Bone tumors. 1ed. St. Louis: Mosby; 1997.

2. Huvos AG. Bone tumors. Diagnosis, treatment and prognosis. 2ed. Philadelphia: WB Saunders; 1991.

3. Unni KK. Dahlin´s bone tumors: general aspects and data on 11,087 cases. 5ed. Philadelphia: Lippincott-Raven, 1996.

4. Cullen JW. Second malignant neoplasms in survivors of childhood cancer. Pediatrician 1991;18:82-9.

5. Meadows AT. Second tumors. Eur J Cancer 2001;37(16):2074-9.

6. Abramson DH, Ellsworth RM, Zimmerman LE. Nonocular cancer in retinoblastoma survivors. Tr Am Acad Ophthalmol & Otol 1976;81:454-7.

7. Abramson DH, Ellsworth RM, Kitchin FD. Osteogenic sarcoma of the humerus after cobalt plaque treatment for retinoblastoma. Am J Ophthalmol 1980;90:374-6.

8. Abramson DH, Ellsworth RM, Kitchin FD, Tung G. Second nonocular tumors in retinoblastoma survivors. Are they radiation induced. Ophthalmology 1984;91:1351-5.

9. Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. Br J Cancer 1986;53:661-71.

10. Hawkins MM, Draper GJ, Kingston JE. Incidence of second primary tumours among childhood survivors. Br J Cancer 1987;56:339-47.

11. Meadows AT, Baum E, Fossati-Bellani F, Green D, Jenkin RDT, Marsden B et al. Second malignant neoplasms in children: an update from the late effects study group. J Clin Oncol 1985;3:532-8.

12. Roarty JD, McLean I, Zimmerman LE. Incidence of second neoplasms in patients with bilateral retinoblastoma. Ophthalmology 1988;95:1583-7.

13. Schwarz MB, Burgess LPA, Fee WE, Donaldson SS. Postirradiation sarcoma in retinoblastoma. Induction of predisposition? Arch of Otolaryngol, Head Neck Surg 1988;114:640-4.

14. Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA et al. A cancer family syndrome in twenty four kindreds. Cancer Res 1988;48:5358-62.

15. Strong LC, Stine M, Norsted TL. Cancer in survivors of childhood soft tissue sarcomas and their relatives. J Nat Cancer Inst 1987;79:1213-20.

16. Goto M, Miller RW, Ishikawa Y, Sugano H. Excess of rare cancers in Werner syndrome (adult progeria). Cancer Epidemiol, Biomarkers Prevention 1996;5:239-46.

17. Ishikawa Y, Miller RW, Machinami R, Sugano H, Goto M. Atypical osteosarcomas in Werner syndrome (adult progeria). Jpn J Cancer Res 2000;91(12):1345-9.

18. Tsuji Y, Kusuzaki K, Kanemitsu K, Matsumoto T, Ishikawa Y, Hirasawa Y. Calcaneal osteosarcoma associated with Werner syndrome. A case report with mutation analyis. J Bone Joint Surg Am Vol 2000;82A:1308-13.

19. Vennos EM, Collins M, James WD. Rothmund-Thomson syndrome: review of the world literature. J Am Acad Dermatol 1992;27:750-62.

20. Bacci G, Delepine N, Bertoni F, Picci P, Mercuri M, Bacchini P et al. Predictive factors of histologic response to primary chemotherapy in osteosarcoma of the extremity: study of 272 patients preoperatively treated with high dose methotrexate, doxorubicin and cisplatin. J Clin Oncol 1998;16:658-63.

21. Hauben EI, Weeden S, Pringle J, Van Marck EA, Hogendoorn PCW. Does the histological subtype of high-grade central osteosarcoma influence the response to treatment with chemotherapy and does it affect overall survival? A study on 570 patients of two consecutive trials of the European Osteosarcoma Intergroup. Eur J Cancer 2002; 38:1218-1225.

22. Ferrari S, Bertoni F, Mercuri M, Picci P, Giacomini S, Longhi A et al. Predictive factors of disease-free survival for non metastatic osteosarcoma of the extremity: an analysis of 300 patients treated at the Rizzoli Institute. Ann Oncol 2001;12:1145-50.

23. Hisada M, Garber JE, Fung CY, Fraumeni JF, Fraumeni FP. Multiple primary cancers in families with Li-Fraumeni syndrome. J Nat Cancer Inst 1998;90:606-11.

24. Coebergh JW, Neumann HA, Vrints LW, van der Heijden L, Meijer WJ, Verhagen-Teulings MT.
Trends in the incidence of non-melanoma skin cancer in the SE Netherlands 1975-1988: a registry-based study. Br J Dermatol 1991;125:353-9.

25. Ewing J. A review of the classification of bone tumors. Bull Am Coll Surg 1939;24:290-5.

26. Macdonald I, Budd JW. Osteogenic sarcoma I. A modified nomenclature and a review of 118 five year cures. Surg Gynecol Obstet 1943;76:413-21.

27. Sim FH, Unni K, Beabout JW, Dahlin DC. Osteosarcoma with small cells simulating Ewing´s tumor. J Bone Joint Surg (Am) 1979;61a:207-15.

28. Simmons CC. Bone sarcoma. Factors influencing the prognosis. Surg Gynecol Obstet 1939;68:67-75.

29. Unni K, Dahlin DC, Beabout JW. Periosteal osteogenic sarcoma. Cancer 1976;37:2476-85.

30. Schajowics F, Sobin LH. World health organization. International histological classification of tumours. Histological typing of bone tumours. 2ed. Berlin: Springer-Verlag; 1993.

31. Pratt CB, Meyer WH, Luo X, Cain A, Kaste S, Pappo AS et al. Second malignant neoplasms occuring in survivors of osteosarcoma. Cancer 1997;80:960-5.

32. Longhi A, Gamberi G, Bacci G. Brain tumors as second malignant neoplasms in patients with osteosarcoma treated with adjuvant and neoadjuvant chemotherapy: report of 2 cases. J Chemother 2000;12:261-2.

33. Ferrari C, Bohling T, Benassi MS, Ferraro A, Gamberi G, Bacci G et al. Secondary tumors in bone sarcomas after treatment with chemotherapy. Cancer Detection Prevent 1999;23:368-74.

34. Nicholson HS, Mulvihill JJ, Byrne J. Late effects of therapy in adult survivors of osteosarcoma and Ewing´s sarcoma. Med Pediatric Oncol 1992;20:6-12.

35. Green DM, Zevon MA, Reese PJ, Lowrie GS, Gaeta JF, Pearce JI et al. Second malignant tumors following treatment during childhood and adolescence for cancer. Med Pediatric Oncol 1994;22:1-10.

36. Hawkins MM, Wilson LM, Burton HS, Potok MHN, Winter DL, Stovall M et al. Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. J Nat Cancer Inst 2001;88:270-8.

37. Li FP. Second malignant tumors after cancer in childhood. Cancer 1977;40:1899-902.

38. Neglia JP, Friedman DL, Yasui Y, Mertens AC, Hammond S, Stovall M et al. Second malignant neoplasms in five year survivors of childhood cancer: childhood cancer survivor study. J Nat Cancer Inst 2001;93:618-29.

39. Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A et al. Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. Proc Nat Acad Sci 1985;82:6216-20.

40. Dryja TP, Rapaport JM, Epstein J, Goorin AM, Weichselbaum R, Koufos A et al. Chromosome 13 homozygosity in osteosarcoma without retinoblastoma. Am J Hum Genet 1986;38:59-66.

41. Newton WA, Meadows AT, Shimada H, Bunin GR, Vawter GF. Bone sarcomas as second malignant neoplasms following childhood cancer. Cancer 1991;67:193-201.

42. Porter DE, Steel CM, Cohen BB, Wallace MR, Reid R. A significant proportion of patients with osteosarcoma may belong to Li-Fraumeni cancer families. J Bone Joint Surg (Br) 1992;74:883-6.

43. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH et al. Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-8.

44. Malkin D, Jolly KW, Barbier N, Look T, Friend SH, Gebhardt MC et al. Germline mutations of the p53 tumor-suppressor gene in children and young adults with second malignant neoplasms. N Engl J Med 1992;326:1309-15.

4

Clinico-histologic parameters of osteosarcoma patients with late relapse

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Abstract

Primary high-grade intramedullary osteosarcoma of the extremities is a clinically aggressive bone tumour. There is an ongoing effort to further improve efficacy of neo-adjuvant chemotherapy and reduce chemotoxicity by trying to identify osteosarcoma patients who are at risk of treatment failure as well as to identify those who can do with less chemotherapy. In only 5% of patients, first distant metastasis or local relapse occurs 5 years or more after initial treatment for osteosarcoma. Patients and physicians can therefore easily erroneously consider a patient with osteosarcoma cured if he or she is disease-free for more than five years following diagnosis and treatment. To investigate if these rare late relapsing patients are characterised by specific clinico-pathological features, we examined clinical and histological variables of late relapse (first local recurrence or metastasis 5 years are more after initial diagnosis) out of a total of 2243 patients, with a special interest in the histological osteosarcoma subtype.

In total, 33 patients had a documented relapse 5 years or more after diagnosis. The study found no statistically significant relationship between involved bone or subtype with late relapse. Half of the patients had good response (≥90% necrosis) to pre-operative chemotherapy and the other half a poor response (<90% necrosis) and late relapses seemed to be more frequently proportionately in those who had a good initial response to chemotherapy. The occurrence of late relapse does not appear to be associated with age or gender. Although not statistically significant there was a trend for patients with a chondroblastic subtype of osteosarcoma or a location of osteosarcoma in the tibia or fibula to have a higher risk for late relapse.

Introduction

Osteosarcoma is the most frequent non-haematogenic malignant bone tumour (1-3). A number of osteosarcoma subtypes are recognised dependent on the site of the involved bone, and the histomorphologic features. Among the high-grade central osteosarcomas conventional, telangiectatic and small cell subtypes exist (4). The conventional subtype comprises an osteoblastic, a chondroblastic and a fibroblastic variant. For research purposes, and comparison with data on osteosarcoma subtype in the literature, we restricted the term conventional to the osteoblastic variant, and the chondroblastic and fibroblastic variant are registered separately. Primary high-grade intramedullary osteosarcoma of the extremities is a highly aggressive bone tumour. Half of the patients are dead from disease five years after initial treatment for those with poor response (commonly defined as less than 90% necrosis) to pre-operative chemotherapy (5-7). On the contrary, the five year overall survival ranges between 70 and 87% for patients with histological good response to chemotherapy (≥ than 90% necrosis) (6-9). There is an ongoing effort to further improve efficacy of chemotherapy and reduce chemotoxicity by identifying at the time of diagnosis, those patients who will be at risk of treatment failure as well as those who need less intensive chemotherapy. Such prognostic factors are under investigation in the clinical, pathologic and molecular field. Information has been obtained on prognostic factors for overall and event-free survival (10-12), the risk for local relapse (13) and the predictive factors for outcome after relapse (14,15) from several large randomised studies on osteosarcoma using different chemotherapy regimes. In the group of osteosarcoma patients with relapse, up to 5% still have their first distant metastasis or local relapse 5 years after their initial, seemingly successful treatment (14,16). Prognosis in case of relapse is poor. The post relapse 5-year survival estimate is only in the range of 25% (14,16). Here, we have looked at clinical and histological variables that could be predictive for patients at risk for late relapse, with a special interest in the histological osteosarcoma subtype.

Material and Methods

In both the control and the study population, we included patients who were aged under 40 years at time of diagnosis. All patients had a biopsy showing high-grade intramedullary osteosarcoma of the extremities, with no evidence of metastasis at time of diagnosis, no previous history of other primary malignancy and no previous treatment with chemotherapy or radiotherapy. Late relapse was defined as first local recurrence or first metastasis occurring at least 5 years after initial diagnosis. The date of diagnostic biopsy was considered to be date the date of diagnosis.
Patient data were retrieved from three sources: [1] EORTC 80831/MRC BO02 (5) and EORTC 80861/MRC BO03 (7) which were two randomised controlled trials run by the European Osteosarcoma Intergroup (EOI), [2] all patients under the age of forty at time of diagnosis with a primary, localised, high-grade central extremity osteosarcoma who were entered into the Cooperative OsteoSarcoma Study Group (COSS) database between the end of 1979 and October 1997, and [3] from a single surgical centre cohort in the UK, Birmingham.

Table1. Late relapse patients

Patient	Age	Gender	Location	Site	Subtype	Response	IV to R (months)	Relapse	Site	Follow up since relapse (years)	Status at last follow up date
B1	16	M	Femur	D	Chondroblastic	Poor	81	Meta	Lung	14.1	Alive
B2	9	F	Tibia	D	Fibro/Osteobl	Unknown	110	Meta	Lung	5.2	Alive
B3	30	M	Femur	D	Fibroblastic	Poor	90	Local		4.5	Alive
B4	9	F	Femur	M	Pleomorphic	Poor	77	Meta	Lung	1	Dead
B5	9	F	Femur	D	Telangiectatic	Poor	66	Local		3	Dead
C1	10	F	Humerus	P	Conventional	Poor	73	Meta	Bone	16.8	Alive
C2	9	M	Ulna	D	Conventional	Good	72	Meta	Bone	4.3	Alive
C3	8	M	Tibia	D	Small cell	Good	63	Local + Meta	Bone	0.6	Alive
C4	26	M	Femur	D	Chondroblastic	Poor	103	Local		4.7	Dead
C5	13	M	Tibia	P	Fibroblastic	Good	66	Local		3.9	Dead
C6	22	M	Tibia	P	Conventional	Good	72	Meta	Lung	0.3	Alive
C7	15	M	Tibia	P	Unknown	Poor	74	Meta	Lung, bone, LN	0.9	Dead
C8	17	F	Fibula	P	Conventional	Good	123	Meta	Lung	2.7	Alive
C9	12	F	Tibia	P	Conventional	Good	101	Meta	Lung	0.4	Alive
C10	10	M	Tibia	P	Conventional	Good	65	Meta	Lung + CNS	0.2	Alive
C11	20	M	Tibia	P	Chondroblastic	Poor	118	Meta	Lung	2.9	Dead
C12	18	F	Tibia	D	Unknown	Good	89	Meta	Lung	6.6	Alive
C13	12	F	Femur	D	Unknown	Good	87	Meta	Lung	3.1	Dead
C14	13	M	Femur	D	Unknown	Good	84	Meta	Lung	11.6	Alive
C15	13	F	Tibia	D	Conventional	Good	75	Meta	Lung	3.8	Alive
C16	15	M	Femur	D	Unknown	Poor	71	Meta	Lung	2.9	Dead
C17	15	M	Fibula	P	Chondroblastic	Poor	69	Meta	Lung	3.3	Alive
C18	35	F	Femur	D	Conventional	Poor	69	Meta	Lung	4.1	Alive
C19	15	M	Humerus	P	Conventional	Good	65	Meta	Lung	0.0	Alive
C20	18	M	Tibia	P	Conventional	Poor	65	Meta	Lung	0.0	Alive
C21	23	M	Tibia	P	Chondroblastic	Poor	61	Meta	Lung	3.4	Alive
C22	17	M	Fibula	P	Chondroblastic	Good	90	Meta	Lung, bone	1.1	Dead
E1	18	M	Femur	D	Conventional	Poor	88	Meta	Lung	1.2	Dead
E2	15	M	Tibia	P	Conventional	Unknown	177	Meta	Lung	0.5	Dead
E3	13	F	Femur	D	Conventional	Good	62	Meta	Bone	1.2	Dead
E4	14	F	Tibia	D	Conventional	Poor	80	Meta	Bone	0.0	Dead
E5	32	M	Humerus	P	Conventional	Good	183	Meta	Lung	5.7	Alive
E6	19	M	Tibia	p	Fibroblastic	Unknown	65	Meta	Unknown	0.0	Dead

