Diagnostics and treatment options in low-grade central chondrosarcomas

Suzan H.M. Verdegaal

DIAGNOSTICS AND TREATMENT OPTIONS IN LOW-GRADE CENTRAL CHONDROSARCOMAS

SUZAN H.M. VERDEGAAL

Voor Marijke, Lucas en Matthijs

Suzan H.M. Verdegaal
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	Prof. dr. P.C.W. Hogendoorn
Leden:	Prof. dr. J.L. Bloem
	Prof. dr. R.G. Forsyth, Vrije Universiteit Brussel, België
	Prof. dr. A.J. Gelderblom
	Dr. P.C. Jutte, Universitair Medisch Centrum, Groningen

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Chapter 1

General introduction

General introduction

Enchondromas are common intra-osseus benign cartilaginous neoplasms that develop in close proximity of the growht plate cartilage.
Chondrosarcoma are malignant bone neoplasms, characterized by the production of cartilage instead of bone.
When multiple enchondromas are present, this condition is called enchondromatosis, also known as Ollier disease. The condition in which enchondromatosis is associated with soft tissue hemangiomas is known as Maffucci syndrome.

Nomenclature/ synonyms:

Enchondroma
Chondrosarcoma:
- Borderline chondrosarcoma/ low-grade chondrosarcoma/ grade I chondrosarcoma/ atypical cartilaginous tumour
- Chondrosarcoma grade II
- Chondrosarcoma grade III

Ollier disease: dyschondroplasia/ multiple cartilaginous enchondromatosis/ enchondromatosis Spranger type I/ multiple enchondromas/ dyschondroplasia
Maffucci syndrome: dyschondrodysplasia with hemangiomas/ enchondromatosis with multiple cavernous hemangiomas/ Kast syndrome/ hemangiomatosis chondrodystrophica/ enchondromatosis Spranger type II.

Atypical cartilaginous tumour/grade I central chondrosarcoma

Introduction

Within the group of chondrosarcomas, different subtypes are defined. In the context of this thesis, we focused on conventional central chondrosarcoma. These neoplasms may arise *de novo* in the medulla of the bone (primary) or in a pre-existent enchondroma (secondary).[1] Atypical cartilaginous tumour (ACT)/ grade I central chondrosarcoma form approximately 85% of all conventional chondrosarcomas. Over the course of time the definitions of chondrosarcoma have been adapted based upon clinico-pathological and radiological correlations. Given the fact that a grade I chondrosarcoma in the extremities when bona-fide sampled will never metastasize unless dedifferentiation occurs and behaves like a locally agressive lesion, the WHO committee introduced the concept of atypical cartilaginous tumour,[2] in analogy with lipomatous tumours.
The most important prognostic factors in predicting the risk for metastasis are grading of the tumour and age of onset above 50 years. Grading is based on cellularity, nuclear size, nuclear staining, hyperchromasia and mitoses.[3,4] While grade I and II rarely metastasize (respectively 0% and 10%), grade III chondrosarcomas do so in about 70% of the cases. The 5-years survival in grade I chondrosarcoma is 84%, in grade II 64% and in grade III 29%. The main reason for the lower than expected 100% survival in ACT/ grade I central chondrosarcoma is the problem of local control in lesions of the skull, scapula or pelvis.[5,6]

Diagnosis

The presence of persistent pain not related to mechanical problems lasting for a period of several weeks should raise attention. Swelling might not at all be the presenting symptom. Even more puzzling is that a substantial number of these tumours are found as an accompanying event by radiological examination for another reason. The diagnosis should be made in a multidisciplinary setting based on clinical, radiological and histological findings. Specific clinical practice guidelines for diagnosis are published by the ESMO working group[7].

Treatment

Surgery is the treatment of choice for malignant cartilage neoplasms, with the extend of the margins depending on the tumour grade and location.[6,7] Radiotherapy and chemotherapy have no substantial role in the treatment of chondrosarcomas.[7,8]
Until the 90's, for all grades of central chondrosarcoma, wide resections and reconstruction (often with the use of a joint-replacing prosthesis), were performed. Due to the fact that morbidity had been high, but mortality low, less agressive surgical methods were introduced in patients with low-grade central chondrosarcoma. Since 1994, the Leiden University Medical Center treated this patient group by intralesional curettage. After instillating the walls of the cavity with phenol 85%, ethanol 96% is used for washing out the phenol. Finally, the bone cavity is filled up with deep-frozen, non-irradiated allograft bone chips derived from donor femoral heads (Bio Implant Services, Leiden, The Netherlands).

Phenol

Liquefied phenol, containing 82 to 86.5% w/w phenol in water, is a colourless or faintly coloured liquid. It may be used as a preservative in pharmaceuticals and chemicals. Liquefied phenol causes cell-wall disruption, precipitation, denaturation of proteins and coagulation necrosis. Liquefied phenol is readily absorbed via inhalation, ingestion and dermal contact, causing both local and systemic toxity. The elimination half-time ranges from 1-14 hours.[12] It is eliminated in the urine, mainly as sulfate and glucuronide conjugates. Clinical symptoms of phenol ingestion may include local corrosion with pain, nausea, vomiting and diarrhea. Systemic toxity may consist of CNS depression, circulatory and respiratory failure, pulmonary edema and hepatic and renal injury. Applied tot the skin, phenol causes blanching and corrosion in a concentration of 1-2%, depending on the exposure time.[13-16]

Macroscopy

Chondrosarcoma displays a translucent, lobular, blue-grey or white surface due to the presence of hyaline cartilage. Yellow-white, chalky areas of calcium deposit are commonly present. There may be areas containing mucoid material and cystic changes. In higher grades, cortical destruction or extended growth into the soft tissue may occur.[2]

Histopathology

The distinction between enchondroma and ACT/ grade I central chondrosarcoma can be difficult, and is subjected to a higher inter-observer variability.[3]
In ACT/ grade I central chondrosarcoma, the chondrocytes are atypical, varying in size and shape and contain enlarged, hyperchromatic nuclei. Binucleation is frequently seen, but mitosis is absent.[4]

Follow-up

In the postoperative period up to three months, plain radiographs are performed to detect bone repair of the bone window, or any complications like fractures. From 6-12 months after surgery, dynamic MR images with the use of Gadolinium are acccomplished periodically to detect any residual or recurrent cartilage tumour.

Ollier disease and Maffucci syndrome

Introduction

While most enchondromas and/or conventional chondrosarcoma are solitary, some occur multiple in the context of a syndrome; enchondromatosis. The two best-known are Ollier disease and Maffucci syndrome.[17-19] Both are characterized by the presence of multiple enchondromas. The difference is that in Maf-

fucci syndrome, also benign vascular lesions (hemangiomas) and/or lymphangioma are present. Both syndromes are non-heriditary. Often one site of the body is affected. The unilateral distibution of enchondromas could point in the direction of an early mutation event in embryogenesis, resulting in mosaïcism.

Diagnosis

Diagnosis is based on clinical features and plain radiographs of the bone. Usually, these syndromes manifest in early childhood; 75% are diagnosed before the age of twenty years.[20]
Besides the short and long tubular bones, also flat bones of the scapula and pelvis can be affected. Due to the asymmetrical distribution of enchondroma, often bowing deformities and/or limb lenght deformities occur.[20,21]

Treatment

There is no medical treatment for enchondromatosis. Surgery is indicated in case of complications, such as growth defects, pathological or pending fractures or malignant transformation.

Microscopy

Histological grading in enchondromatosis is more difficult, as increased cellularity and some nuclear atypia are not sufficient to diagnose low-grade chondrosarcoma. The distinction between benignity and malignity should be made within a multidisciplinary team. Also, radiological features should be taken into consideration (e.g. cortical destruction, soft tissue extension).