B: Birmingham; C: COSS; E: EOI; D: distal; M: midshaft; P: proximal; IV to R: Inteval to relapse in months; Meta: metastasisLN: Lymphnode; CNS: Central nerve system

Patients in the Birmingham cohort had received chemotherapy similar to the EOI trials but had not formally been entered in the EOI trial, thus there was no duplication of patient entry. The EOI patients contributed to the late relapses and control group, the COSS and Birmingham cohort patients contributed only to the late relapses.
Therefore, for the EOI there were a total of 557 osteosarcoma patients who where randomised to the trials between July 1983-December 1986 (BO02/80831: 179 patients) (5) and September 1986-December 1991 (studyBO03/80861: 391 patients) (7). This number excludes 13 patients without a reported progression but less than 5 years follow-up. Six (1%) of these patients had a late relapse and are considered cases of this paper, the remaining 551 form the control group. The median follow-up in patients last alive is 14 years (min 5.2 years, max 20.5 years). For COSS this is a total of 1136 patients with a median follow up of 6 years (min 31 days, max 22.6 years) for all patients and 8.3 years (min 0.3-22.6 years) for 796 survivors. In the files from Birmingham there were a total of 550 patients.
Data analysed were age, gender and location of the tumour, histological subtype and response to chemotherapy. Since the COSS database does not record the histological subtype this was retrieved from the original pathology reports by one of the authors (EH). The subtype was specified in the report in 17 (77%) patients. The criteria for subtyping and the method of assessment of necrosis have been extensively described elsewhere (17,18). In short, an osteosarcoma was classified as one or other subtype if ≥ 90% of the lesion showed histological features specific for a given subtype. The exception was chondroblastic osteosarcoma, which was classified as such if more than 30% of the lesion was composed of a chondroid matrix. As for necrosis, in the EOI this was reported as a percentage of the total tumour. In the COSS database necrosis is graded according to Salzer-Kuntschick criteria (18) as follows: 1: no viable tumour, 2: solitary viable cells or one islet of less than 0.5 cm, 3: less than 10% of viable tumour, 4: 10-50% of viable tumour, 5: more than 50 % viable tumour, 6: no effect of chemotherapy. For analysis, this was converted in accordance with the EOI data to good responders (≥ 90%) for Salzer-Kuntschick grade 1, 2 and 3, and poor responders (<90%) for Salzer-Kuntschick grade 4 and higher.
In summary, patients with first relapse 5 years or more after diagnosis from two EOI trials, COSS database and the Birmingham cohort are included. Controls are patients with at least 5 years follow-up but no late relapse reported in the two EOI trials. With regard to evaluation of necrosis, data on the resected specimen was available in 364 cases.

Results

Out of a total of 1136 osteosarcoma patients in the COSS cohort 22 patients (2%) had a first relapse or metastasis 5 years or more after their initial diagnosis. In the EOI group there were 6 (1%) cases from a total of 557 and in the patient group from Birmingham 5 (0.9%) cases on a total of 550. This is a total of 33 patients. Twenty-eight patients (85%) relapsed with metastases, 4 (12%) with a local relapse and 1 (3%) with simultaneous local relapse and metastasis. Patient details are summarised in Table 1. The control group comprised the patients from the EOI trials, of whom 245 had 5 or more years of follow-up and no relapse and 306 who relapsed within 5 years.

Table 2.
Number of osteosarcoma patients by age group

	Late relapses (all sources)			EOI control: EOI early relapses			EOI control: EOI no relapse			EOI control: EOI overall		
Age	M	F	T	M	F	T	M	F	T	M	F	T
0-4	0	0	0	1	1	2	2	0	2	3	1	4
5-9	2	3	5	11	11	22	11	9	20	22	20	42
10-14	3	6	9	55	47	102	34	36	70	89	83	172
15-19	10	2	12	87	31	118	63	30	93	150	61	211
20-24	3	0	3	31	7	38	32	11	43	63	18	81
25-29	1	0	1	11	3	14	3	4	7	14	7	21
30-34	2	0	2	2	4	6	1	3	4	3	7	10
35-40	0	1	1	3	1	4	5	1	6	8	2	10
Total	21	12	33	201	105	306	151	94	245	352	199	551
%	64	36	100	66	34	100	62	38	100	64	36	100

M: male, F: female, T: Total

Table 3.
Location of osteosarcoma and subtype

	Late relapses (all sources)		EOI control: early relapse		EOI control: no relapse		EOI control: overall	
	N	%	N	%	N	%	N	%
Location								
Femur	11	33%	169	55%	133	54%	302	55%
Tibia	15	45%	73	24%	70	29%	143	26%
Fibula	3	9%	13	4%	14	6%	27	5%
Humerus	3	9%	48	16%	21	9%	69	13%
Radius/Ulna	1	3%	1	0%	5	2%	6	1%
Other	0	0%	2	1%	2	1%	4	1%
Subtype								
Data Missing	5		1		1		2	
Data available	28	100%	305	100%	244	100%	549	100%
Conventional	15	54%	220	72%	171	70%	391	71%
Chondroblastic	6	21%	27	9%	25	10%	52	9%
Fibroblastic	3	11%	27	9%	22	9%	49	9%
Anaplastic	1	3%	13	4%	10	4%	23	4%
Telangiectatic	1	3%	5	2%	5	2%	10	2%
Small cell	1	3%	3	1%	0	0%	3	1%
Other	1	3%	10	3%	11	5%	21	4%
Total	33		306		245		551	

Table 4.
Subtype in relation to involved bone

	Humerus and Femur		Tibia, Fibula, Ulna	
	N	%	N	%
Conventional	6	45	9	53
Non-Conventional	5	54	8	47
Not available	3		2	
Total	14		19	

Table 5.
Gender, localisation and subtype of high-grade intramedullary osteosarcoma in some large osteosarcoma study groups

Study group	Coss 80[24]		Coss 86[22]		Rizolli 1-5[21]		EOI 1&2[17,23]	
	N	%	N	%	N	%	N	%
Patients	116	100	171	100	510	100	570*	100
Male	69	59	107	63	292	57	361	63
Female	47	41	64	37	218	43	207	37
Location								
Femur	61	53	73	43	277	54	315	55
Tibia	38[!]	33	47	27	142	28	148	26
Humerus	12	10	18	11	56	11	72	13
Fibula	4[!]	3	12	7			27	5
Radius	2	2					3	1
Other			21	12	35	7	3	1
Unknown							2	
Subtype[&]								
Conventional					348	68	71	65
Chondroblastic					63	12	10	11
Telangiectatic					44	9	2	8
Fibroblastic					39	8	9	7
Other							8	8
NOS					16	3		

* Gender not registered in 2 cases
[!] One patient with 2 osteosarcomas
& No details available on subtype in the COSS study database

Late relapse and clinico-pathological features.
Of the patients with late relapse, 21 (64%) were male and 12 (36%) female (Table 2). Age at diagnosis ranged from 9 to 35 years with a peak incidence at 15-19 years. Age and sex distribution were similar in the control groups. Eighteen (54%) of the patients with late relapse had their osteosarcoma located in the tibia or the fibula in apparent contrast with 170 (31%) of osteosarcomas occurring in these locations in the control group (Table 3). There is some evidence that late relapses occurred more in patients with an initial localisation not in the femur or humerus) (Pearson χ^2 5.08 (df=1), p=0.024). Thirteen (46%) of the patients with late relapse had a non-conventional subtype of osteosarcoma compared with 158 (29%) of the patients in the EOI control (Table 3). The main difference was due to the higher proportion of chondroblastic tumours in the study group (21%) compared to the control group (9%). However, there was no good evidence of a difference in the number of patients with or without late relapse by pathological subtype (conventional vs chondroblastic vs other non-conventional) (Pearson χ^2 4.1099 (df=2), P=0.128). There was no evidence of a relationship between subtype of osteosarcoma and localisation in the bone (χ^2= 0.0069 (df=1) P 0.934) (Table 4).

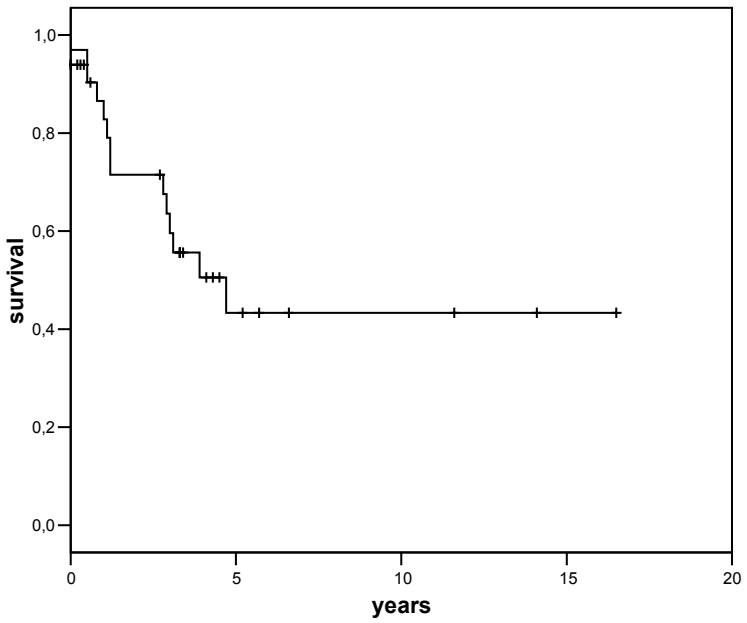
Late relapse and response to pre-operative chemotherapy.
Half of the late relapse patients had good response (≥90% necrosis) to pre-operative chemotherapy and the other half a poor response (<90% necrosis). Only 102 (28 %) patients out of the 364 in the EOI control, for which the resected specimen was available for review, showed good response to chemotherapy. However, among the 1136 eligible COSS patients 580 had good (57%) and 433 (43%) had a poor response to pre-operative chemotherapy, with the rest of the patients having either primary surgery or no data available on response.

Late relapse and survival.
The overall survival for patients with late relapse is depicted in Fig.1. The overall survival at 1, 3 and 5 years following relapse was 83%, 60% and 43% respectively.

Discussion

When studying a disease in which there are multi-factorial influences on progression and outcome such as osteosarcoma, large randomised trials are mandatory. Even then, due to the overall low incidence of osteosarcoma the total number of cases can be too small when examining subsets of factors such as osteosarcoma subtype or studying events as late relapse. For the purpose of our study, which tried to identify predictive factors for late relapse, we were thus obliged to merge data from EOI trial 80831 (BO02) and 80861 (BO03), the patients in the COSS cohort and the patients from Birmingham. Even then there were only a small number of patients with late relapse making it difficult to comment reliably on predictive markers. Merging of data poses the problem of looking at patients who had different chemotherapy regimes with different response to chemotherapy and different outcome in terms of disease free survival (DFS) and overall survival (OS). Patients randomised in protocols with better DFS relapse statistically later than patients in protocols with significant worse DFS (19). This is reflected by the 2% of patients with late relapse in the COSS cohort and 1% of patients with late relapse in the EOI patients. The 5 year disease free survival in both groups is 62% (20) and 46%

Fig 1.
Kaplan-Meier survival curve of patients with first relapse more than 5 years after initial diagnosis and treatment



Kaplan-Meier: actuarial survival (standard error)
2-year 72% (SE 9%)
5-year 43% (SE 11%)

respectively (5,7). It is possible therefore that a "good" response to chemotherapy may in fact simply delay the onset of metastases in some patients.
With regard to age, location of the osteosarcoma and subtype no significant differences were expected between the EOI and COSS study groups since these variables show similar distributions in diverse randomised trials (17,21-24) (Table 5). In our study group of osteosarcoma patients with late relapse, the peak incidence of diagnosis was between 15 and 19 years with a male predominance, which was the same as in the control group and data from the literature (3,23). Normally, the bones around the knee and the proximal humerus are mostly involved with the distal femur being the site of predilection. Though not statistically significant there was an observed shift towards tibial or fibular involvement in patients with late relapse. There was also an over-representation of subtypes other than conventional osteoblastic in the late relapsing patients. This is probably largely due to the presence of a chondroblastic component, but the evidence is not as yet statistically significant. The histological subtype of osteosarcoma has been shown to be a predictive factor for response to chemotherapy (17,21,25). Generally,

chondroblastic osteosarcomas show poorer response to pre-operative chemotherapy as was already demonstrated by Kersjes in 1987 (26). In his report, a mean chondroid ground substance of 21% was seen in patients with poor response to pre-operative chemotherapy. In contrast a chondroblastic subtype tends to be related with better overall survival (17). Whether patients with a chondroblastic subtype of osteosarcoma thus represent a high-risk or low-risk subgroup of osteosarcoma patients is open for further discussion and research. To determine with certainty whether chondroblastic osteosarcoma represents a separate entity with regard to clinical behaviour requires further investigation. However, the rarity of osteosarcoma and even more the diverse subtypes of osteosarcoma hamper this.

In conclusion, relapse 5 years after initial treatment of osteosarcoma is low, arising in between 1 to 2% of all osteosarcoma patients, and is consistently so in the three osteosarcoma study groups on which this study has been based. There is no evidence that late relapse is related to age or gender, but there is a trend for it to arise more commonly in chondroblastic subtypes, in patients with a primary in the tibia and fibula and seems to be proportionately more common in patients with initial good response to chemotherapy. These patients thus should be considered as at risk for late relapse, for which long term follow up for detection of relapse is warranted.

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Reference List

1. Dorfman HD, Czerniak B. *Bone tumors*. St. Louis: Mosby, 1997.

2. Huvos AG. Bone tumors. Diagnosis, treatment and prognosis. Philadelphia: WB Saunders, 1991.

3. Unni KK. Dahlin´s bone tumors: general aspects and data on 11,087 cases. Philadelphia: Lippincott-Raven, 1996.

4. World Health Organization Classification of tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone Lyon: IARC Press, 2002.

5. Bramwell VHC, Burgers M, Sneath RJ *et al.* A comparison of two short intensive adjuvant chemotherapy regimens in operable osteosarcoma of limbs in children and young adults: the first study of the European Osteosarcoma Intergroup. J Clin Oncol 1992;10:1579-1591.