Genetics

Both Ollier disease and Maffucci syndrome are non-heriditary. Mutations in the gene encoding for isocitrate dehydrogenase 1 (IDH1) and IDH2 were detected

in solitary cartilaginous tumours as well in patients with enchondromatosis. These mutations might represent early postzygotic genetic events and account for the initiation of the disease process. Mutations of the gene encoding for parathyroid hormone receptor 1 (PTHR1) are found in a small subset (~10%) of patients with Ollier disease.[22-25]

Malignant transformation

The risk to develop secondary chondrosarcoma is 40% in Ollier disease (range 5-50) and up to 53% in Maffucci syndrome.[20]

Aim of the thesis

Regarding cartilage neoplasms of the bone, this thesis could be devided in two different parts;
The **first aim** was trying to identify clinical characteristics in a large group of patients suffering from Ollier disease or Maffucci syndrome in order to predict the risk of secondary development of chondrosarcoma and its subsequent mortality.
The **second aim** was to prove the assumption that phenol indeed has an effective role as local adjuvant in patients suffering from central grade I chondrosarcoma/ atypical cartilaginous tumour of the long bones.
Therefore we investigated two aspects;
First, the *in-vitro* cell-killing potential of phenol was studied. Secondly, phenol-concentrations during surgery were measured.
To prove the clinical effect on patients, we studied a group of patients treated with phenol as adjuvant therapy following intralesional curettage.
Also, the role of MRI imaging in the follow-up in these patients was studied.

Outline of the thesis:

In **chapter two**, an international multicenter study was performed to gain more insight in the clinical behaviour and characteristics of enchondromas in patients with Ollier diseae and Maffucci syndrome. An important aim was to es-

timate the cumulative probability of secondary transformation of enchondroma to chondrosarcoma over a lifetime. Variables who significantly were associated with the transformation to chondrosarcoma and mortality were defined.
In **chapter three**, the cytotoxic action of phenol on different cell lines were studied in order to provide a rationale for its use as an adjuvant. We investigated potentially effective concentrations *in vitro*.
Besides the expected cytotoxic effect of phenol on cell lines, we also analysed the independent effect of ethanol at different concentrations to assess cytotoxicity.
In **chapter four**, the surgical technique was outlined in more detail regarding the use of phenol and ethanol as adjuvant therapy following intralesional curettage of the cartilage tumour. Firstly, the initial local concentration of phenol in the cavity wall was measured. Secondly, we investigated the dilution of phenol 85% by ethanol 96%, and the role of the size of the cavity in the degree and speed of dilution.
Chapter five describes the results of intralesional curettage, followed by phenol application as adjuvant therapy and bone grafting. Aim was to describe clinical outcomes in a retrospective study.
Chapter six concerns the follow-up of patients suffering from ACT/ grade I central chondrosarcoma and those that were treated by intralesional curettage as described above. Already studies were performed on the predictive values of (dynamic) Gd-MR imaging in the preoperative diagnosis and grading of the lesion. So far, no results were published on the postoperative follow-up in these groups of patients. The outline of the study was to identify MR imaging features of normal postoperative changes and local residual or recurrent disease. Next to this, we tried to identify characteristics by using postoperative MR images to design a flow chart for different follow-up and treatment options.

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1

2

Chapter 2

Incidence, predictive factors and prognosis of chondrosarcoma in patients with Ollier disease and Maffucci syndrome: an international multicenter study of 161 patients

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S.H.M. Verdegaal[a], J.V.M.G. Bovée[a], T.C. Pansurya[a], R.J. Grimer[b], H. Ozger[c], P.C. Jutte[d], M. San Julian[e], D.J. Biau[f], I.C.M. van der Geest[g], A. Leithner[h], A. Streitbürger[i], F.M. Klenke[i], F.G. Gouin[k], D.A. Campanacci[l], P. Marec-Berard[m], P.C.W. Hogendoorn[a], R. Brand[a], A.H.M. Taminiau[a]

[a]Leiden University Medical Center, Leiden, The Netherlands
[b]Royal Orthopaedic Hospital, Birmingham, United Kingdom
[c]Istanbul Tip Fak Capa-FATIH, Istanbul, Turkey
[d]University Medical Centre Groningen, Groningen, The Netherlands
[e]Clinica Universitaria de Navarra, Pamplona, Spain
[f]Hôpital Cochin, Paris, France
[g]Radboud University Medical Centre, Nijmegen, The Netherlands
[h]Department of Orthopaedic Surgery, Medical University of Graz, Graz, Austria
[i]Klinik und Poliklinik für Allgemeiner Orthopädie Uniklinik Münster, Münster, Germany
[i]Inselspital, University of Bern, Bern, Switzerland
[k]Hotel-Dieu, Nantes, France
[l]Orthopaedic Oncology Unit, Azienda Ospedaliera Universitaria Careggi, Firenze, Italy
[m]Institut d'Hématologie et d'Oncologie Pediatrique, Lyon, France

Abstract

Background

Enchondromatosis is characterized by the presence of multiple benign cartilage lesions in bone. While Ollier disease is typified by multiple enchondromas, in Maffucci syndrome these are associated with hemangiomas. Studies evaluating the predictive value of clinical symptoms for development of secondary chondrosarcoma and prognosis are lacking. This multi-center study evaluates the clinical characteristics of patients, to get better insight on behavior and prognosis of these diseases.

Method

A retrospective study was conducted using clinical data of 144 Ollier and 17 Maffucci patients from 13 European centers and one national databank supplied by members of the European Musculoskeletal Oncology Society.

Results

Patients had multiple enchondromas in the hands and feet only (group I, 18%), in long bones including scapula and pelvis only (group II, 39%), and in both small and long/flat bones (group III, 43%), respectively. The overall incidence of chondrosarcoma thus far is 40%. In group I, only 4 patients (15%) developed chondrosarcoma, in contrast to 27 patients (43%) in group II and 26 patients (46%) in group III, respectively. The risk of developing chondrosarcoma is increased when enchondromas are located in the pelvis (odds ratio: 3.8; p=0.001).

Conclusions

Overall incidence of development of chondrosarcoma is 40%, but may, due to age-dependency, increase when considered as a lifelong risk. Patients with enchondromas located in long bones or axial skeleton, especially the pelvis, have a seriously increased risk of developing chondrosarcoma, and are identified as the population that needs regular screening on early detection of malignant transformation.

Introduction

Ollier disease[1] and Maffucci syndrome[2] are both rare, nonhereditary disorders in which patients develop multiple enchondromas, which are benign cartilaginous tumours in the bone.[3-5]

The diagnosis of Ollier disease, with a prevalence of one in 100,000,[6] is mainly based on clinical, radiological, and histological evaluation.[7] There is asymmetrical involvement of the extremities, with one side of the skeleton being affected with enchondromas either exclusively or predominantly.[6,8] Throughout their lives, patients experience a variety of different kinds of symptoms. Medical problems like leg length discrepancies or bowing deformities resulting from skeletal deformities caused by the enchondromas become prominent during childhood and adolescence. These deformities, mainly developing as a consequence of the asymmetrical distribution of the enchondromas, often require surgical correction.[9,10] If the enchondromas are located in the small tubular bones, the function of the hands and feet may be disabled to varying degrees depending on the severity of enlargement and deformities. The most severe complication is malignant transformation of enchondromas toward secondary chondrosarcomas, for which the reported incidence is highly variable, in the range of 5%-50% in the literature.[11-15] In addition, gliomas, acute myeloid leukemia, and juvenile granulosa cell tumours have been found in patients with Ollier disease.[3,16]

In Maffucci syndrome, cutaneous, soft tissue, or visceral hemangiomas are found in addition to multiple enchondromas.[11,17,19] Deformities of the bones resulting from asymmetrical involvement of the extremities are seen, as in Ollier disease. According to the existing literature, a large number of other malignancies, particularly pancreatic and hepatic adenocarcinoma, mesenchymal ovarian tumours, brain tumours such as glioma and astrocytoma, and various kinds of sarcomas are observed with this disease.[3,15,17,20–22]

Mutations of the gene encoding for parathyroid hormone receptor 1 (PTHR1) are found in a small subset (10%) of patients with Ollier disease.[3,23–25] Recently, mutations in the gene encoding for isocitrate dehydrogenase 1 (IDH1) and IDH2 were detected in solitary cartilaginous tumours as well as in patients with multiple enchondromas. These mutations might represent early postzygotic genetic events and account for the initiation of the disease process.[16]

No specific therapy yet exists to cure these potentially disabling diseases. Thus far, surgical therapy is the only available option when complications occur, for example,

pathological fractures, growth defects, or malignant transformation.
When properly diagnosed, osteochondromas do not appear in patients with Ollier disease or Maffucci syndrome. The combination of multiple enchondromas and osteochondroma-like lesions is known as metachondromatosis.[3]
To gain more insight into and a better understanding of the clinical behavior and characteristics of enchondromas in patients with Ollier disease and Maffucci syndrome, this European retrospective, multicenter study aimed at better defining the presentation and characteristics of enchondromas in patients with Ollier disease and Maffucci syndrome, estimate the cumulative probability of secondary transformation of enchondroma over a lifetime, and find variables significantly associated with this latter outcome and mortality. Data were collected by the European Musculoskeletal Oncology Society (EMSOS), a multidisciplinary society with great interest in bone and soft tissue tumours.

Methods

Data collection

The objectives of this retrospective cohort study were formulated and discussed at the EMSOS annual meeting in Porto, Portugal, in 2007. A questionnaire was designed (by S.H.M.V., J.V.M.G.B., T.C.P., P.C.W.H., and A.H.M.T.) to collect clinical data on patients with Ollier disease and Maffucci syndrome. The questionnaire was digitally sent as an Excel file to 130 EMSOS members at 76 hospitals in 26 countries in Europe and the Russian Federation. The digital file was sent with an accompanying manual. The questionnaire was completed by the participating physicians using patients' clinical files, radiological test results, and, when relevant, surgical and histological reports.
Each worksheet was used to record the available information of the included patients. Requested patient characteristics included: gender, age, family history, comorbidity, leg length discrepancies, and bowing deformities. Abnormalities of the spine were scored to exclude other, rare enchondromatosis subtypes.[3] Requested radiological characteristics included: estimated number and location of enchondromas, local cortical destruction, soft tissue extension, scalloping, and fractures. Requested tumour characteristics included: site and distribution of enchondromas, development of secondary chondrosarcomas, histological grading in cases with bi-

opsy or surgery, and location and histology of vascular lesions in cases of Maffucci syndrome. Requested surgical information included: any surgery that had been performed to correct deformities or leg length discrepancies, surgery for benign lesions, type and extent of surgery performed for malignancies, follow-up time after treatment and prognosis with respect to metastasis, dedifferentiation, and survival.
When more than one surgery was performed for a chondrosarcoma at a specific location, this was recorded as a single tumour with local recurrence rather than as a second chondrosarcoma.

Statistical analysis

Data were collected by different institutions using the unified Excel spreadsheets, which contained data validation and explicit definitions of all items asked. The spreadsheets were then converted and merged to one SPSS data file for analysis (SPSS, Inc., Chicago, IL).
Descriptive analyses consisted of tabular overviews of means, medians, percentiles and standard deviations. Bivariate associations of discrete variables were tested in crosstabulations using the likelihood ratio test or Fisher's exact test (in the case of low counts).
Estimates related to the occurrence in time of a specific event, for example, death, were obtained in a survival analysis framework. The primary approach was Kaplan-Meier estimation. The primary outcome of interest was patient survival, defined as the time from birth to the time of death from any cause.
A logistic regression framework was used to estimate the probability of 'being diagnosed with chondrosarcoma' as a function of the occurrence of enchondromas at various locations in the body. To this end, binary variables were constructed as indicators of the presence of enchondromas on plain x-rays in the scapula, humerus, ulna, radius, carpus, metacarpus, phalanges of the hand, pelvis, femur, tibia, fibula, tarsus, metatarsus and phalanges of the feet. They were entered into the model and a backward elimination of multivariately nonsignificant predictors was performed.
A discriminant analysis was used for the same purpose, but only as verification using another statistical method.
To calculate the cumulative incidence of enchondromas in combination with chondrosarcomas over time, a competing risk framework was used. The competing risk in this case was death resulting from any cause. The starting point of these cumulative incidence curve estimates was the date of birth of the patient. Because of the

construction of this dataset, the probability estimates should never be interpreted as 'life-long probabilities' since birth. This is not a cohort study following patients from birth but a study population highly selected on the occurrence of disease, and hence all probabilities (or proportions) have only a descriptive meaning conditional on the disease having been diagnosed.

Results

In total, 14 bone tumour referral centers and centralized national databanks in nine different European countries contributed patient data for the study, resulting in 161 patients. Apart from non-response, the primary reason for nonparticipation was a lack of patients who clinically fit the study's profile.

General characteristics

In total, 144 patients with Ollier disease and 17 patients with Maffucci syndrome were included in the study. Information regarding comorbidities, the development of other malignancies, and family history was provided for <3% of the patients, and therefore no evaluation of these data was performed. No positive family history or aberrations of the spine were reported (Table 1).[3]

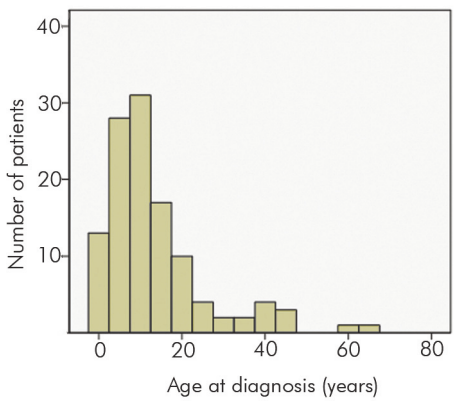

Figure 1.
Age when disorder was first discovered and a diagnosis of Ollier disease or Maffucci syndrome was made (n = 116). Twenty-three percent of the patients were diagnosed between age 0 and age 5 years and 45% were diagnosed before the age of 10 years. At 20 years of age, 75% of all patients had been diagnosed. Mean age at diagnosis, 13.38 years; standard deviation, ± 12.58 years.

Table 1. Characteristics of patients with Ollier disease		
characteristic	Ollier disease	Maffucci syndrome
n of patients	144	17
male versus female	74 versus 70	11 versus 6
mean age (range) at first diagnosis, yrs	13 (0-59)	12 (1-65)
mean age (range) at time of this study, yrs	31.3 (4-64)	37.9 (13-66)
unilateral versus bilateral, %	59 versus 41	37.5 versus 62.5
development of chondrosarcoma, % of patients	40%	53%
occurrence of chondrosarcomas, according to the distribution patterns of enchondromas (n of chondrosarcomas developed) Unifocal chondrosarcomas Multifocal chondrosarcomas	57/144 (40%) 74% 26%	9/17 (53%) 67% 33%
mean age (range) at surgery for first chondrosarcoma, yrs	33 (10-59)	30 (14-51)
other reported malignancies	hepatocellular carcinoma (n=1), glioma (n=2)	none reported
disease-related deaths	n=7 (5%)	n=1 (6%)

Overview of characteristics of patients with Ollier disease and Maffucci syndrome. The mean ages at first surgery were, respectively, 33 years and 30 years; noting that the mean age at the time of the study was 32 years, the incidence of chondrosarcoma in our patient cohort is expected to increase in the future.

Age at diagnosis of Ollier disease or Maffucci syndrome

The mean age of patients at the time of diagnosis of Ollier disease in this series was 13 years (range, 0-59 years; data from 105 of 144 patients with Ollier disease). For Maffucci syndrome, the mean age was 12 years (range, 1-65 years; data completed for 11 of 17 patients with Maffucci syndrome). 75% of the patients were diagnosed before the age of 20 years, both for Maffucci syndrome and for Ollier disease (Figure 1).