6. Provisor AJ, Ettinger LJ, Nachman JB *et al.* Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children´s Cancer Group. J Clin Oncol 1997;15:76-84.

7. Souhami RL, Craft AW, Van der Eijken JW *et al.* Randomises trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. *Lancet* 1997;350:900-901.

8. Bacci G, Ferrari S, Longhi A *et al.* High-dose ifosfamide in combination with high dose methotrexate, adriamycin and cisplatin in the neoadjuvant treatment of extremity osteosarcoma: preliminary results of an Italian Sarcoma Group/Scandinavian Sarcoma Group pilot study. J Chemother 2002;14:198-206.

9. Bacci G, Ferrari C, Longhi A *et al.* Neoadjuvant chemotherapy for high grade osteosarcoma of the extremities: long-term results for patients treated according to the Rizzoli IOR/OS -3b protocol. J Chemother 2001;13:93-99.

10. Bielack SS, Kempf-Bielack B, Delling G *et al.* Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocol. J Clin Oncol 2002;1:776-790.

11. Ferrari S, Bertoni F, Mercuri M *et al.* Predictive factors of disease-free survival for non metastatic osteosarcoma of the extremity: an analysis of 300 patients treated at the Rizzoli Institute. Ann Oncol 2001;12:1145-1150.

12. Zunino JH, Johnston JO. Prognostic value of histologic tumor necrosis assessment in osteogenic sarcoma of bone. Am J Orthop 2000;29:369-372.

13. Bacci G, Ferrari S, Mercuri M *et al.* Predictive factors of local recurrence in osteosarcoma: 540 patients with extremity tumors followed for minimum 2.5 years after neoadjuvant chemotherapy. Acta Orthop Scand 1998;69:230-236.

14. Ferrari S, Briccoli A, Mercuri M *et al.* Postrelapse survival in osteosarcoma of the extremities: prognostic factors for long-term survival. J Clin Oncol 2003;21:710-715.

15. Tabone MD, Kalifa C, Rodary C, Raquin M, Valteau-Couanet D, Lemerle J. Osteosarcoma recurrences in pediatric patients previously treated with intensive chemotherapy. J Clin Oncol 1994;12:2614-2620.

16. Kempf-Bielack B, Bielack SS, Jürgens H *et al.* Osteosarcoma relapsing after combined modality therapy. An analysis of unselected patients in the Cooperative Osteosarcoma Study Group (COSS). J Clin Oncol 2005;23:559-568

17. Hauben EI, Weeden S, Pringle J *et al.* Does the histological subtyp of high-grade central osteosarcoma influence the response to treatment with chemotherapy and does it affect overall survival? A study on 570 patients of two consecutive trials of the European Osteosarcoma Intergroup. Eur J Cancer 2002;38:1218-1225.

18. Salzer-Kuntshick M, Brand G, Delling G. Bestimmung der morphologischen Regressionsgrades nach Chemotherapie bei Malignen Knochentumoren. Pathologe 1983;4:135-141.

19. Bacci G, Ferrari S, Longhi A *et al.* Pattern of relapse in patients with osteosarcoma of the extremities treated with neoadjuvant chemotherapy. Eur J Cancer 2001;37:32-38.

20. Bielack SS, Kempf-Bielack B, Schwenzer D *et al.* Neoadjuvant therapy for localized osteosarcoma of extremities. Results from the Cooperative Osteosarcoma Study group COSS of 925 patients. Klin Padiatr 1999;211:260-270.

21. Bacci G, Ferrari S, Bertoni F *et al.* Histologic response of high-grade nonmetastatic osteosarcoma of the extremity to chemotherapy. Clin Orthop 2001;386:186-196.

22. Fuchs N, Bielack SS, Epler D *et al.* Long-term results of the co-operative German-Austrian -Swiss osteosarcoma study group´s protocol COSS-86, of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. Ann Oncol 1998;9:893-899.

23. Hauben EI, Arends J, Vandenbroucke JP *et al.* Multiple primary malignancies in osteosarcoma patients. Incidence and predictive value of osteosarcoma subtype for cancer syndromes related with osteosarcoma. Eur J Hum Genet 2004;11:611-618.

24. Winkler K, Beron G, Kotz R *et al.* Neoadjuvant chemotherapy for osteogenic sarcoma: Results of the cooperative German/Austrian study. J Clin Oncol 1984;2:617-624.

25. Bacci G, Delepine N, Bertoni F *et al.* Predictive factors of histologic response to primary chemotherapy in osteosarcoma of the extremity: study of 272 patients preoperatively treated with high dose methotrexate, doxorubicin and cisplatin. J Clin Oncol 1998;16:658-663.

26. Kersjes W, Heise U, Winkler K *et al.* Comparison of quantitative ground substance analysis in biopsy and resected tumour in osteosarcomas. Virchows Arch A 1987;412:155-160.

5

Adamantinoma-like Ewing sarcoma and Ewing-like adamantinoma. The t(11;22), t(21;22) status.

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Abstract

Adamantinoma of the long bones and Ewing sarcoma are two malignant tumours between which at first sight, there seems to be no morphological and clinical relationship. Both tumours, however, are known to express cytokeratins. Adamantinoma is a tumour of true epithelial nature, predominantly expressing cytokeratins 14 and 19. Ewing sarcoma, believed to be from neuroectodermal origin, like other mesenchymal tumours, can aberrantly express cytokeratin 8 and 18. In the literature there are some reports of tumours showing clinical and/or morphologic overlap between adamantinoma and Ewing sarcoma, suggesting a possible relationship. These studies are mostly based on the epithelioid configuration of these lesions and their cytokeratin expression on immunohistochemistry. This raises the question of whether there is occasionally a morphological similarity between adamantinoma and Ewing sarcoma, or whether there is a common genetic background. The Ewing sarcoma/primitive peripheral neuroectodermal tumour (PNET) family is characterised in 90-95 % of cases by a t(11;22) and in 5-10 % of cases by t(21;22). In the few reports in the literature on cytogenetic investigations on adamantinoma, these translocations were never found using classical karyotyping. This study investigated the putative presence of t(11;22) and t(21;22) in 14 cases of adamantinoma by RT-PCR. These translocations were not found in any of these cases. The results support the view that these tumours are genetically and clinically distinct, but may eventually show overlapping morphological and immunohistochemical features.

Introduction

Small cell tumours of the Ewing sarcoma/primitive peripheral neuroectodermal tumour family are highly malignant skeletal and extra skeletal tumours, believed to be from neuroectodermal origin. Ewing sarcoma (1) is defined as a primitive malignant tumour characterised by uniform densely packed small cells, without distinct cytoplasmic borders or prominent nucleoli (2). Adamantinoma of the long bones is a low-grade malignancy with a predilection for the tibial cortex, and is composed of epithelial cells in a fibrous or osteofibrous stroma (3,4).
Since Ewing sarcoma is a small blue cell tumour and adamantinoma displays clear epithelioid features, there is apparently no problem in differential diagnosis on histology, and no reason to suspect a relationship between these two entities.
However, in 1975, Van Haelst et al. (5) reported a case of a tumour in the metatarsus in a 13-year old girl, which posed a differential diagnosis between adamantinoma and atypical Ewing sarcoma. Since then, a few reports have been published of adamantinoma looking like Ewing sarcoma and visa versa (6-8).
Fukunaga et al., in 1998, presented a case of a tumour in a 15-year-old male localised in the tibia and composed of strands and trabeculae of epithelioid cells immunoreactive for Leu7 and synaptophysin (9). There was strong diffuse and membranous immunoreactivity for O13, but the lesional cells were also immunoreactive for pancytokeratin and EMA. Based on the epithelioid morphology and immunohistochemistry, together with the localisation in the tibia, they postulated that this tumour was a variant of adamantinoma, possibly related to Ewing sarcoma.
The Ewing sarcoma/primitive peripheral neuroectodermal tumour group is characterised in 90 to 95 % of cases by a t(11;22)(q24;q12) (10-12), resulting in the fusion of the EWS gene on chromosome 22 with the FLI-1 gene on chromosome 11 (13). In order to look for a possible relationship between Ewing sarcoma and adamantinoma of the long bones, reverse transcription polymerase chain reaction (RT-PCR) for the detection of the t(11;22) and t(21;22) was performed on 14 cases of biopsy-proven adamantinoma from which frozen material was available.

Material and Methods

Patient material
From 14 patients out of 37 with a confirmed diagnosis of adamantinoma of the long bones, registered in the data base of the Netherlands Committee on Bone Tumours, snap frozen material, stored at –80°C was available. The diagnosis was based on histological, clinical, radiological and immunohistochemical data. All cases have been reviewed previously and clinicopathologic data have been reported (14,15).

Table 1
Primers used for translocation analysis and housekeeping gene analysis

EWS 22.8	5′-CCCACTAGTTACCCACCCCAAA-3′
FLI-1 11.11	5′-TGTTGGGCTTGCTTTTCCGCTC-3′
ERG 11	5′-TGTTGGGTTTGCTCTTCCGCTC-3′
HPRT hum 1	5′-ACCGGCTTCCTCCTCCTGAGCAGTC-3′
HPRT hum 2	5′- AGGACTCCAGATGTTTCCAAACTCAACTT-3′

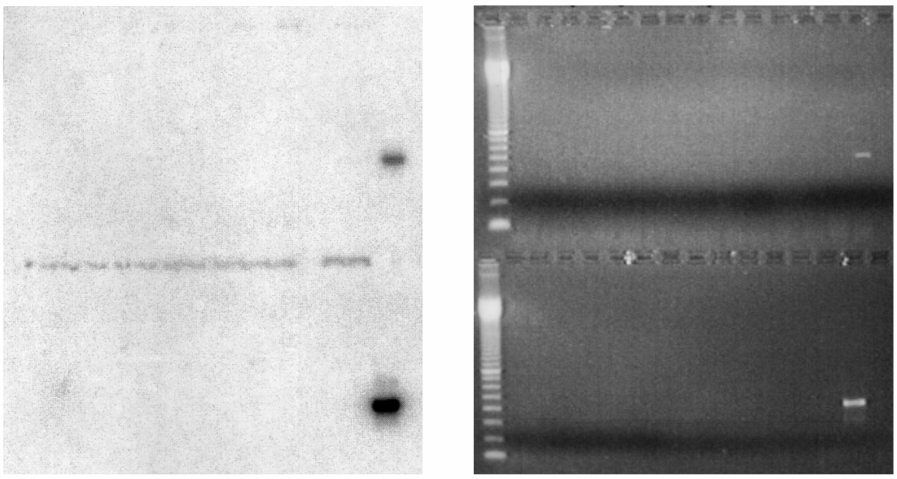
RNA extraction and RT-PCR

The protocol used for the extraction of RNA, the reverse transcription and the polymerase chain reaction has been described previously (16). In short, after extraction of RNA and reverse transcription, the polymerase chain reaction was performed using the primers in Table 1, according to published sequences (17,18). After electrophoresis on agarose gel, the amplification product was visualised by ethidium bromide. To improve sensitivity, electrophoresis was followed by transferring the PCR products to nitro-cellulose, which was subsequently subjected to hybridisation. The hybridisation product was performed with a ^{32}P-labeled oligonucleotide (5′-CCGTCATTCTTGAACTCCCCGTTGG-3′) which is complementary to a highly homologous sequence within the FLI-1- and ERG-gene. A Ewing sarcoma cell culture with cytogenetically proven t(11;22)(q24;q12) or frozen material from a Ewing sarcoma with documented t(21;22)(q22;q12) was used as a positive control. H_2O was used as a template to exclude the possibility of contamination. A transcript of the housekeeping gene HPRT was amplified simultaneously to confirm that each RNA sample tested could potentially yield products after RT-PCR.

Results

In 2 of the 14 cases of adamantinoma, there was no amplification of the housekeeping gene HPRT. Light microscopic examination of the frozen material showed substantial freezing artefacts and low cellularity, based upon the presence of neocortical bone only. These two cases were excluded from further studies.

Fig 1.
Southern bloth analysis and gel electrophoresis following RT-PCR



At the right gel electrophoresis following RT-PCR, showing the absence of a reaction product in all adamantinomas tested. RNA integrity was controlled by positive reactivity for the housekeeping gene HPRT in 12/14 cases. The positive controls for EWS/ERG (upper) or EWS/FLI-1 (lower) showed a positive product at the expected molecular weight. At the left Southern blot analysis showing the true absence of reaction product and appropriate reactivity for the controls.

In none of the other 12 cases of adamantinoma EWS/FLI1 or EWS/ERG fusion transcripts were detected on agarose gel and after hybridisation. The positive controls showed appropriate signal both on agarose gel as well as after hybridisation. The H_2O control was negative. The results are depicted in Figure 1.

Discussion

Ewing sarcoma, a "small blue cell tumour" of the bone and soft tissues, is a tumour of neuroectodermal origin. Its diagnosis in routine practice has been based on its histochemical and immunohistochemical characteristics. The cells of Ewing sarcoma are known to contain glycogen and to be immunoreactive for neuron specific enolase (NSE) and vimentin, and variably reactive for synaptophysin and Leu 7.
Ewing sarcoma and PNET show a high expression of the MIC2 gene product, a 30/32 kD surface antigen (19,20). The detection of this surface protein by the antibody CD99 or O13, though not specific, is very characteristic when there is strong membranous immunoreactivity in the large majority of the cells.
The translocation (11;22) is specific for the Ewing sarcoma/PNET tumour family (10-12), although it has occasionally been reported in other tumour types (21-24). The demonstration of this translocation, which is present in nearly 90-95% of Ewing sarcoma/PNET has nowadays become an invaluable diagnostic marker, especially since RT-PCR and FISH can also be applied on formalin-fixed and paraffin-embedded tissue (25-28).
Adamantinoma of the long bones, primarily occurring in the tibia, is a lesion of epithelial nature. This was proved by electron microscopy in 1969 and confirmed by immunohistochemistry in 1982 (29,30). The epithelial nature was further revealed by the work of Hazelbag et al. (31), showing the expression of basal cell type cytokeratins in the cells of adamantinoma. More specifically, there is expression of cytokeratins 14, 19, 5 and also in a lesser degree, CK 17.
Although adamantinoma and Ewing sarcoma seem to be of a different nature, a few reports have been published of cases in which the differential diagnosis proved to be problematic (5-9).
Fukunaga et al. (9) used the epithelioid features, defined both morphologically and immunohistochemically, as an argument for a possible relationship between adamantinoma and Ewing sarcoma.
Recent articles have drawn the attention to the fact that although it is not typical, cytokeratin expression is not unusual in Ewing sarcoma (32,33). In the publication by Gu et al. (32) 20% of the 50 cases of cytogenetically proven Ewing sarcoma tested by immunohistochemistry for cytokeratin with antibody Cam 5.2 (detection of CK 8,18 and 19) and the pan CK antibody CK AE1/AE3, proved to be cytokeratin positive. Vakar-López et al. (33) revealed immunoreactivity for CK Cam 5.2, CK AE1/AE3 or CK 18 in 18% of their 33 cases. As early as 1987, Moll et al. (34) demonstrated the expression of predominantly CK18 and CK 8 in Ewing sarcoma. This is in contrast to the cytokeratin profile of adamantinoma in which no expression of CK 18 and 8 is found (31). This strongly underlines the need for caution when comparing cytokeratin expression in tumours, without looking at the specific subtypes expressed.
In 1999, Bridge et al. (35) published two historical cases originally diagnosed as adamantinoma and a new one arising in the fibula of a 13-years-old male diagnosed as atypical Ewing, because of epithelioid features. RT-PCR on this three lesions and FISH on the lesion in the fibula revealed the Ewing/PNET specific (11;22) translocation. They concluded that these lesions were a variant of Ewing sarcoma and

proposed the term adamantinoma-like Ewing sarcoma. Given the very restricted distribution pattern of adamantinoma, which is not the case in Ewing sarcoma, a diagnostic problem could arise in individual cases in the tibial shaft. In these cases, radiology, immunohistochemistry and ultimately molecular analysis could render a specific diagnosis. We do, however, support the view of Gaffeny (36) that simply finding the t(11;22) is not enough in a small cell tumour to classify it as a Ewing sarcoma or PNET, since this translocation can be found in other small cell tumours (21-24).