Location and distribution of enchondromas

Eighty-nine patients (55%) had cartilaginous lesions on one side of the body and 68 patients (42%) had bilateral disease (four patients had missing data). Enchondromas were predominantly found in the femur (affected in 59% of patients), tibia (af-

fected in 47% of patients), humerus (affected in 32% of patients), fibula (affected in 27% of patients) and pelvis (affected in 25% of patients). The small tubular bones of the hands were more often affected with enchondromas (carpal bones, 11%; metacarpals, 35%; phalanges of the hands, 45%) than the small tubular bones of the feet (tarsal bones, 10%; metatarsals, 19%; phalanges of the feet, 21%).
We distinguished three patterns of distribution of enchondromas. In 18% of cases, only the hands and/or feet were affected (designated as group I). 40% of patients had enchondromas in the long tubular and/or flat bones (group II). In 42% of patients, both the long and the flat bones as well as the small tubular bones of the hands and/or feet were affected (group III) (Table 2).

Skeletal deformities

44 Patients with Ollier disease (31%) and three patients with Maffucci syndrome (18%) had bowing or leg length deformities, particularly as a result of asymmetric distribution of enchondromas in the metaphysis and diaphysis of the long bones. The types of surgery performed included mainly lengthening procedures in the case of length discrepancies, osteotomies and local surgery like debulking or amputation to correct disabling enlargement of the fingers and toes. With respect to the site of surgery, 80% involved the long bones of the lower extremities, 13% involved the long bones of the upper extremities and 7% of the procedures were related to the metacarpals or phalanges of the hands.

Radiology

The radiological features of enchondromas and chondrosarcomas were compared on conventional radiographs (95 patients versus 66 patients). When both cortical destruction and soft tissue extension were present, the chance of dealing with a chondrosarcoma instead of an enchondroma was increased by a factor of 2.3 (95% confidence interval [CI], 1.28-4.8; p=0.019).

Table 2. Distribution patterns of enchondroma

distribution of enchondromas over the body, divided into three groups	total n of patients in each group	distribution of enchondromas according groups, n of patients		patients developing chondrosarcoma, n of patients (%)		disease-related death[a], n of patients	
		Ollier disease	Maffucci syndrome	Ollier disease	Maffucci syndrome	Ollier disease	Maffucci syndrome
group I: Enchondromas only in short tubular bones in hands and feet	29	27	2	4 (15%)	0	0	0
group II: Enchondromas only in long tubular bones and flat bones	64	62	2	28 (45%)	1 (50%)	2	1
group III: Enchondromas in short, long, and flat bones	68	55	13	25 (46%)	8 (62%)	5	0

the local distribution pattern of enchondromas correlated with the risk for developing chondrosarcoma.
[a]total deaths: eleven. DOD (death of disease; these patients died as a result of the disease): eight patients, pulmonary metastasis secondary to chondrosarcoma; three patients, nondisease related (glioma, n=2; hepatic carcinoma, n=1).

Hemangiomas in patients with Maffucci syndrome

One or more skin lesions were present in 12 of 17 patients with Maffucci syndrome. The location was mainly in the upper extremities (forearm, *n*=4; hands, *n*=6) and lower extremities (leg, *n*=3; lower leg, *n*=2; foot, *n*=2).
Excision of the lesion was performed in eight patients. Histological analysis identified spindle cell hemangioma in all cases.

Development of chondrosarcomas

66 Patients (41%) developed one or more secondary chondrosarcomas (Ollier disease, *n*=57; Maffucci syndrome, *n*=9). The mean age at which they first underwent surgery for chondrosarcoma was 33 years for patients with Ollier disease (range,10-59 years) and 30 years for patients with Maffucci syndrome (range, 14-51 years) (Figure 2).

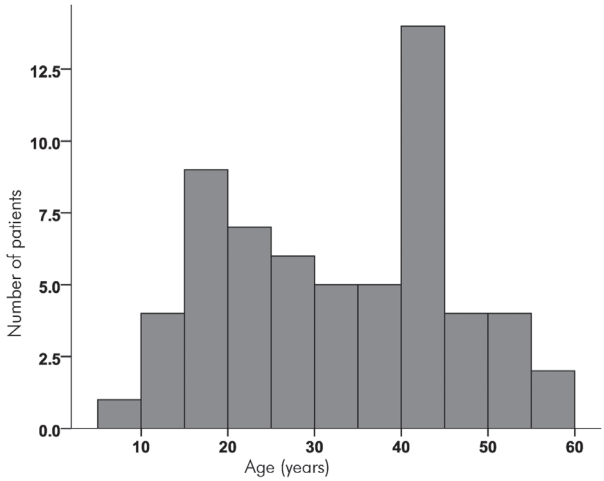

Figure 2.
Distribution of age at first surgery for chondrosarcoma over time. Only 50% of the patients had their first event before the age of 35 years. Mean age at first event, 33.0 years; standard deviation, 13.2 years

Of these 66 patients, 48 developed one chondrosarcoma whereas 18 developed two to four chondrosarcomas. Of these 18 patients, 33% had synchronous and 56% had metachronous chondrosarcomas (unknown, *n*=2 (11%)).

Altogether, 89 chondrosarcomas were diagnosed in the 66 patients. The primary locations affected in the long bones were the humerus (*n*=10, femur (*n*=18), and tibia (*n*=10). Nineteen chondrosarcomas were found in the flat bones (scapula, *n*=8; pelvis, *n*=11). Of the small tubular bones, the metacarpals and metatarsals were less often involved than the phalanges of the hands and feet (*n*=9 and *n*=14, respectively), which contradicts a nonsyndromal distribution.[26]

Chondrosarcomas developed in 45% and 46% of patients in Ollier disease in group II and group III, respectively (Table 2). In contrast, in group I where enchondromas were restricted to the small tubular bones of the hands and feet, the risk for developing chondrosarcoma was lower, at 15%.

In patients with Maffucci syndrome, both the short and the long tubular bones were affected more often with enchondromas (group III) (Table 2).

Using a logistic regression model for estimation of the probability of having chondrosarcoma as a function of the location of enchondromas, the only indicator remaining was the presence of an enchondroma in the pelvis. The other locations of enchondroma did not contribute significantly to the outcome. The odds ratio associated with enchondroma of the pelvis was 3.8, with a 95% CI of 1.8–8.0 (p=.001).

In the previously mentioned group of 47 patients with surgery for skeletal deformities, 19 also developed one or more chondrosarcomas in the course of their disease. In three patients with Ollier disease and one patient with Maffucci syndrome, chondrosarcomas developed at the site of previous surgery. All four demonstrated unilateral disease and had been operated on for deformities of the femur.

Histology of chondrosarcomas

Histological data were provided for 90% of the chondrosarcomas. 52% Were found to be grade I, 32% were grade II, 6% were grade III, and 10% were of unknown grade (Table 3).

Surgery for chondrosarcomas

Eighty-nine surgeries were performed for chondrosarcomas in 66 patients. In five cases with grade I chondrosarcomas, no surgery was performed after the biopsy. In 24 cases, intralesional curettage was performed (grade I, *n*=17; grade II, *n*=4; unknown grade, *n*=3). In eight of these cases (33%), local adjuvant therapy was used with intralesional curettage (phenol/ethanol, *n*=4; cryosurgery, *n*=3; radiofrequency ablation and phenol, *n*=1). Resection was performed in 46 cases (grade I, *n*=17; grade II, *n*=20; grade III, *n*=3; unknown grade, *n*=6). In two patients, surgery was followed by radiation therapy (intralesional curettage of grade II lesion of the skull, *n*=1; resection of grade II lesion of the humerus, *n*=1).
Amputation was performed in 13 cases. Sites of amputation were the phalanges of the hands and feet (eight patients; grade I, *n*=5; grade II, *n*=1; unknown grade, *n*=1), metacarpals (grade II, *n* =1), tarsal bone (grade II, *n* =1), femur (two patients; grade II, *n*=1; unknown grade, *n*=1), and humerus (grade III, *n*=1).

Table 3. Histological grade of chondrosarcoma

location	grade I	grade II	grade III	unknown	total
skull	1	1			2
scapula	3	4			7
pelvis	6	5			11
humerus	3	5	2		10
radius	1	1			2
femur	9	8			17
tibia	5	1	2	1	9
fibula	3			2	5
metacarpals	2	1	1	1	5
phalanges, hand	3	1		4	8
tarsals	1	1			2
metatarsals	2	1			3
phalanges, foot	6				6
total	45	29	5	8	87

Based on the histological grade of chondrosarcomas as diagnosed in the center of origin. Eighty-seven chondrosarcomas developed in 66 patients. Fifty percent of the lesions were low-grade chondrosarcoma.

Mortality

The disease-related mortality rate of the 161 patients included in this series was 6.8% (11 patients). Three patients died as a result of malignancies other than chondrosarcoma (Table 2 and Figure 4). Seven of the eight disease-related deceased patients were diagnosed with Ollier disease and died as a result of pulmonary metastases secondary to chondrosarcomas. The chondrosarcomas were primarily located in the humerus (n=1), radius (n=1), pelvis (n=1), femur(n=3), and tibia (n=1).
Three of these eight deceased patients with Ollier disease also developed a second chondrosarcoma located in the fibula (grade I), tarsus (grade II), and phalanges of the foot (grade I).
One patient with Maffucci syndrome died as a result of metastases of a chondrosarcoma located in the humerus. On average, there were 57 months between the first surgery for chondrosarcoma and the time of death. The mean age at the time of death in the deceased patients was 44.5 years (range, 29.2–58.9 years) (Figure 3).

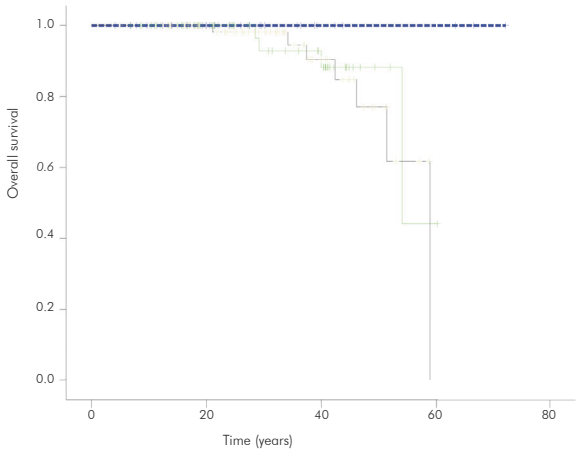

Figure 3.
Kaplan–Meier overall survival curves for group I (blue), group II (green), and group III (black) demonstrating excellent prognosis when enchondromas are restricted to the small bones of the hands and feet (group I). However, as soon as enchondromas are located in the long and flat bones, with or without disease in the small bones as well (group II and group III, respectively), the overall survival time is shorter. The difference between the curves is borderline significant (log-rank trend test, p.08). Note that there are only zero, four, and seven events in the three groups, respectively, which explains the rather low power of the test. Point estimates (95% confidence intervals) at time 40 years were: group II, 88% (75%–100%); group III, 85% (70%–100%)

Three non-chondrosarcoma-related deaths concerned patients with Ollier disease-who died at an average age of 29 years (range, 21–37 years) from hepatic carcinoma and glioma (*n*=2). The patient who died from hepatic carcinoma had previously undergone a surgical resection of a grade I chondrosarcoma in the pelvis; no evidence of local recurrence or distant metastasis was found after 91 months of follow-up (Table 1).

Discussion

Enchondromatosis, of which Ollier disease and Maffucci syndrome are the most common subtypes, is a rare disorder, and descriptive clinical studies are sparse[3]. This study was performed to gain more insight into and a better understanding of the clinical behavior of these disabling diseases.
The reported incidence in previous studies of malignant transformation in enchondromas is variable, and it is estimated to occur in 5–50% of cases. The cause of the

wide variation in the incidence of secondary chondrosarcomas and other related tumours, especially in patients with Maffucci syndrome, is the small numbers of patients in the series published so far.[11-15] The present study recorded the development of one or more chondrosarcomas in 40% of patients. The fact that our data were mainly collected from referral centers for musculoskeletal oncology may have led to a selection bias and the true incidence of malignancy may be slightly lower. It is also unclear whether patients were only referred to these reference centers because they had malignancy suspected or because of the underlying condition. On the other hand, this is the selected group of patients that we deal with in a specialized center,

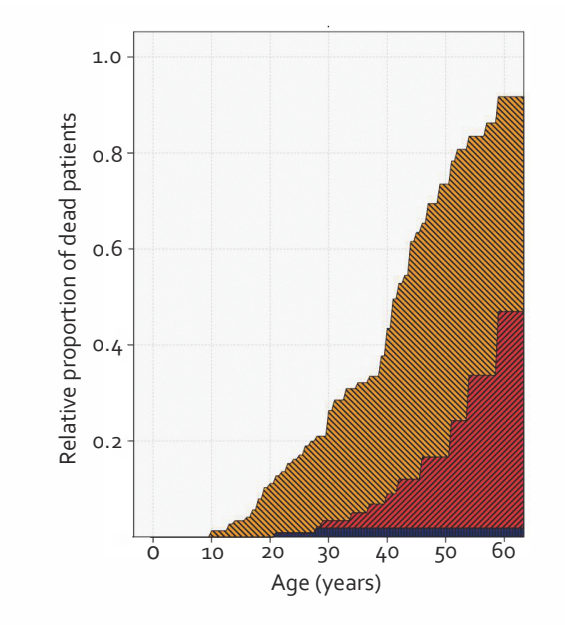

Figure 4.
Survival analysis using a competing risk model. This analysis only aims at describing relative proportions (the composition of the study population) for specific patient ages. It therefore only pretends to give a (an unbiased) graphical description for each possible number of years since birth of how many patients at that moment were actually already dead or alive with enchondroma (EC) or chondrosarcoma (CHS) or alive without detection of EC or CHS. More precisely, at each point in time (patient age), it shows the relative proportion of study patients already dead without EC or CHS (the blue pattern); the proportion already dead after detection of EC or CHS (the red or inclining pattern); the proportion at that age alive with EC or CHS (the yellow or declining pattern); and the proportion alive at that age without EC or CHS. Note that the separation between the two shaded areas (red and orange) is in fact the overall survival duration of this group of patients; the border between the silver and the orange area is the survival interval free from EC and CHS. Again, the interpretation is a description of the age at onset of EC or CHS death among these specific patients; it is useful as a description of the population and gives insight into the interplay of the competing risks (dying versus acquiring EC or CHS). It is not a predictive model per se because this is not a cohort followed from birth but a set of patients defined retrospectively on the basis of the occurrence of disease.

and this study represents what actually happens once the patient visits the hospital. As a result of the fact that we did not perform a cohort study in which all patients are followed until death, and because a substantial percentage of patients had their first surgery before the actual mean age in our study population, combined with the fact that we did observe malignancies among those patients substantially older than the mean age (32 years), we may expect the young patients in our study group to survive a substantial number of years and thus indeed develop additional malignancies, the probability of which is clearly not negligible.

Fiorenza et al.[27] performed multivariate analysis on independent risk factors for rate of survival in patients with solitary chondrosarcomas of bone. Extracompartimental spread, the development of local recurrence, and high histological grade were defined. Cumulative rates of death in 153 patients at 10 years and 15 years were 30% and 37%, respectively. With respect to mortality in that study, eight patients (5%) died as a result of chondrosarcoma with high-grade malignancy. Compared with the above-mentioned study, the percentage in our series is relative low. Considering the age at time of the study, however, a higher number of deaths can be expected in the future.