In all our 12 cases of adamantinoma analysed by RT-PCR on frozen material, the t(11;22)(q24;q12) or the t(21;22)(q22;q12) could not be detected. We admit that the RT-PCR employed only recognises 95% of the EWS translocations, but given the nature of adamantinoma, it would be very unlikely that they would show one of the rarer variants of EWS translocation. FISH analyses looking for EWS-gene rearrangements could be used to search for variant translocations. Adamantinoma sections are, however, often heavily calcified because of the osteofibrous component. This makes FISH on tissue sections technically difficult. Moreover, a previous cytogenetic analysis on five adamantinomas from the Netherlands Committee on Bone tumours (15) revealed trisomy of chromosomes 7, 8, 12 and 19, one case with a t(10;12) and one case with a translocation (2;11)(p23;q14)inv(11)(p14;q14), but no (11;22) translocation specific for the Ewing/PNET group of tumours. Nor was there any of the other less frequent translocations seen in Ewing sarcoma/PNET, such as the t(21;22)(q22;q12), t(7;22)(p22;q12), t(17;22)(q12;q12) and t(2;21;22)(q33;q22;q12) all involving the EWS gene on chromosome 22 [18;37-39]. In fact, none of the chromosomal aberrations in the five cases of adamantinoma investigated by Hazelbag involved chromosome 22.

We conclude that there is no cytogenetic relationship between adamantinoma and Ewing sarcoma. Any morphologic overlap seems to be indeed only morphologic, since even the cytokeratin profiles of both lesions are completely different. In the rare case of doubt, immunohistochemistry for assessment of the cytokeratin profile and cytogenetic analysis should lead to a correct diagnosis.

References

1. Ewing J. Diffuse endothelioma of bone. Proc NY Path Soc 1921; 21:17-24.

2. Schajowicz F, Sobin LH. World health organization. International histological classification of tumours. Histological typing of bone tumours. Berlin: Springer-Verlag, 1993

3. Fischer B. Über ein primäres Adamantinom der Tibia. Frankf Z Pathol 1913; 12:422-441.

4. Moon NF, Mori H. Adamantinoma of the appendicular skeleton--updated. Clin Orthop 1986; 204:215-237.

5. Van Haelst UJGM, de Haas van Dorsser AH. A perplexing malignant bone tumor. Highly malignant so-called adamantinoma or non-typical Ewing's sarcoma. Virchows Arch [A] 1975; 365:63-74.

6. Ishida T, Kikuchi F, Oka T, et al. Case report 727: Juxtacortical adamantinoma of humerus (simulating Ewing tumor). Skeletal Radiol 1992; 21:205-209.

7. Lipper S, Kahn LB. Case report 235. Ewing-like adamantinoma of the left radial head and neck. Skeletal Radiol 1983; 10:61-66.

8. Meister P, Konrad E, Hubner G. Malignant tumor of humerus with features of "adamantinoma" and Ewing's sarcoma. Path Res Pract 1979; 166:112-122.

9. Fukunaga M, Ushigome S. Periosteal Ewing-like adamantinoma. Virchows Arch 1998; 433:385-389.

10. Aurias A, Rimbaut C, Buffe D, Dubousset J, Mazabraud A. Chromosomal translocations in Ewing´s sarcoma. N Engl J Med 1983; 309:496-497.

11. Turc-Carel C, Philip I, Berger MP, Philip T, Lenoir GM. Chromosomal translocations in Ewing´s sarcoma. N Engl J Med 1983; 309:497-498.

12. Turc-Carel C, Aurias A, Mugneret F, et al. Chromosomes in Ewing´s sarcoma. I. An evaluation of 85 cases and remarkable consistency of t(11;22)(q24;q12*)*. Cancer Genet Cytogenet 1988; 32:229-238.

13. Zucman J, Delattre O, Plougastel B, et al. Cloning and characterization of the Ewing´s sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chromosomes Cancer 1992; 5:271-277.

14. Hazelbag HM, Taminiau AHM, Fleuren GJ, Hogendoorn PCW. Adamantinoma of long bones. A clinicopathological study of thirty-two cases with emphasis on histological subtype, precursor lesion and biological behaviour. J Bone Joint Surg [Am] 1994; 76A:1482-1499.

15. Hazelbag, H. M., Wessels, J. W., Mollevangers, P., Van den Berg, E., Molenaar, W. M., and Hogendoorn, P. C. W. Cytogenetic analysis of adamantinoma of long bones. Further indications for a common histogenesis with osteofibrous dysplasia. Cancer Genet Cytogenet 1997; 97, 5-11.

16. Bovee JVMG, Devilee P, Cornelisse CJ, Schuuring E, Hogendoorn PCW. Identification of an EWS-pseudogene using translocation detection by RT-PCR in Ewing's sarcoma. Biochem Biophys Res Commun 1995; 213:1051-1060.

17. Delattre O, Zucman J, Melot T, et al. The Ewing family of tumors. A subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med 1994; 331:294-299.

18. Zucman J, Melot T, Desmaze C, et al. Combinatorial generation of variable fusion proteins in the Ewing family of tumours. EMBO J 1993; 12:4481-4487.

19. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H, Salzer-Kuntschik M. MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors: Evidence for a common histogenesis of Ewing's sarcoma and peripheral primitive neuroectodermal tumors from MIC2 expression and specific chromosome aberration. Cancer 1991; 67:1886-1893.

20. Fellinger EJ, Garin-Chesa P, Triche TJ, Huvos AG, Rettig W.J. Immunohistochemical analysis of Ewing's sarcoma cell surface antigen p30/32^{MIC2}. Am J Pathol 1991; 139:317-325.

21. Noguera R, Navarro S, Triche TJ. Translocation (11;22) in small cell osteosarcoma. Cancer Genet Cytogenet 1990; 45:121-124.

22. Thorner P, Squire J, Chilton-MacNeill S, et al. Is the EWS/FLI-1 Fusion transcript specific for Ewing sarcoma and peripheral primitive neuroectodermal tumor? A report of four cases showing this transcript in a wider range of tumor types. Am J Pathol 1996; 148:1125-1138.

23. Burchill SA, Wheeldon J, Cullinane C, Lewis IJ. EWS-FLI1 fusion transcripts identified in patients with typical neuroblastoma. Eur J Cancer 1997; 33:239-243.

24. Mastik MF, Molenaar WM, Plaat BE, et al. Translocation (11;22)(q24;q12) in a small cell tumor of the thigh in a 2-year-old boy : immunohistology, cytogenetics, molecular genetics, and review of the literature. Hum Pathol 1999; 30: 352-355.

25. Hisaoka M, Tsuji S, Morimitsu Y, et al. Molecular detection of EWS-FLI1 chimeric transcripts in Ewing family tumors by nested reverse transcription-polymerase chain reaction: application to archival paraffin-embedded tumor tissues. Apmis 1999; 107:577-84.

26. Nagao K, Ito H, Yoshida H, et al. Chromosomal rearrangement t(11;22) in extraskeletal Ewing´s sarcoma and primitive neuroectodermal tumour analysed

by fluorescence in situ hybridisation using paraffin-embedded tissue. J Pathol 1997; 181:62-6.

27. Adams V, Hany MA, Schmid M, Hassam S, Briner J, Niggli FK. Detection of t(11;22)(q24;q12) translocation breakpoint in Paraffin-embedded tissue of the Ewing´s sarcoma family by nested reverse transcription polymerase chain reaction. Diagn Mol Pathol 1996; 5:107-13.

28. Kumar S, Pack S, Kumar D, et al. Detection of EWS-FLI-1 fusion in Ewing´s sarcoma peripheral primitive neuroectodermal tumor by fluorescence in situ hybridization using formalin-fixed paraffin-embedded tissue. Hum Pathol 1999; 30:324-30.

29. Rosai J. Adamantinoma of the tibia: Electron microscopic evidence of its epithelial origin. Am J Clin Pathol 1969; 51:786-92.

30. Rosai J, Pinkus GS. Immunohistochemical demonstration of epithelial differentiation in adamantinoma of the tibia. Am J Surg Pathol 1982; 6**:**427-34.

31. Hazelbag HM, Fleuren GJ, Van den Broek LJCM, Taminiau AHM, Hogendoorn PCW. Adamantinoma of the long bones: keratin subclass immunoreactivity pattern with reference to its histogenesis. Am J Surg Pathol 1993; 17:1225-33.

32. Gu M, Antonescu CR, Guiter G, Huvos AG, Ladanyi M, Zakowski MF. Cytokeratin immunoreactivity in Ewing´s sarcoma. Prevalence in 50 cases confirmed by molecular diagnostic studies. Am J Surg Pathol 2000; 24:410-6.

33. Vakar-López F, Ayala AG, Raymond AK, Czerniak B. Epithelial phenotype in Ewing´s sarcoma/primitive neuroectodermal tumor. Int J Surg Pathol 2000; 8:59-65.

34. Moll R, Lee I, Gould VE, Berndt R, Roessner A, Franke WW. Immunocytochemical analysis of Ewing's tumors. Patterns of expression of intermediate filaments and desmosomal proteins indicate cell type heterogeneity and pluripotential differentiation. Am J Pathol 1987; 127:288-304.

35. Bridge JA, Fidler ME, Neff JR, et al. Adamantinoma-like Ewing´s sarcoma: Genomic confirmation, phenotypic drift. Am J Surg Pathol 1999; 23:159-65.

36. Gaffney EF. Like-but oh, how different. Am J Surg Pathol 2000; 24, 322

37. Kaneko Y, Yoshida K, Handa M, et al. fusion of an ETS-family gene, E1AF to EWS by t(17;22)(q12;q12) chromosome translocation in an undifferentiated sarcoma of infancy. Genes Chromosomes Cancer 1996; 15:115-21.

38. Jeon IS, Davis JN, Braun BS, et al. A variant Ewing´s sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1. Oncogene 1995; 10:1229-34.

39. Peter M, Couturier J, Pacquement H, et al. A new member of the ETS family fused to EWS in Ewing tumors. Oncogene 1997; 14:1159-64.

6

Desmoplastic fibroma of bone: An immunohistochemical study including β-catenin expression and mutational analysis for β-catenin.

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Abstract

Desmoplastic fibroma of bone is a very rare primary bone tumour morphologically resembling desmoid-type fibromatosis, its much more common counterpart of soft tissue. The aim of this study is to investigate the immunohistochemical profile and the involvement of the β-catenin pathway in desmoplastic fibroma as it is known in desmoid-type fibromatosis. Immunohistochemistry was performed on 13 cases of desmoplastic fibroma for muscle-specific markers, oestrogen and progesterone receptors, CD117, β-catenin, and the potential downstream target of β-catenin, namely, cyclin D1. In all 13 cases, DNA sequencing was performed for the detection of activating β-catenin gene mutations. There was no immunoreactivity of CD117, oestrogen, and progesterone receptors. Seven cases were immunoreactive for one or more muscle-specific markers. In 6 cases, there was overexpression of β-catenin in the cytoplasm; in one of these cases, there was also accumulation of β-catenin in the nucleus. In 6 cases in which DNA sequencing was successful, no β-catenin mutations were detected. Search in a national pathology database showed that not a single case over a frame of 23 years was associated with the occurrence of colon cancer in the same patient. The epidemiological, histological and immunohistochemical findings in desmoplastic fibroma are suggestive of desmoplastic fibroma being the bony counterpart of the more common desmoid-type fibromatosis of soft tissue. However, the β-catenin pathway does not seem to have the same essential role in the tumourigenesis of desmoplastic fibroma as it has in desmoid-type fibromatosis.

Introduction

Desmoplastic fibroma, of which an example is seen in Fig. 1, is an exceedingly rare benign primary tumour of bone, first described by Jaffe (1) in 1958, which can be locally aggressive. It is considered to be the bony equivalent of the desmoid-type fibromatosis (aggressive fibromatosis of soft tissue) (2), based on its comparable histological and clinical features.

Desmoid-type fibromatosis occurs mostly sporadic but is also seen in Gardner syndrome (3), a hereditary syndrome with familial adenomatous polyposis coli (FAP), osteomas, fibromas, epidermal or sebaceous cysts and a variety of malignant tumours. The basic defect in FAP is a mutation in the adenomatous polyposis (APC) gene. The APC gene product is part of the Wnt signalling pathway and is involved in the breakdown and regulation of the cellular level of β-catenin (4). In the absence of Wnt signalling, β-catenin levels are regulated by a multiprotein complex, which phosphorylates β-catenin, thus marking it for ubiquitination and degradation. The β-catenin degradation protein complex is composed of the APC tumour suppressor protein, AXIN an glycogen synthase kinase 3β. Mutations in the APC gene or stabilizing mutations in the gene for β-catenin (CTNNB1) (5) result in the accumulation of β-catenin in the cytoplasm, which can subsequently translocate to the nucleus where it is able to bind to and thereby activate transcription factors of the T-cell factor and lymphoid enhancer factor family (6). Potential targets for transcriptional activation by β-catenin/T-cell factor are c-*myc* (7) and cyclin D1 (8). Germ line mutations in the APC gene are restricted to patients with FAP or familial hereditary desmoids (9). The nature and position of the APC mutation determines the clinical presentation and course of the disease (10). However, somatic mutations in the APC gene have been described in sporadic cases of desmoid-type fibromatosis (9,11,12).

In sporadic desmoids, CTNNB1 mutations occur more frequently than somatic mutations in the APC gene (13). A correlation between the nuclear expression of β-catenin and overexpression of cyclin D1 has been demonstrated in cases of sporadic desmoid type fibromatosis (14,15).

In contrast to the desmoid-type fibromatosis, information on the immunohistochemical profile, coexistence with the occurrence of colon cancer, and possible involvement of the APC/β-catenin pathway in desmoplastic fibroma is missing, which is the subject of this study.

Material and methods

Patient material

In the period from 1953 to 2000, 9 patients in the database of the Netherlands Committee on Bone Tumours had a histologically confirmed diagnosis of desmoplastic fibroma. This database contains 16 000 cases of bone tumours and tumourlike lesions collected over the time interval given. From 5 patients, the paraffin blocks were still available. A search of the Pathological Anatomy National Automated Archive (PALGA) revealed after histological review of potential patients no extra cases. One additional case was retrieved from the Department of Pathology, University Hospital Antwerp and 7 from the consultancy files from the Institute of Pathology of the Bone Tumor Reference Center Basel, Switzerland. All material was decalcified, Bouin- (2 cases) or formalin-fixed (11 cases) and paraffin- embedded.

Table 1.
Antibodies used with their specifications

Primary antibody	Manufacturer	Antigen retrieval	Dilution	External Control
SMA	Progen[1]	None	1:4000	Intestine
Actin	Neomarkers[2]	None	1:30	Intestine
Desmin	Dako[3]	Citrate	1:50	Intestine
Oestrogen receptor	Neomarkers	Citrate	1:200	Breast
Progesterone receptor	Dako	Citrate	1:100	Breast
Catenin-β	Tranduct[4]	Citrate	1:800	Skin
Cyclin D1 M	Dako	Citrate	1:4000	Tonsil
Cyclin D1 R	Neomarkers	Citrate	1:75	Tonsil
CD117	Dako	None	1:200	GIST

GIST: Gastro-intestinal stromal tumour
M: Mouse monoclonal antibody, R: Rabbit monoclonal antibody
1 Progen Biotechnik, Heidelberg, Germany
2 Neomarkers Klinipath, Duiven, the Netherlands
3 Dako Diagnostica GMBH, Freiburg, Germany
4 Transduction, Alphen aan de Rijn, the Netherlands

From all cases, haematoxylin-eosin stained 4μm sections were available for review. Immunohistochemistry, following the avidin-biotin method, was performed using the antibodies, as listed in Table 1. Immunohistochemistry was scored semi quantitatively as 0, +, ++ and +++.
To investigate a potential relation within the context of FAP, we searched the PALGA database (year interval 1973-1996) for the coexistence of a diagnosis desmoplastic fibroma and/or multiple adenomatous polyps or colon cancer in 1 patient.

DNA isolation, PCR, and sequencing for identification of CTNNB1 activating mutations
DNA was isolated from paraffin-embedded formalin- or Bouin-fixed tissue sections of 10 μM. Sections were resuspended in 250 μl PK-1 lysis buffer (50 mM KCL, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP40, 0.45% Tween 20, 0.1 mg/ml gelatine) containing 5% Chelex beads (Biorad, Hercules, USA) and 10 μl proteinase K (10 mg/ml) and incubated overnight at 56 ^{o}C. Subsequently, the suspension was incubated for 10 min at 100 ^{o}C, centrifuged and the supernatant carefully decanted.
To detect β-catenin activating mutations, exon 3 of the CTNNB1 gene was amplified using primers described by Koch et al. (16). Plymerase chain reactions (PCRs) consisted of 50μl containing 1 μl DNA, 5 μl PCRII buffer (Roche Diagnostics, Almere, The Netherlands), 2 mM dNTPS, 5 pMol of each primer, 1 mM $MgCl_2$ and 1 Unit AmpliTaq (Roche Diagnostics).

Table 2.
Patients characteristics

Patient number	Localization	Age at diagnosis	Gender
1	Clavicle	33	Male
2	Femur	22	Male
3	Frontal bone	26	Female
4	Radius	22	Male
5	Humerus	26	Female
6	Femur	68	Female
7	Os ileum	30	Female
8	Mandible	44	Female
9	Mandible	13	Female
10	Femur	48	Male
11	Tibia	25	Male
12	Femur	21	Female
13	Radius	17	Female

Fig 1.

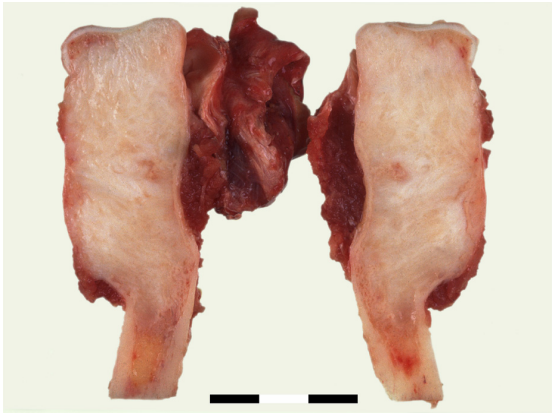

Fig.2.

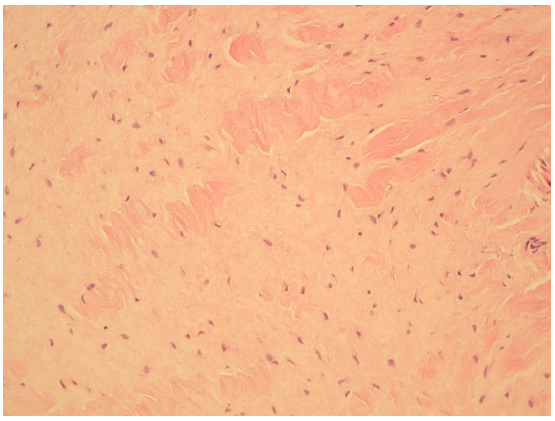

Fig. 3.

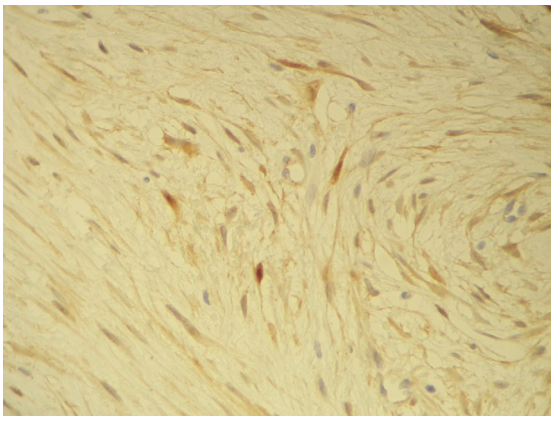

Fig 1. Macroscopic view of a desmoplastic fibroma of the distal ulna
Fig 2. Light micrograph showing a typical example of desmoplastic fibroma showing slender fibroblasts set in a copious collagenous stroma, with focally dense collagen bundles (haematoxylin-eosin stain, original magnification x200).
Fig 3. Light micrograph showing immunoreactivity for β-catenin with strong nuclear immunoreactivity in some of the fibroblast-like cells (original magnification x400)

Results

Patients
The patient's characteristics are summarized in Table 2. Search of our national pathology database (PALGA) did not reveal a single case of desmoplastic fibroma and colon carcinoma and/or adenomatous polyps of the colon.

Histology
All 13 cases were composed of slender fibroblastic to stellate cells set in an abundant collagenous matrix (Fig. 2). Lesions ranged from cell-poor to cell-rich. The cells were arranged in fascicles or whorls and mitotic figures were extremely rare (less than 1/10HPF). Between the cells there were variable amounts of mast cells in 11 cases. The lesions were moderately vascular with capillaries and small- to medium- sized well developed arterioles regularly dispersed through the tumour. Perivascularly, there were small amounts of iron. Focally, in some lesions there was microcyst formation with some osteoclastic giant cells in the cyst wall. There was no osteoid or chondroid matrix deposition.

Immunohistochemistry
All external positive controls in Table 1 were strongly positive for the respective antigen. In the desmoplastic fibromas, there was no nuclear immunoreactivity for oestrogen and progesterone receptor, and no immunoreactivity for CD117, whereas the mast cells present in 11 of the 13 cases showed moderate to strong cytoplasmic staining, thereby functioning as internal positive control. The results for smooth muscle actin, panactin and desmin are summarized in Table 3, those for cyclin D1 and β-catenin in Table 4. In all, 7 cases were immunoreactive for one or more muscle-specific markers in more than 10% of the cells. Six cases showed cytoplasmic β-catenin in most cells (>50%); in 1 case (case 9), there was also strong nuclear staining in more than 50% of the cells (Fig. 3). In accordance with the literature (14), the cut-off value for cyclin D1 overexpression was set at 5% or more of the cells showing immunoreactivity. Combining the results for the reactivity for the mouse monoclonal antibody as well as the rabbit monoclonal antibody, 5 cases were positive. The case with nuclear accumulation of β-catenin did not overexpress cyclin D1.

Exon 3 CTNNB1 mutation screening
Mutation detection was validated on DNA from colorectal cell line SW48, which has been reported to have a cytosine to adenine mutation resulting in affecting codon 33 of CTNNB1 (6) and on 2 desmoid tumours with a guanine to adenine mutation in codon 41. All mutations were detected both on the forward and the reverse strand. DNA isolated from placenta or fromperipheral blood lymphocytes did not show any sequence alterations in exon 3. Of the 13 desmoplastic fibroma cases, 6 yielded PCR products that gave an interpretable result for sequencing of CTNNB1 exon 3. Sequencing of both DNA strands revealed no mutations. The other samples failed, three because the DNA did not yield any PCR product (cases 4, 5 and 9), 2 because the DNA sequence was not readable (case 3 and 6) and two because of a high degree of DNA fragmentation due to Bouin fixation (case 1 and 13).

Table 3.
Immunoreactivity for smooth muscle actin, panactin and desmin

Patient number	Smooth muscle actin		Panactin		Desmin	
	Intensity of staining	Percentage of positive cells	Intensity of staining	Percentage of positive cells	Intensity of staining	Percentage of positive cells
1	++	<10	-		+	<10
2	+	<10	+	<10	-	
3	+	<10	+	<10	-	
4	+	<10	-		+	<10
5	+++	<10	-		-	
6	+++	10-25	+++	<10	-	
7	++	26-50	-		+	10-25
8	+	10-25	++	<10	+	<10
9	+++	>50	++	>50	++	26-50
10	+	<10	-		-	
11	++	>50	++	>50	-	
12	+++	26-50	++	10-25	+	10-25
13	+++	26-50	-		-	

Table 4.
Immunoreactivity for β-catenin and Cyclin-D1

Patient number	B-catenin cytoplasm		B-catenin nucleus		Cyclin-D1 M		Cyclin-D1 R	
	Intensity of staining	Percentage of positive cells	Intensity of staining	Percentage of positive cells	Intensity of staining	Percentage of positive cells	Intensity of staining	Percentage of positive cells
1	+	>50	-		+	>50	-	
3	+	<10	+	<10	-		+	>50
4	+	<10	-		+++	5-50	*	
5	-		-		-		-	
6	+	<10	+	<10	+	<5	+	<5
7	+	>50	+	<10	+	<5	++	<5
8	+++	10-50	+	<10	+	5-50	+	5-50
9	+++	>50	+++	>50	-		-	
10	+	<10	+	<10	-		-	
11	+++	>50	+	<10	+	<5	+	<5
12	+++	>50	+++	<10	-		+	<5
13	-		-		-		-	

* No more slides available for staining

Discussion

Desmoplastic fibroma is a very rare, fibrogenic, benign primary bone tumour. It can occur at any age and at any site, but is most frequently seen between 15 and 40 years, in the meta-epiphyseal region of the femur and tibia, and in the pelvis (2). Although our series has a slight female predominance (8/13), there is no sex predilection in the literature.

The lesions are composed of slender to sometimes more reticular cells embedded in a copious collagenous stroma. Cellularity is variable. The cells are mostly arranged in long sweeping fascicles; sometimes the pattern is more whirling. The epidemiology and histology are identical to extra-abdominal desmoid-type fibromatosis, with the exception of a female predominance in desmoid-type fibromatosis. Similar to desmoid-type fibromatosis, desmoplastic fibroma of bone is a locally non-metastasizing aggressive tumour with high risk for local recurrence.

Of our 13 cases of desmoplastic fibroma, 7 were positive for one or more muscle-specific markers in more than 10% of the cells. This is in accordance with desmoid-type fibromatosis, which is generally positive for actin and smooth muscle actin and, sometimes, also for desmin. In contrast, there was no immunoreactivity for oestrogen or progesterone receptor. Presence of the oestrogen receptor has been documented in approximately one third of the cases of desmoid-type fibromatosis by Lim et al (18).

Genetically, trisomy 8 and/or 20 is described in desmoid-type fibromatosis (19-24). Bridge et al (25) demonstrated, using fluorescence in situ hybridisation analysis, trisomy 8 in 1 of 3 cases and trisomy 20 in 2 of 3 cases of desmoplastic fibroma.

The involvement of the APC/β-catenin pathway in desmoid type-fibromatosis as well in the setting of Gardner syndrome and in hereditary desmoid-type fibromatosis as in sporadic desmoid-type fibromatosis is well documented. Miyoshi et al (5) and Tejpar et al (13), demonstrated β-catenin mutations in approximately 50% of their cases of sporadic desmoid-type fibromatosis. The search for mutations of β-catenin in our cases of desmoplastic fibroma of bone was hampered because all the samples had been decalcified, resulting in a higher degree of fragmentation of the DNA. The poor quality of the DNA samples inhibited us from screening for mutations in the APC gene because this is a very large gene with a coding sequence of 8911 base pairs, which is inactivated by mutations that are scattered over the entire sequence. In contrast, the activating mutations in CTNNB1 are restricted to exon 3, consisting of 228 base pairs. In addition, it has been reported that in sporadic desmoid-type fibromatosis mutations in CTNNB1 are more frequent then in APC, that is, 22 versus 9 in 42 patients (13). Inactivation mutations of the gene for the AXIN protein, also part of the degradation protein complex of β-catenin, have been described in cases of hepatocellular carcinoma (26), ovarian endometrioid carcinoma (27) , hepatoblastoma (28,29) and medulloblastoma (30,31). To our knowledge nothing is known on the mutational status of AXIN in desmoid tumors and was not the scope of our study.

In the series of desmoid-type fibromatosis by Tejpar et al. (13) and Saito et al. (14,15), nearly all of the cases showed nuclear and cytoplasmic expression of β-catenin by immunohistochemistry. In contrast, 6 of our cases showed cytoplasmic overexpression of β-catenin; in 1 case, there was also nuclear accumulation (case 9). Unfortunately, this case yielded no PCR product for sequencing. Furthermore, the coupling of nuclear β-catenin expression with cyclin D1 overexpression described in sporadic desmoid-type fibromatosis (14,15) could not be demonstrated in our cases of desmoplastic fibroma of the bone. On the base of our results, involvement of the

APC/ β-catenin signaling pathway, be it by inactivating APC gene mutation or an activating CTNNB1 mutation, is thus not completely excluded in the tumorigenesis of desmoplastic fibroma, but seems to be unlikely and in any case is far less essential than in desmoid-type fibromatosis.