The difference in skeletal deformities of 31% for patients with Ollier disease and 18% for patients with Maffucci syndrome is not statistically significant (Fisher's exact test, p=.40). The odds ratio was two (95% CI, 0.56-7.5). The variability in the estimates of the percentage of deformities is so large that a difference of both a factor of two lower and a factor of seven higher are compatible with the data. Hence, the difference between 31% and 18% is well within the change fluctuation.

In this study, we tried to define characteristics of enchondromas in patients with Ollier disease and Maffucci syndrome. We discovered that the distinction of a solitary enchondroma from a solitary low-grade central chondrosarcoma is notoriously difficult when analyzing conventional radiographs.[28] Normally, no cortical destruction and soft tissue extension are seen with enchondromas on conventional radiographs. This study shows, however, that in the case of Ollier disease and Maffucci syndrome, the behavior of the enchondroma is locally more aggressive, and cortical destruction and/or soft tissue extension are seen in 44% of cases. In addition, the aforementioned distinction is also difficult at a histological level[29] and is, as in histological grading, subject to a high level of interobserver variability.[30] In the case of Ollier disease or Maffucci syndrome, the distinction is even more difficult because objective criteria for determining the occurrence of these diseases are lacking and therefore, in general, more worrisome histological features are tolerated within this context.

This study is hampered by the fact that no central review of radiographs and histolo-

gy was performed. Comparable studies carried out previously within EMSOS have shown that, in practice, it is too difficult to try to perform this in a retrospective, multicenter study because of, among other reasons, different national regulations regarding tissue handling.[31,32]

To estimate the cumulative probability of secondary transformation over a lifetime, it is important to distinguish the distribution patterns of enchondroma. Patients with enchondromas restricted to the small bones of their hands and/or feet, have a relatively low chance (14%, group I) of developing malignancies. In contrast, when enchondromas are found in the long bones or axial skeleton, there is a higher risk (44%–50%) for developing chondrosarcomas (group II and III). The only variable that was significantly associated with a higher risk for developing chondrosarcoma was the occurrence of enchondromas in the pelvis. Patients who have enchondromas located in the pelvis had a 3.8 higher risk for developing chondrosarcoma anywhere in their skeleton. Most importantly, disease-related mortality only occurred in patients with chondrosarcoma of the long or flat bones.

In the literature, various other malignancies have been reported in patients with Maffucci syndrome, such as pancreatic and hepatic adenocarcinoma, mesenchymal ovarian tumours, brain tumours (glioma and astrocytoma), acute myeloid leukemia[16], and various kinds of sarcomas (reviewed by Pansuriya et al.[3]). Only one case has been described in which autopsy-based molecular genetic tests were performed on a 34-year-old man with Ollier disease.[33] Therefore, several authors have advocated an abdominal computed tomography (CT) scan upon the diagnosis of Maffucci syndrome.[34] For Ollier disease, the spectrum of associated malignancies is much smaller, mainly consisting of gliomas and juvenile granulosa cell tumours.[3] Brain tumours in patients with Ollier disease are almost exclusively of glial origin, and patients are almost 10 years younger than patients with Maffucci syndrome when developing brain tumours.[21,35] Our results are in line with this; two patients (1.2%) developed and died from gliomas and one patient died as a result of hepatic carcinoma. In our series, in 17 patients with Maffucci syndrome, no other malignancies were reported. Therefore, minimal neurological complaints or abdominal symptoms should warrant a cerebral or abdominal CT.

This study found that metastases mainly arose in the lungs, which is in line with conventional chondrosarcoma and should be a guidance for follow-up.

Following the results of this study and summarizing data from the literature, we would like to recommend our opinion in the grading and follow-up of patients with multiple enchondromas. In cases when two or more enchondromas are detected in a patient, the patient should be staged by a Technetium scan. X-rays of every single

enchondroma should be performed to have a point of departure for the future. If any hemangioma is detected, the patient is diagnosed with Maffucci syndrome, otherwise the patient has Ollier disease. According to the locations of the enchondromas, patients can be divided into one of the above-mentioned groups to assess the risk for developing chondrosarcoma in the future.

In follow-up, random periodical x-rays of enchondromas usually give little information. In cases in which patients have dozens or hundreds of enchondromas, in particular, local situations can change at any moment. Patients with enchondromas of the long and/or flat bones and especially those with enchondromas of the pelvis, should be screened more carefully radiologically using plain x-rays when any complaints of pain, swelling, or neurological disorders appear or increase, whereas for patients with only enchondromas of the short tubular bones of the hands and feet, longer intervals can be used. When cortical and/or soft tissue extension on a plain radiograph is new or increases, a gadolinium (Gd) magnetic resonance imaging (MRI) scan should be performed.[28,36]

When malignant transformation is suspected, a biopsy should be completed. To prevent sampling error resulting from tumour heterogeneity, Gd-MRI can be helpful to increase tissue characterization.[36-39] Depending on the number and the location of the lesions, a biopsy and additional surgical therapy should be carried out. As a result of the fact that the patients with Ollier disease or Maffucci syndrome reported in literature had a higher risk for other malignant tumours, an additional CT scan of the brain or abdomen should always be considered when patients have symptoms.[16]

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3

Chapter 3

The cytotoxic effect of phenol and ethanol on the chondrosarcoma-derived cell line OUMS-27, an *in vitro* experiment

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S.H.M. Verdegaal[a], W.E. Corver[b], P.C.W. Hogendoorn[b], A.H.M. Taminiau[a]

[a]Department of Orthopaedic Surgery
[b]Department of Pathology
Leiden University Medical Center, Leiden,The Netherlands

Summary

Surgery is considered to be the most effective treatment for cartilaginous tumours. In recent years, a trend has emerged for patients with low-grade tumours to be treated less invasively using curettage followed by various forms of adjuvant therapy. We investigated the potential for phenol to be used as an adjuvant. Using a human chondrosarcoma-derived cartilageproducing cell line OUMS-27 as an *in vitro* model we studied the cytotoxic effect of phenol and ethanol. Since ethanol is the standard substance used to rinse phenol out of a bone cavity, we included an assessment of ethanol to see whether this was an important secondary factor with respect to cell death. The latter was assessed by flow cytometry.
A cytotoxic effect was found for concentrations of phenol of 1.5% and of ethanol of 42.5%. These results may provide a clinical rationale for the use of both phenol and ethanol as adjuvant therapy after intralesional curettage in low-grade central chondrosarcoma and justify further investigation.

Introduction

Malignant cartilaginous tumours are the second largest group of primary bone tumours.[1] Approximately 90% of chondrosarcomas are of the conventional type while the remainder in decreasing order of malignancy are dedifferentiated, extra-osseous, juxtacortical, mesenchymal and clear-cell chondrosarcomas.[1] An enchondroma is a hyaline cartilaginous tumour which is found centrally in the medullary cavity and may contain some secondary calcification and/or ossification. The malignant counterpart is conventional chondrosarcoma, which is subdivided into a peripheral and central subtype according to the clinicoradiological presentation and the oncogenic pathway.[2]
A primary intramedullary presentation and the production of a hyaline cartilaginous matrix are the hallmarks of a central chondrosarcoma. Most arise *de novo* but a small subset can appear secondary to a pre-existing enchondroma (secondary chondrosarcoma).[3] Central chondrosarcomas constitute about 75% of all chondrosarcomas, of which most are also low grade (grade I, 55%; grade II, 37%; grade III, 8%).[4] These tumours primarily occur in adults between the ages of 30 years and 70 years with an equal gender distribution.[5] Grade-I tumours are characterised by local destruction and a tendency to recur locally after excision without adequate margins.