We conclude that despite the morphological overlap between desmoplastic fibroma and desmoid-type fibromatosis with regard to epidemiology, histology, immunohistochemical profile, and genetics, the likely absence of involvement of the APC/β-catenin pathway and the absence of β-catenin/cyclin D1 overexpression coupling, or the absence of concordant occurrence of FAP or colon cancer brings in to question if desmoplastic fibroma of bone is, in fact, the bony counterpart of desmoid-type fibromatosis.

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References

1. Jaffe HL. Desmoplastic fibroma and fibrosarcoma. In: Jaffe HL, ed. Tumors and tumorous conditions of bones and joints. Philadelphia: Lea Febiger, 1958:298-303.

2. Desmoid tumor of Bone (desmoplastic fibroma or aggressive fibromatosis) In: Bone tumors. Clinical, radiological and pathologic correlations. Philadelphia: Lea & Febiger, 1989:735.

3. Gardner EJ, Richards RC. Multiple cutaneous and subcutaneous lesions occurring simultaneously with hereditary polyposis and osteomatosis. Am J Hum Genet 1953;5:139-47.

4. Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 2003;1653:1-24

5. Miyoshi Y, Iwao K, Nawa G, Yoshikawa H, Ochi T, Nakamura Y. Frequent mutations in the beta-catenin gene in desmoid tumors from patients without familial adenomatous polyposis. Oncol Res 1998;10:591-4.

6. Morin PJ, Sparks AB, Korinek V, Clevers H, Vogelstein B, Kinzler KW. Activations of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997;275:1752-3.

7. He TC, Sparks AB, Rago C et al. Identification of c-MYC as a target of the APC pathway. Science 1998;281:1438-41.

8. Tetsu O, McCormick F. Beta-catenin regulates expression of Cyclin D1 in colon carcinoma cells. Nature 1999;398:422-6.

9. Giarola M, Wells D, Mondini P et al. Mutations of adenomatous polyposis coli (APC) gene are uncommon in sporadic desmoid tumours. Br J Cancer 1998;78:582-7.

10. Fodde R, Khan PM. Genotype-phenotype correlations at the adenomatous polyposis coli (APC) gene. Crit Rev Oncog 1995;6:291-303

11. Alman BA, Li C, Pajerski ME, Diaz-Cano S, Wolfe HJ. Increased Beta-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors). Am J Pathol 1997;151:329-34.

12. Bridge JA, Meloni AM, Neff JR et al. Deletion 5q in desmoid tumor and fluorescence in situ hybridization for chromosome 8 and/or 20 copy number. Cancer Genet Cytogenet 1996;92:150-1.

13. Tejpar S, Nollet F, Li C et al. Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 1999;18:6615-20.

14. Saito T, Oda Y, Tanaka K et al. Beta-Catenin nuclear expression correlates with cyclin D1 overexpression in sporadic desmoid tumours. J Pathol 2001;195:222-8.

15. Saito T, Yoshinao O, Kawaguchi K et al. Possible association between higher β-Catenin mRNA expression and mutated β-Catenin in sporadic desmoid tumors: Real-time semiquantitative assay by TaqMan polymerase chain reaction. Lab Invest 2002;82:97-103

16. Koch A, Denkhaus D, Albrecht S, Leuschner I, von Schweinitz D, Pietsch T. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. Cancer Res 1999;59:269-73.

17. Greer CE, Peterson SL, Kiviat NB et al. PCR amplification from paraffin-embedded tissues: Effects of fixative and fixation time. Am J Clin Pathol 1991;95:117-24

18. Lim CL, Walker MJ, Mehta RR et al. Estrogen and antiestrogen binding sites in desmoid tumors. Eur J Cancer Clin Oncol 1986;22:583-7

19. Dal Cin P, Sciot R, Aly MS et al. Some desmoid tumors are characterized by trisomy 8. Genes Chromosomes Cancer 1994;10:131-5.

20. Dal Cin P, Sciot R, Van Damme B, De Wever I, Van den Berghe H. Trisomy 20 characterizes a second group of desmoid tumors. Cancer Genet Cytogenet 1995;79:189.

21. De Wever I, Dal Cin P, Fletcher CDM et al. Cytogenetic, clinical and morphologic correlations in 78 cases of fibromatosis: a report from the CHAMP study group. Mod Pathol 2000;13:1080-5.

22. Fletcher JA, Naeem R, Xiao S, Corson JM. Chromosome aberrations in desmoid tumors. Trisomy 8 may be a predictor of recurrence. Cancer Genet Cytogenet 1995;79:139-43.

23. Mertens F, Willén H, Rydholm A et al. Trisomy 20 is a primary chromosome aberration in desmoid tumors. Int J Cancer 1995;63:527-9.

24. Qi H, Dal Cin P, Hernández JM et al. Trisomies 8 and 20 in desmoid tumors. Cancer Genet Cytogenet 1996;92:147-9.

25. Bridge JA, Swarts SJ, Buresh C et al. Trisomies 8 and 20 characterize a subgroup of benign fibrous lesions arising in both soft tissue and bone. Am J Pathol 1999;154:729-33.

26. Satoh S, Daigo Y, Furukawa,T et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus mediated transfer of AXIN1. Nat Genet 2000;24:245-250

27. Wu R, Zhai Y, Fearon ER et al. Diverse mechanisms of beta-catenin deregulation in ovarian endometrioid adenocarcinoma. Cancer Res 2001;61:8247-8255

28. Oda H, Imai Y, Nakatsuru Y et al. Somatic mutations of the APC gene in sporadic hepatoblastoma. Cancer Res 1996;56:3320-3323

29. Taniguchi K, Roberts LR, Aderca IN et al. Mutational spectrum of beta-catenin, AXIN1, and AXIN 2 in hepatocellular carcinomas and hepatoblastomas. Oncogene 2002;21,4863-4871

30. Dahmen RP, Koch A, Denkhaus D et al. Deletions of AXIN1, a component of the WNT/wingless pathway in sporadic medulloblastomas. Cancer Res 2001;61:7039-7043

31. Yokota N, Nishizawa S, Ohta S et al. Role of Wnt pathway in medulloblastoma oncogenesis. Int J Cancer 2002;101:198-201

7
Summary and conclusion

1. Non-haematogenic primary bone tumours

Non-haematogenic tumours arising primarily in the bone are rare. Based on their histomorphology they are classified as chondrogenic, osteogenic, fibrogenic, histiocytic, vascular, neurogenic, lipogenic, notochordal and of unknown histologic type (Table 1, **Chapter 1**). The group of the osteofibrous tumours is characterised by variable amounts of bone or osteoid and fibrous tissue. The spectrum of osteofibrous tumours ranges from benign, exclusively fibrous lesions as desmoplastic fibroma to the high-grade intramedullar osteosarcoma. Within the spectrum of osteofibrous tumours there is histological variability in a given entity as well as similarities between entities. The purpose of this thesis is to reveal the meaning of the phenotypic spectrum of osteofibrous tumours. More specific, the aim was to see if phenotypic differences in a given entity reflect different biological behavior and if similarities between entities justifies that these entities are grouped together in one disease process or not. Correct classification, reclassification of known entities on new insights and sub-classification on phenotypic differences, when related with biological behavior, has implications for clinical practice and disease management, and contributes to optimal patient care.

2. Osteosarcoma and its histological subtypes

Osteosarcoma is the most frequent non-haematogenic malignant bone tumour. It can occur de novo (primary osteosarcoma), in a pre-existing abnormality (secondary osteosarcoma) or in a setting of hereditary cancer syndromes. Primary osteosarcoma affects mostly adolescents and has a high morbidity and mortality. Overall 5 year survival for patients with non-metastatic osteosarcoma of the limbs ranges from 55 to 75%. Osteosarcoma is currently treated by preoperative chemotherapy, surgery, and post-operative chemotherapy. There is an ongoing effort to further improve efficacy of chemotherapy and reduce chemotoxicity by identifying osteosarcoma patients who will be at risk of treatment failure as well as those who need less chemotherapy. As such prognostic factors are investigated in the clinical, pathologic and molecular field. This is done in large randomised trials since these are mandatory when one wants to examine the effect of different aspects (such as the treatment modality) of a pathological condition on the overall outcome.
One putative variable is the histological subtype of osteosarcoma. An overview of osteosarcoma subtypes is given in **Chapter 1**, Table 1.

In **Chapter 2** data on 570 patients with biopsy-proven primary central osteosarcoma of an extremity included in two consecutive studies of the European Osteosarcoma Intergroup (EOI) were analysed to evaluate the validity of osteosarcoma subtyping on the biopsy specimen and to evaluate the relation between the histological subtype and histological response to chemotherapy, and the relation between the histological subtype and overall survival. Subtyping on the biopsy specimen proved to be highly representative for the subtype of the whole tumour. In 102 patients for which subtyping was performed on the biopsy and the resected specimen there were only 2

discrepancies. The proportion of patients responding well (defined as ≥ 90 % necrosis on the resected specimen) to chemotherapy differed significantly between subtypes (chi-square test statistics = 11.44, P = 0, 01 on 3 degrees of freedom (df)). In comparison with the conventional osteoblastic subtype there was a higher proportion of good responders in the fibroblastic group and a lower proportion of good responders in the chondroblastic group. Good responders had significantly better survival than patients who responded poorly to preoperative chemotherapy (log rank statistic = 7.68, P < 0.01 on 1 df). Survival did not differ significantly according to subtype (log rank statistic = 2.72, P = 0.44 on 3 df), although there was a suggestion that patients with chondroblastic tumours, despite their poor response to preoperative chemotherapy, experience better long-term survival.

Chapter 3 is a population wide study on the incidence of osteosarcoma associated with other malignancies and on the predictive value of histological subtype for a genetic predisposition to malignancy in osteosarcoma patients. Between January 1975 and May 2000 a total of 938 patients were entered in the Dutch National Pathology Database Palga with a confident diagnosis of osteosarcoma. Of these patients 66 had another malignancy. When compared with a control group of 23 252 tonsillectomy patients, the osteosarcoma patients had a relative risk of 2.4 (95% confidence interval 1.88 - 3.07) to develop another malignancy. Especially women seem to be more susceptible for the development of multiple primaries. In all, 5% of the male osteosarcoma patients and 10% of the female osteosarcoma patients had another primary malignant tumour. The patient group suspected for a hereditary cancer syndrome was composed by excluding patients older than 46 years at the diagnosis of the first malignancy and patients with a chemo- or radiation induced secondary tumour. In this syndrome suspected study group all patients had an osteosarcoma subtype other than conventional osteoblastic. The osteosarcoma patients from the European Osteosarcoma Intergroup served as control for subtype. These patients presented only in 29% of the cases with a non-conventional osteoblastic subtype. A non-conventional subtype of osteosarcoma should thus draw attention to a possible genetic predisposition of the patient involved.

In **Chapter 4** those osteosarcoma patients who are at risk of first relapse five years after initial treatment are identified, and we tried to answer the question of these unusual cases (less then 2% of cases in three independent databases) had a characteristic clinicopathological presentation. Patients with high-grade intramedullar osteosarcoma of the extremities and with a first local or systemic relapse 5 years after diagnosis were retrieved from the data files of the European Osteosarcoma Intergroup study 80831 and 80861, from the Cooperative Osteosarcoma Studies running between 1979 and 1997 and from a single surgical centre cohort in the UK, Birmingham. Age, sex, localisation and subtype of osteosarcoma were compared with this of the whole osteosarcoma patient population of the European Osteosarcoma Intergroup study 80831 and 80861. Patients with a first relapse 5 years after diagnosis tended to have their osteosarcoma more often localised in the fibula or tibia, and more often presented with a non osteoblastic especially a chondroblastic subtype. Thus patients with a chondroblastic subtype of osteosarcoma or an osteosarcoma localised in the tibia or fibula should be considered as at risk for late relapse, for which long term follow up for detection of relapse is warranted.
In contrast, the overall very low percentage of relapse of non chondroblastic osteosarcomas 5 years after initial treatment makes it acceptable from a clinical

managerial point of view to consider these patients as cured after 5 years of disease free survival. And for the patients with a non chondroblastic osteosarcoma follow up later on could therefore be focussed on late effects of therapy (functionality, cardio toxicity, secondary malignancies) rather then detection of relapse.

3. Ewing like adamantinoma

Adamantinoma of the long bones is a rare malignant osteofibrous lesion of the bone with variable amounts of epithelial cells. It expresses basal cell type cytokeratins. More specifically, there is expression of cytokeratins 14, 19, 5 and also in a lesser degree, cytokeratin 17. The osteofibrous subtype differs from osteofibrous dysplasia only by the presence of epithelial cells, sometimes only in very small amounts. The considerable clinical and histological overlap between osteofibrous dysplasia of the long bones and adamantinoma of the long bones suggest that osteofibrous dysplasia and adamantinoma represent the opposite ends of a spectrum of one tumour entity.
There have been also descriptions of rare cases with overlapping features between adamantinoma of long bones and Ewing sarcoma suggesting a relationship between these two entities.
Ewing sarcoma/PNET is classically described as a small blue cell tumour and as such does not belong to the osteofibrous tumours of bones. It shares its small cell morphology with lymphoma, small cell cancer, mesenchymal chondrosarcoma, solid variant of alveolar rhabdomyosarcoma and medulloblastoma. The main differential diagnosis in the bone is with small cell osteosarcoma. The main diagnostic criterion is the presence of a highly specific translocation (11;22)(q24;q12) in 90-95% of cases of Ewing/PNET. Variant translocations are: t(21;22)(q22;q12), t(7;22)(p22;q12), t(17;22)(q12;q12) and t(2;21;22)(q33;q22;q12)

Chapter 5 describes 14 cases of known adamantinoma of the bone in which no t(11;22)(q24;q12) or t(21;22)(q22;q12) could be detected by RT-PCR. Together with the epithelial nature of adamantinoma this unequivocally proves that there is no relation what so ever between Ewing sarcoma and adamantinoma. Correct classification of a tumour with overlapping features of adamantinoma and Ewing sarcoma is of utmost importance, since adamantinoma is a low-grade malignancy that is treated by complete local excision, whereas Ewing sarcoma is a high-grade malignancy, for which a combined treatment of chemotherapy, resection and radiotherapy is necessary. These treatment modalities are not without risk for acute and long-term complications.

4. Desmoplastic fibroma of bone, the bony counterpart of desmoid-type fibromatosis of soft tissue

In **Chapter 6,** thirteen cases of desmoplastic fibroma are characterised by immunohistochemistry and DNA-sequencing. The results are compared with the known characteristics of desmoid-type fibromatosis of the soft tissues. The histology of desmoplastic fibroma and desmoid-type fibromatosis is identical suggesting that desmoplastic fibroma is the bony counterpart of desmoid tumour. In contrast with desmoid-type fibromatosis there was no nuclear immunoreactivity for oestrogen and progesterone receptor in the 13 cases of desmoplastic fibroma. Seven cases were immunoreactive for one or more muscle markers consistent, as in desmoid-type fibromatosis, with the myofibroblastic nature of the lesional cells. In desmoid-type

fibromatosis the APC/β-catenin pathway is involved. Six cases of desmoplastic fibroma showed cytoplasmic β-catenin overexpression, with in one case also nuclear accumulation. Cyclin D1 was overexpressed in four cases, but not in the case with nuclear β-catenin accumulation.
DNA sequencing for activating β-catenin mutations yielded no product in 3 cases, including the case with nuclear β-catenin over expression, and resulted in an incomplete or bad product in 2 cases. In the remaining 8 cases no mutations were found in the β-catenin gene. Though activating β-catenin mutations in our 13 cases of desmoplastic fibroma were not seen, the one case with strong cytoplasmic and nuclear expression of β-catenin on immunohistochemistry does not exclude the involvement of the APC/ β-catenin pathway in the morphogenesis or tumourigenesis of desmoplastic fibroma, though it is much less essential as in desmoid type fibromatosis. Moreover, the coupling present between nuclear β-catenin accumulation and cyclin D1 overexpression as documented in desmoid-type fibromatosis could not be demonstrated in desmoplastic fibroma. These finding raise doubt as to the question if desmoplastic fibroma of bone is in fact the bony counterpart of desmoid-type fibromatosis. The clinical implication is that that a patient presenting with a desmoplastic fibroma of bone is not to be suspected to have Gardner syndrome, and is not likely to have a higher risk for colonic cancer.

5. Conclusion

In this thesis the phenotypic differences in a given tumour entity and morphologic similarities between different entities have been studied. This revealed that these differences and similarities are more than just a whim of nature. In retrospect, the histological subtype of osteosarcoma is a predictive factor for response to chemotherapy, late relapse and the risk of a hereditary cancer syndrome. This has direct consequences for the treatment and follow-up for osteosarcoma patients, and when a hereditary cancer syndrome is suspected, also for the blood relatives. For further validation, these findings should be considered in the design of future studies on osteosarcoma. Since osteosarcomas in general, and some of the histological subtypes in specific, are rare tumours, large randomised trials are mandatory when examining the effect of different aspects of osteosarcoma on overall outcome. Even then numbers can be too small to come to a definite conclusion, as was demonstrated in our studies on osteosarcoma subtype and overall survival and subtype and late relapse. Different osteosarcoma intergroups performing randomised trials have experienced the same problems in evaluating different chemotherapy regimens and several groups among which the European Osteosarcoma Intergroup, the Childrens Oncology Group, the Cooperative OsteoSarcoma Study group and the Scandinavian Sarcoma Group have decided to collaborate together in a transatlantic concortium named EURAMOS to develop and conduct large randomised trials for osteosarcoma. This type of collaboration makes it possible to validate, refine and define factors that are relevant for patients outcome and clinical management. Further research lies also in revealing the molecular drive of the complex process of the recruitment, proliferation, and differentiation of mesenchymal stem cells and the loss of regulatory control resulting into a certain tumour type, with its characteristic morphology and behavior. This is possible by cDNA micro-array studies which make it possible to simultaneously analyse thousands of genes. Comparative gene-expression analysis between the different subtypes of osteosarcoma might thus reveal the genes coding for phenotype and clinical behaviour. Gene expression profiles not only helps us to better

classify and diagnose tumours, but can also reveal candidate genes predictive for prognosis and genes coding for certain processes that can be a potential target for treatment. Very recently the first reports are published on gene-expression profiles in osteosarcoma predictive for response to chemotherapy.
Overlapping histological and/or clinical parameters between certain tumour entities does not justify to classify these tumours as part of one disease entity as has been documented by the study on desmoplastic fibroma of bone and the study on Ewing like adamantinoma. As has been shown in these two studies, the continuously acquirement of knowledge in tumour biology, together with improvement and further development in ancillary techniques as immunohistochemistry and molecular biological techniques makes it possible to (re-)classify tumours on more than morphology alone. Not only in the classification of tumours, but even more so in the diagnostic field and dealing with small biopsy specimens, cytogenetic analysis is virtually indispensable to make a correct diagnosis in some case, especially when dealing with entities with overlapping histological features, so that the patient can benefit from optimal care.

Samenvatting en conclusie

1. Niet hematogene primaire bottumoren

Primaire tumoren uitgaande van het bot zijn zeldzaam. Gebaseerd op hun histologisch uitzicht worden primaire bottumoren geklasseerd als kraakbenig, benig, fibreus, histiocytair, vasculair, neurogeen, lipogeen, notochordaal of als ongekend (Tabel 1. **Hoofdstuk 1**). De groep van de osteofibreuze tumoren wordt gekenmerkt door variabele hoeveelheden bot of osteoid en fibreus weefsel. Het spectrum van de osteofibreuze tumoren varieert van goedaardige tumoren uitsluitend bestaande uit een fibreus stroma zoals het desmoplastische fibroom tot kwaadaardige tumoren zoals het intramedullair osteosarcoom. Binnen dit spectrum is er zowel histologische variabiliteit van één enkele tumorentiteit, als gelijkenis tussen entiteiten onderling. Het doel van deze thesis was de betekenis te achterhalen van dit spectrum van uiterlijke kenmerken van de osteofibreuze tumoren. Meer specifiek: is het verschil in uitzicht (fenotype) tussen tumoren binnen één entiteit een weerspiegeling van verschil in biologisch gedrag en is het gerechtvaardigd tumoren met eenzelfde uitzicht te groeperen onder eenzelfde entiteit of juist niet? Een juiste classificatie, herclassificatie op basis van nieuw verworven inzichten en subclassificatie daar waar fenotype gerelateerd is met biologisch gedrag, heeft consequenties voor het klinische beleid en draagt bij tot een optimale behandeling en patiëntenzorg.

2. Histologische subtypen van osteosarcoom

Het intramedullair osteosarcoom is de meest frequente maligne tumor van het bot. Het kan de novo ontstaan (primair osteosarcoom), in een vooraf bestaande abnormaliteit van het bot (secundair osteosarcoom), en ook in het kader van een familiaal kanker syndroom.
Primair osteosarcoom is een tumor van jongeren met een hoog sterftecijfer. De 5-jaars overleving voor patiënten met niet uitgezaaid osteosarcoom op het ogenblik van de diagnose varieert naargelang de studiegroep van 55 tot 75%. De behandeling bestaat uit preoperatieve chemotherapie gevolgd door heelkunde en postoperatieve chemotherapie. Het is een blijvende doelstelling de efficiëntie van de chemotherapie te verbeteren en de toxische effecten hiervan te verminderen door deze patiënten te identificeren die een hoog risico hebben op falen van de huidig bestaande behandelingsschema's, alsook deze te herkennen die het kunnen stellen met minder intensieve of toxische chemotherapie. Hiervoor worden voorspellende factoren onderzocht zowel op klinisch, pathologisch als moleculair vlak. Dit gebeurt in grote gerandomiseerde studies aangezien dit soort studies aangewezen zijn wanneer men verschillende aspecten en hun invloed op het klinische verloop wil onderzoeken van een bepaalde ziekte. Eén van deze voorspellende factoren is het histologische subtype van osteosarcoom. Een overzicht van de osteosarcoom subtypen wordt gegeven in **Hoofdstuk 1**. Tabel 1.

In **Hoofdstuk 2** worden de gegevens geanalyseerd van 570 patiënten met een primair intramedulair osteosarcoom van de ledematen die opgenomen zijn in twee opeenvolgende studies van de Europese Osteosarcoma Intergroep (EOI). Hierbij werd de waarde geëvalueerd van het subtyperen van het osteosarcoom op de biopsie. Ook werd de relatie onderzocht tussen het osteosarcoom subtype en de respons op

chemotherapie, en de relatie tussen het histologische subtype en de overleving. De bepaling van het subtype op de biopsie bleek sterk representatief te zijn voor het subtype van het osteosarcoom bij resectie. Van de 102 patiënten waarvan het subtype bepaald werd op zowel de biopsie als de resectie bleek slechts in 2 gevallen het subtype op de resectie anders te zijn dan dit van de biopsie. Het aandeel van de patiënten met goede respons op de preoperatieve chemotherapie (gedefinieerd als ≥ 90 % necrose) verschilde significant tussen de verschillende osteosarcoom subtypen (chi-kwadraat test statistiek = 11.44, P = 0,01 over 3 vrijheidsgraden). In tegenstelling met het conventionele osteoblastaire subtype, was het aantal patiënten met goede respons groter in de groep van de fibroblastaire variant en lager in de groep van de chondroblastaire variant. Patiënten met een goede respons hadden een significant betere overleving dan deze met slechte respons (logrank statistic = 7.68, P < 0.01 over 1 vrijheidsgraad). De overleving verschilde echter niet significant met het subtype (logrank statistic = 2.72, P = 0.44 over 3 vrijheidsgraden), hoewel er een tendens was voor patiënten met een chondroblastair subtype om ondanks het slechter beantwoorden aan de chemotherapie toch een betere overleving te hebben.

Hoofdstuk 3 is een bevolkingsstudie naar het voorkomen van osteosarcoom geassocieerd met ander maligne tumoren en naar de voorspellende waarde van het histologische subtype voor een erfelijke voorgeschiktheid tot kanker in osteosarcoom patiënten. Tussen januari 1975 en mei 2000 werden er in de gegevensbank van het Nederlandse Nationale Register voor Pathologie Palga, 938 patiënten geregistreerd met een betrouwbare diagnose van osteosarcoom. Zesenzestig van deze patiënten had een tweede kwaadaardige tumor. Een groep van 23 252 patiënten die een verwijdering van de keelamandel ondergingen in dezelfde periode diende als controle. In vergelijking met deze controlegroep hebben osteosarcoom patiënten een relatief risico van 2.4 (95% betrouwbaarheidsinterval 1.88 - 3.07) op het ontwikkelen van nog een kwaadaardige tumor. Vooral vrouwen blijken hiervoor gevoeliger te zijn dan mannen. Vijf procent van de mannelijke osteosarcoom patiënten en 10% van de vrouwelijke osteosarcoom patiënten hebben nog een andere tumor. De studiegroep van patiënten verdacht voor een erfelijk kanker syndroom werd geselecteerd door patiënten ouder dan 46 jaar bij de diagnose van hun eerste tumor en door patiënten met een radiotherapie of chemotherapie geïnduceerde maligniteit uit te sluiten. Alle osteosarcoom patiënten in deze studie groep hadden een niet-conventioneel subtype in vergelijking met de controle groep bestaande uit de patiënten opgenomen in de studies van de Europese Osteosarcoom Intergroep, waarin slecht 29% van de patiënten een niet-conventioneel subtype heeft. Een niet conventioneel subtype dient ons dus bedacht te maken op de mogelijkheid van een genetische voorgeschiktheid voor kanker bij de betrokken patiënt en zijn familie.

In **Hoofdstuk 4** werden die osteosarcoom patiënten geïdentificeerd met risico op eerste herval vijf jaar na diagnose en eerste behandeling en trachten we de vraag te beantwoorden of deze uitzonderlijke gevallen (minder dan 2% van de gevallen in drie onafhankelijke databanken) een karakteristieke klinisch pathologische presentatie hebben. Patiënten met herval na 5 jaar werden geselecteerd uit de gegevensbanken van studie 80831 en 80861 van de Europese Osteosarcoma Intergroep, de studies lopende van 1979 tot 1997 van de Coöperatieve Osteosarcoma Studie Group en de dossiers van het Royal Orthopaedic Hospital te Birmingham. Leeftijd, geslacht, locatie en subtype van het osteosarcoma werden vergeleken met deze van de gehele groep osteosarcoom patiënten opgenomen in de Europese Osteosarcoma Intergroep

studie 80831 and 80861. Patiënten met een eerste herval 5 jaar na de diagnosis hadden meer frequent een osteosarcoom gelokaliseerd ter hoogte van de fibula en tibia, en hadden ook meer frequent een niet-conventioneel subtype. Patiënten met een niet-conventioneel subtype van osteosarcoom gelokaliseerd ter hoogte van fibula of tibia dienen dus beschouwd te worden als hoogrisicopatiënten, waarvoor lange termijn opvolging aangewezen is. Het algemeen lage percentage van herval echter van patiënten met een niet chondroblastair subtype 5 jaar na initiële therapie laat toe deze patiënten als genezen te beschouwen na een ziektevrij interval van 5 jaar. De opvolging van deze patiënten later door kan zich dus toespitsen op late effecten secundair aan de therapie (functionaliteit, cardiotoxiciteit, secundair maligniteit e.a.) in plaats van op herval.

3. Op Ewing sarcoom gelijkend adamantinoom

Adamantinoom van de lange pijpbeenderen is een zeldzame maligne osteofibreuze tumor met een variabele hoeveelheid epitheliale cellen. Deze brengen basaal cel type cytokeratines tot expressie, meer specifiek cytokeratines 14, 19 en 5, en in minder mate ook cytokeratine 17. Het osteofibreuze subtype van het adamantinoom verschilt enkel van osteofibreuze dysplasie van de lange beenderen door de aanwezigheid van epitheliale cellen die soms slechts in kleine aantallen aantoonbaar zijn. De sterke overlap tussen adamantinoom en fibreuze dysplasie zowel klinisch als histologisch is sterk suggestief voor één en dezelfde tumor entiteit waarbij het adamantinoom en de osteofibreuze dysplasie de twee uitersten vormen.
Er zijn ook zeldzame gevallen beschreven van botlaesies met overlappende histologische kenmerken van Ewing sarcoma en adamantinoom suggestief voor een verband tussen deze twee entiteiten.
Ewing sarcoma/PNET wordt klassiek beschreven als een kleincellige blauwe tumor en behoort als dusdanig niet tot de osteofibreuze laesies van het bot. Het histologische kenmerk van een kleincellige laesie wordt gedeeld met maligne lymfoom, kleincellig carcinoom, mesenchymal chondrosarcoom, solide variant van alveolair rhabdomyosarcoom en medulloblastoma. De belangrijkste differentiaal diagnose stelt zich met het kleincellig osteosarcoom. Het belangrijkste diagnostische hulpmiddel is de aanwezigheid van de sterk specifieke (11;22)(q24;q12) translocatie aanwezig in 90-95% van de gevallen van Ewing sarcoom/PNET. Beschreven variante translocaties zijn: t(21;22)(q22;q12), t(7;22)(p22;q12), t(17;22)(q12;q12) and t(2;21;22)(q33;q22;q12)

Hoofdstuk 5 beschrijft 14 gevallen van adamantinoom. In deze gevallen kon er geen t(11;22)(q24;q12) of t(21;22)(q22;q12) aangetoond worden door middel van RT-PCR. Samen met het gekende keratineprofiel van adamantinoom, dat niet tot expressie komt in Ewing's sarcoom, toont dit zonder twijfel aan dat er geen verband is tussen Ewing's sarcoom en adamantinoom. Een juiste classificatie van een tumor met overlappende kenmerken tussen Ewing sarcoom en adamantinoom is van het grootste belang voor de patiënt. Het adamantinoom van de lange pijpbeenderen is namelijk een maligne tumor van lage maligniteitsgraad die behandeld wordt met complete lokale excisie. Het Ewing sarcoom echter is een maligne tumor van hoge maligniteitsgraad die dient behandeld te worden met een combinatie van chemotherapie, heelkunde en radiotherapie. Behandelingen die ook niet zonder risico zijn op korte en lange termijn.