They do not seem to metastasise or behave in a lethal manner except for those located at the base of the skull and in the pelvis.[4]

Surgery remains the most effective treatment for chondrosarcomas. Additionally, there is strong evidence that disease-free survival is closely related to the grade of the tumour and to the adequacy of the resection margins.[4] With the exception of proton-beam irradiation for small lesions located at the base of the skull,[6] it has not been shown that radiation therapy has any effect on chondrosarcomas.[7] However, the type of surgical procedure required may vary according to the grade of malignancy and the extent of the tumour.[8] Patients with grade I central chondrosarcoma may benefit from curettage given that this avoids resection and reconstruction, thus minimising functional impairment. This approach is only applicable for tumours which will not metastasise and thus only require local control.

Curettage without adjuvant therapy results in a high rate of recurrence of up to 40% for patients with grade I tumours.[4] Different forms of adjuvant therapy have been reported. Polymethylmethacrylate (PMMA) was first proposed by Persson and Wouters[9] during the mid-1970s based on the hypothesis that it may kill residual tumour cells. Another advantage of using PMMA was the possibility of early weight-bearing. The maximum peripheral limit of a thermal lesion induced by PMMA varies from 2 mm to 5 mm in cancellous bone and 0 mm to 5 mm in cortical bone.[10] Cryosurgery uses low temperature to induce tissue necrosis with reported cycles of freezing and thawing.[11] Cryosurgery can affect tissue at least 7 mm to 12 mm beyond the surgical margin.[12] The side-effects of cryosurgery are temporary nerve damage and a risk of fracture.[13] By contrast, an 85% solution of phenol (Figure 1) may be applied as adjuvant therapy with subsequent washing of the cavity with a 96% solution of ethanol. Despite the fact that several unpublished series have demonstrated a positive effect on the local recurrence rate in the treatment of cartilaginous tumours, the direct anti-tumour effect of phenol has been debated.[14,15]

We have studied the cytotoxic action of phenol on different cell lines by flow cytometry in order to provide a rationale for its use as an adjuvant and to identify potentially effective concentrations *in vitro*, which may be extended to clinical use. Given the standard use of ethanol to wash the phenol out of the bone cavity, we assessed whether this was an important independent factor with respect to cell necrosis. Ethanol was tested at different concentrations on chondrosarcoma cell lines to assess its effect.

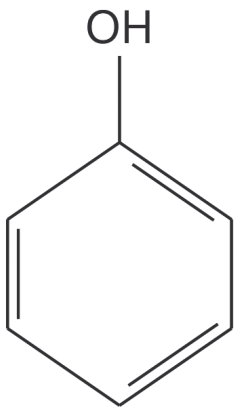

Figure 1.
Diagram of the chemical formula of phenol which is a benzene ring in which one H+ is replaced by a hydroxyl group. Its chemical formula is C6H5OH and its structure is that of a hydroxyl group (-OH) bonded to a phenyl ring. Phenol is a carbolic acid, a colourless crystalline solid. One of its properties is its limited solubility in water and it is known for its capacity to denaturate proteins.

Materials and methods

Cell lines and culture conditions

We used a chondrosarcoma-derived cell line (OUMS-27) as well as a cervical carcinoma cell line (SiHa). The characteristics of these cell lines have been reported previously.[16,17] All the cells were cultured in monolayers in Dulbecco's modified Eagle's medium (Invitrogen Life Technologies, Carlsbad, California) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen Life Technologies), 2 mM L-glutamine, 50 IU/ml of penicillin and 50 µg/ml of streptomycin (ICN Biomedicals, Aurora, Ohio) at 37°C in 5.0% CO_2. Detachment and dissociation of the cells before for flow cytometric analysis were performed when the cultures had just reached confluence. The cells were harvested using Hank's Balanced Salt Solution (HBSS; Sigma-Aldrich, St. Louis, Missouri) buffered in 5 mM EDTA/0.25% trypsin (Invitro-gen Life Technologies) at pH 7.2 and at 37°C, as described previously.[17] 14 different concentrations of phenol diluted in phosphate-buffered saline (PBS) ranging from 0% to 1.5% and 14 concentrations of ethanol ranging from 0% to 47.5% were used.

Phenol and Ethanol treatment

Two sets of experiments were undertaken. In the first the SiHa cell line was used to investigate the effective range of concentration of phenol (Liquid Phenol, Ph Ned Ed VI quality; BUFA bv, Pharmaceutical Products, Uitgeest, The Netherlands) (Table 1) and ethanol (Merck, Darmstadt, Germany) (Table 2). Initially, concentrations of phenol from 0.04% to 10.63% were used, but the ranges were narrowed after cell death was observed at low concentrations. For ethanol, concentrations ranging from 0% to 96% were applied.

Table 1. Tests performed on the SiHa cell line. Virtually all cells were killed in a solution of 1.33% phenol. Because of the total disintegration of the nucleotides, flow cytometry was no longer able to count the killed cells, and the percentage of cells killed decreased

phenol (%)	SiHa cell death (%)
0	2.6
0.65	56.9
1.33	97.1
2.66	98.5
5.32	97.7
10.63	88.1

The second set of experiments was undertaken on the OUMS-27 cell line using the concentrations defined in the first experiment. Harvested cells were subjected to 14 different concentrations of phenol diluted in phosphate-buffered saline (PBS) ranging from 0% to 1.5% and 14 concentrations of ethanol ranging from 0% to 47.5%.

Harvested cells were washed twice with HBSS and cell concentrations were determined using a Bürker counting chamber (Omnilabo, Breda, The Netherlands), 500 000 cells pelleted at 500 g (centrifugal force), for five minutes at 4°C. The supernatant was decanted and 50 µl of PBS were added to the pellet. Next, 50 µl of a phenol or ethanol dilution were added to the cells while gently stirring. The cells were subsequently incubated on ice. After five minutes, 1000 µl of PBS were added and the cells were deposited by centrifugation. They were then rewashed with PBS and finally resuspended in 500 µl of PBS containing 1.0 µM propidium iodide to stain for dead and necrotic cells.[19] This is a cell-membrane-impermeable fluorescent dye which binds to RNA and DNA thereby allowing the presence of dead and vi-

able cells to be determined by flow cytometry. After incubation for 30 minutes on ice, the cells were ready for flow cytometric analysis (Figure 2). Each experiment was repeated four times.

Table 2. Initial tests performed to determine the dose-effect curves of ethanol on a SiHa cell line showed that a concentration of ethanol of 42.5% killed all cells

ethanol (%)	SiHa cell death (%)
0	2.52
0.65	2.20
1.33	1.46
2.66	1.38
5.31	1.80
10.63	1.86
21.25	27.80
42.5	99.54
63.75	99.78
85	99.76
96	99.38

Flow cytometry

The flow cytometric analysis of stained cells was performed as described previously.[18] Briefly, for each measurement 10 000 events were collected using a standard FACS-calibur (BD Biosciences, San Jose, California) flow cytometer. Autofluorescence was measured using the green fluorescent parameter (FL1, 530/30 nm). Propidium iodide fluorescence was collected using the deep-red fluorescence detector (FL3, > 670 nm). Simultaneously, forward-scatter and side-scatter data were monitored and collected. Listmode files were analysed using WinList 6.0 software (Verity Software House Inc, Topsham, Maine).