4. Desmoplastisch fibroom van het bot, de benige tegenhanger van het desmoid type van fibromatosis

In **Hoofdstuk 6,** werden 13 gevallen van desmoplastisch fibroom vergeleken met de desmoid tumor van de weke weefsels door middel van immunohistochemie en DNA-sequencing. De histologie van het desmoplastische fibroom en de desmoid tumor is identiek. In de 13 gevallen van desmoplastisch fibroom was er geen immunoreactiviteit in de celkern voor oestrogeen and progesteron receptor, waar dit wel beschreven wordt voor desmoid tumoren. Zeven gevallen toonden immunoreactiviteit voor één of meerder spiermerkers. Dit is in overeenstemming met de myofibroblastaire aard van de cellen, zoals dit ook het geval is in de desmoid tumor. Zes gevallen toonden cytoplasmatische overexpressie voor β-catenine met in één geval ook overexpressie in de kern. Vier gevallen toonden Cyclin-D1 over expressie.
DNA sequencing voor activerende mutaties van het β-catenine gen resulteerde in geen DNA product in 3 gevallen, waaronder ook het geval met β-catenine overexpressie in de kern, en resulteerde in een onvolledig of slecht product in 2 gevallen. In de resterende 8 gevallen werden er geen mutaties gevonden in het β-catenine gen. Hoewel activerende β-catenine mutaties niet werden gezien in onze 13 gevallen van desmoplastisch fibroom, sluit het ene geval met sterke cytoplasmatische en nucleaire immunoreactiviteit voor β-catenine de betrokkenheid van de APC/ β-catenine weg niet uit in de morfogenese of tumorgenese van het desmoplastische fibroom. Deze weg lijkt echter minder essentieel te zijn voor de ontwikkeling van het desmoplastische fibroom dan dit het geval is voor de desmoid tumoren. Bovendien werd er in onze gevallen van desmoplastisch fibroom ook geen verband tussen opstapeling van β-catenine in de kern en cycline-D1 overexpressie aangetoond, waar dit wel gedocumenteerd is voor desmoid tumoren. Deze bevindingen doen de vraag rijzen of het desmoplastische fibroom inderdaad wel de tegenhanger is van de desmoid tumor van de weke delen. Het klinische belang ligt in het feit dat een patiënt met een desmoplastisch fibroom van het bot niet verdacht is op Gardner syndroom, en geen verhoogd risico heeft op kanker van de dikke darm.

5. Conclusie

In deze thesis worden histologische (fenotypische) verschillen binnen een bepaalde tumorentiteit en fenotypische gelijkenissen tussen verschillende entiteiten bestudeerd. Hieruit blijkt dat deze verschillen en gelijkenissen meer zijn dan een gril van de natuur. Retrospectief is het histologische subtype van een osteosarcoom dus een voorspellende factor voor de respons op chemotherapie, het risico op recidief meer dan 5 jaar na initiële behandeling en het risico op een erfelijke voorgeschiktheid tot kanker. Dit heeft directe implicatie voor de behandeling en opvolging van osteosarcoom patiënten en wanneer er verdenking is voor erfelijke voorbeschiktijd tot kanker ook voor hun directe familieleden. Deze bevindingen dienen echter gevalideerd te worden en dienen mee in overweging genomen te worden bij de opzet van toekomstige nieuwe osteosarcoma studies. Gezien osteosarcoom in het algemeen, en sommige subtypes in het bijzonder, zeldzame tumoren zijn, zijn grote gerandomiseerde studies noodzakelijk om de effecten van verschillende klinisch pathologische factoren en behandeling van het osteosarcoom op het algemene verloop van de ziekte te evalueren. Zelfs dan kunnen de absolute aantallen van de diverse gevallen te laag liggen om tot een sluitende en betrouwbare conclusie te komen. Dit

blijkt duidelijk uit ons onderzoek naar het verband tussen subtype van osteosarcoom en algemene overleving en verband tussen subtype en risico op laattijdig recidief. Verschillende osteosarcoma onderzoeksgroepen hebben dezelfde problemen ondervonden bij het evalueren van diverse chemotherapieschema´s. Daarom hebben meerdere onderzoeksgroepen waaronder de European Osteosarcoma Intergroup, de Childrens Oncology Group, de Cooperative OsteoSarcoma Study group and de Scandinavian Sarcoma Group besloten samen te werken onder de benaming van EURAMOS in een transatlantisch consortium. Aldus zullen ze grote gerandomiseerde studies in het onderzoek naar osteosarcoom ontwikkelen en uitvoeren. Dit type samenwerking maakt het mogelijk om verder prognostische factoren en factoren van belang voor de behandeling van patiënten verder te (her)-definiëren en te valideren. Een mogelijk toekomstperspectief ligt ook in het ophelderen van de moleculaire genetische sturing aan de basis van de complexe processen van celwerving, proliferatie, differentiatie en het verlies van controle op deze processen waardoor een maligne tumor kan ontstaan. Dit kan bijvoorbeeld door cDNA micro-array studies, waarbij het mogelijk is simultaan duizenden genen te analyseren. Vergelijkende analyses in genexpressie tussen de verschillende subtypes van osteosarcoom kunnen dus potentieel de genen aantonen verantwoordelijk voor de uiterlijke verschillen en het verschillende biologische gedrag. Dit is trouwens niet alleen geldig voor osteosarcomen maar voor kankeronderzoek in het algemeen. Genexpressie profielen laten ons niet alleen toe tumoren beter te klasseren en diagnosticeren, maar kunnen ook toelaten genen te herkennen die een voorspellende waarde hebben op het klinische verloop en die de basis vormen van processen waarop gericht therapeutisch kan ingegrepen worden. Zeer recent zijn de eerste rapporten verschenen over genexpressie profielen in osteosarcomen die een voorspelde waarde kunnen hebben voor het al dan niet gevoelig zijn aan chemotherapie.
Histologische gelijkenis tussen verschillende tumorentiteiten rechtvaardigt niet deze tumoren te beschouwen als één enkele entiteit of deel uitmakende van eenzelfde ziekteproces as aangetoond in de studies over het op Ewing sarcoma gelijkende adamantinoom of het desmoplastisch fibroom van het bot. Het niet stoppende proces van verwerven van nieuwe inzichten in tumorbiologie, samen met de verbetering en nieuwe ontwikkelingen op het vlak van gespecialiseerde technieken laat toe tumoren te (her-) klasseren op meer dan hun morfologie alleen. Niet alleen in de classificatie van tumoren, maar zeker ook in de diagnostiek, waar men soms slecht over een geringe hoeveelheid materiaal beschikt afkomstig van een naaldbiopt, wordt cytogenetische analyse in sommige gevallen nagenoeg onmisbaar, zeker wanneer men te maken heeft met tumoren met overlappend histologisch uitzicht. Aldus wordt bijgedragen tot een optimale behandeling en zorg voor de patiënt.

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Curriculum vitae

Esther Hauben werd geboren op 2 november 1964 geboren te Leut, België. In juni 1982 behaalde zij het diploma van Hoger Middelbaar Onderwijs, richting Latijn-Wetenschappen, aan het Koninklijk Lyceum te Hasselt. In oktober van dat jaar vatte zij de studie Geneeskunde aan, de eerste 3 jaar aan het Limburgs Universitair Centrum, de daaropvolgende 4 jaar aan de Universitaire Instelling Antwerpen waar ze in juni 1989 haar artsendiploma behaalde. Vanaf augustus 1989 was zij gedurende 1 jaar werkzaam als arts op de afdeling hartrevalidatie van het Universitair Ziekenhuis Antwerpen waarna ze assistent werd op het departement weefselleer en ontleedkunde aan het Limburgs Universitair Centrum. In december 1990 werd gestart met de opleiding Pathologie aan het Universitair Centrum Antwerpen (hoofd: Prof. Dr. E. Van Marck) welke voleindigd werd in december 1995, waarna ze nog tot juli 2000 in dienst was als staflid. Vanaf augustus 2000 is zij staflid in het Laboratorium voor Pathologische Anatomie Stichting PAMM te Eindhoven. Sinds 1999 is ze als referentiepatholoog verbonden aan de European Osteosarcoma Intergroup en voerde zij, naast haar reguliere werk als patholoog respectievelijk te Antwerpen en Eindhoven, het hier beschreven promotieonderzoek uit op de afdeling pathologie van het Leids Universitair Medisch Centrum, onder leiding van Prof. Dr. P.C.W. Hogendoorn en Prof. Dr. E. Van Marck.

List of publications

Mucinous cystadenocarcinoma of the pancreas with liver metastasis. An unusual presentation of a rare tumor.
Shamsi K., Deckers F., De Schepper A., Hauben E., Van Marck E.
Ann Radiol 1993; **36**(4): 328-331

Cavernous hemangioma of the adrenal gland: CT appearance.
Deckers F., De Schepper A., Shamsi K., Hauben E., Van Marck E.
J Comput Assist Tomogr 1993;**17**(3): 506-507

Massive cerebral infarction after completion pneumonectomy for pulmonary torsion.
Hendriks J., Van Schil P., De Backer W., Hauben E., Vanmaele R., Van Marck E.
Thorax 1994; **49**(12): 1274-1275

Histoplasma capsulatum infection in three aids patients living in Africa.
Colebunders R., Vandenabeele K., Hauben E., Verstraeten T., Heremans T., Van Den Ende J., Van Marck E.
Scand J Infect Dis 1995; **27**(1): 89-91

Laparoscopic resection of an adenoma of the urachus in combination with a laparoscopic cholecystectomy.
Hubens G., Vaneerdeweg W., Fierens H., Corthouts B., Hauben E., Van Marck E., Eyskens E.
Surg Endosc 1995; **9**(8): 914-916

Sternal resection for primary presternal and retrosternal mediastinal liposarcoma.
Van Schil P.E.Y., Van Look R., Van Calster E.L.Y., Van Oosterom A.T., Hauben E.I.
Eur J Cardiothorac Surg 1996; **10**(3): 217-219

Subcutaneous diffuse neurofibroma of the neck: a case report.
Janssens de Varebeke S., De Schepper A., Hauben E., Declau F., Van Marck E., Van de Heyning P.
J Laryngol Otol 1996; **110**(2): 182-184

Cytokeratin profiles and mucin secretion in combined hepatocellular-cholangiocarcinoma. A case report.
Hauben E., Struyf N., Michielsen P., Van Marck E.
Pathol Res Pract 1996; **192**(5): 488-491

Rhodococcus equi infection in 3 aids patients.
Colebunders R., De Roo A., Verstraeten T., Van Den Abbeele K., Ieven M., Hauben E., Van Marck E., Schaal K., Portaels F.
Acta Clin Belg 1996; **51**(2): 101-105

Hepatotoxicity after a short course of low-dose pyrazinamide
al Sarraf K.A., Michielsen P.P., Hauben E.I., Lefebure A., Ramon A.M., Van Marck E.A., Pelckmans P.A.
Acta Gastroenterol Belg 1996; **59**(4): 251-253

Serum aminotransferase levels and histological disease in chronic hepatitis C.
Michielsen P.P., Hauben E.I., Ramon A.M., Van Marck E.A., Pelckmans P.A.
Acta Gastroenterol Belg 1997; **60**(1): 11-14

Interferon-alfa and the cure of metastasis of a malignant meningioma in a kidney allograft recipient: a case report.
Bosmans JL., Ysebaert D., De Cock A., Hauben E., Muylle L., Schrijvers D., Van Marck E., Eyskens E., De Broe M.
Transplant Proc 1997; **29**(1-2): 838

Malignant melanoma of the soft parts (clear-cell sarcoma): confirmation of EWS and ATF-1 gene fusion caused by a t(12;22) translocation.
Speleman F., Delattre O., Peter M., Hauben E., Van Roy N., Van Marck E.
Mod Pathol 1997; **10**(5): 496-499

Localised amyloid tumour of the duodenum : a case reports
Hauben E., Fierens H., Heylen H., Van Marck E.
Acta Gastroenterol Belg 1997; **60**(4): 304-305

Two imported cases of Penicillium marneffei infection in Belgium
Depraetere K., Colebunders R., Ieven M., De Droogh E., Pelgrom Y., Hauben E., Van Marck E., Devroey C.
Acta Clin Belg 1998 ; **53**(4): 255-258

Epithelioid sarcoma of the vulva.
Tjalma A.A., Hauben E.I., Deprez S.M.E., Van Marck E.A.E., van Dam P.A.
Gynecol Oncol 1999; **73**(1): 160-164

Unusual cutaneous lesions in two patients with visceral leishmaniasis and HIV infection.
Colebunders R., Depraetere K., Verstraeten T., Lambert J., Hauben E., Van Marck E., Maurer T., Banuls A.L., Dujardin J.C.
J Am Acad Dermatol 1999; **41**: 847-850

Desmoplastic fibroma of bone: MRI features.
Vanhoenacker F.M., Hauben E., De Beuckeleer L.H., Willemen D., Van Marck E., De Schepper A.M.
Skeletal Radiol 2000; **29**(3), 171-175

MR imaging of clear cell sarcoma (malignant melanoma of the soft parts): a multicenter correlative MRI-pathology study of 21 cases and literature review
De Beuckeleer L.H., De Schepper A.M., Vandevenne J.E., Bloem, J.L., Davies A.M., Oudkerk, M., Hauben E., Van Marck E., Somville J., Vanal D., Steinbach L.S., Guinebretière J.M., Hogendoorn P.C.W., Mooi W.J., Verstraete K., Zaloudek C., Jones H.
Skeletal Radiol 2000; **29**(4): 187-195

Adamantinoma-like Ewing's sarcoma and Ewing's like adamantinoma. The t(11;22),t(21;22) status.
Hauben E.I., van Den Broek LC, Van Marck E., Hogendoorn,P.C.W.
J Pathol 2001; **195**(2): 218-221

Does the histological subtype of high-grade central osteosarcoma influence the response to treatment with chemotherapy and does it affect overall survival? A study on 570 patients of two consecutive trials of the European Osteosarcoma Intergroup
Hauben E.I., Weeden S., Pringle J., Van Marck E.A., Hogendoorn P.C.W.
Eur J Cancer 2002; **38**(9): 1218-1225

Multiple primary malignancies in osteosarcoma patients. Incidence and predictive value of osteosarcoma subtype for cancer syndromes related with osteosarcoma.
Hauben E.I., Arends,J., Vandenbroucke J.P., van Asperen C.J., Van Marck E., Hogendoorn P.C.W.
Eur J Hum Genet 2003; **11**(8): 611-618

Desmoplastic fibroma of bone: An immunohistochemical study including β-catenin expression and mutational analysis for β-catenin.
Hauben E.I., Jundt G., Cleton-Jansen A.M., Yavas A., Kroon H. Van Marck E., Hogendoorn P.C.W.
Hum Pathol 2005; **36**(9): 1025-1030