Statistical analysis

For each experiment, the observed data consisted of a series of X and Y values where X was the concentration of phenol or ethanol and Y was the percentage of cells kil-

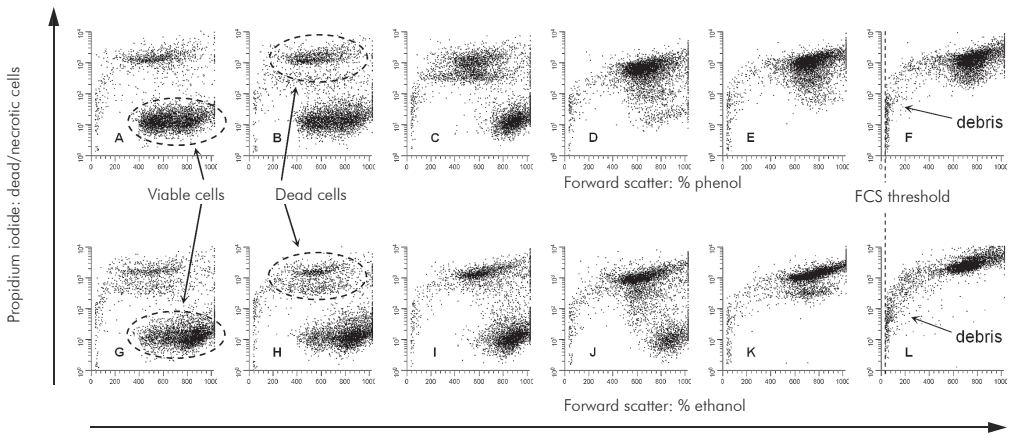

Figure 2.
Flow cytometric dot plots of forward scatter (abscissa) vs propidium iodide fluorescence (dead cells, ordinate) showing the death and disintegration of OUMS-27 cells after exposure to increasing concentrations of phenol and ethanol. Upper row, increasing concentrations of phenol; A=0.2%; B=0.4%; C=0.6%; D=0.9%; E=1.2%; F=1.5%. At a concentration of phenol of 1.5% the cells were killed. Lower row, increasing concentrations of ethanol: G=10%; H=20%; I=25%; J=30%; K=35%; L=45%.

led. A logistic regression curve was applied to these data points, usually around 15 points, using the standard formula: $Y = Y_{min} + (Y_{max} - Y_{min})^* 1/ (1+ EXP (- slope^* (X - X_{half}))$ where Y_{min} was the estimated horizontal asymptote of the curve for very low values of X, Y_{max} was the estimated horizontal asymptote of the curve for very high values of X, slope was the parameter to quantify the curvature of the logistic curve and X_{half} was the concentration where the curve had maximum slope.
The values of lethal dose xx (x) when xx was 50% or 95%, were calculated using the estimated parameters by determining X when xx denoted the relative distance between Y_{min} and Y_{max}.
The experiments showed that both the mean lethal dose xx and its associated 95% confidence interval could be computed, thus summarising the evidence from all experiments together.

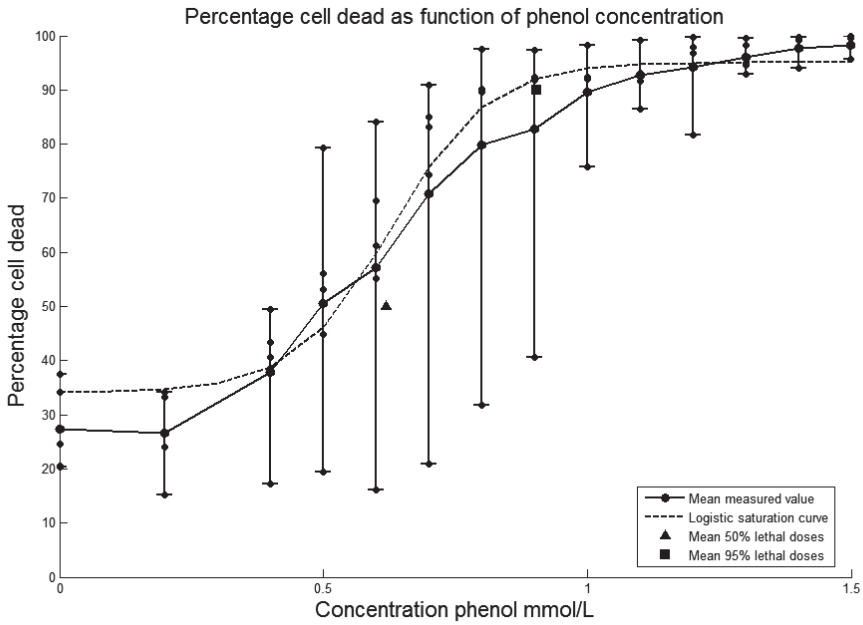

Figure 3.
Graph showing 100% cell death at concentrations of phenol higher than 1.4%. Lethal dose$_{50}$ and lethal dose$_{95}$ were corrected for the relatively high amount of cell death at T_0.

Results

The first series of experiments with different concentrations of phenol and ethanol on SiHa cells showed that a concentration window of 0% to 1.5% for phenol and 0% to 47.5% for ethanol could be used.

In experiment 2, this concentration range was applied to the chondrosarcoma cell line. For phenol, 100% of OUMS-27 cells were killed by a concentration of phenol of 1.5%. This experiment was repeated four times with identical results (Figure 3).

In these tests the proportion of cells dying without exposure to any toxic insult ranged from 20% to 37% because of the relatively high vulnerability during the harvesting of these cells. The data in Figure 2 are presented as logistic saturation curves, which also show the dose effect. Due to the relatively high amount of death of OUMS-27 cells by T_0, the lethal dose$_{50}$ was corrected as explained earlier.

In the 14 different concentrations of ethanol tested on 500 000 cells per test, five repeated tests all OUMS-27 cells were killed by a concentration of ethanol of 42.5%

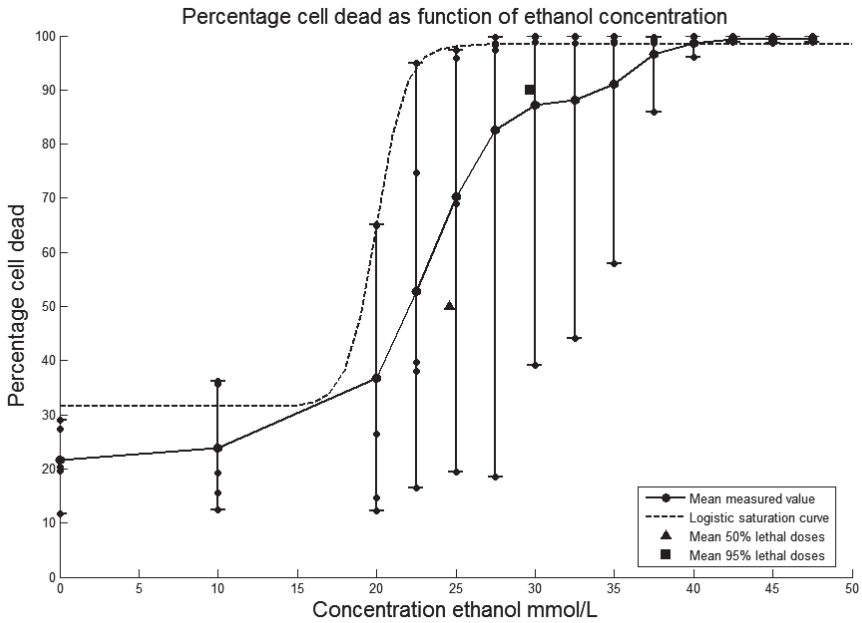

Figure 4.
Graph showing 100% cell death with higher concentrations of ethanol. Lethal dose$_{50}$ and lethal dose$_{95}$ were corrected for the relatively high amount of cell death at T_0.

and higher. The dose-effect curves for ethanol in the tests, which were performed separately, are presented in Figure 4. For ethanol a correction was made in calculating the lethal dose$_{50}$ and lethal dose$_{95}$.

Discussion

In recent years, intralesional curettage of low-grade central chondrosarcoma followed by local adjuvant therapy has become an accepted method of treatment. In chondrosarcoma, the use of cryotherapy has been shown to have particularly good clinical results with regard to local control of the tumour but serious complications may occur.[11] The effect of using phenol as an adjuvant in low-grade chondrosarcoma has been debated in the literature.[14,15] Surprisingly, the effect of ethanol as adjuvant therapy has never been investigated.
Our study has shown that both phenol and ethanol can kill chondrosarcoma cells *in vitro*. For phenol, all the cells were killed by a relatively low concentration of 1.5%.

Ethanol also had the potential to kill cells when used as a neo-adjuvant in the treatment of low-grade chondrosarcoma. In our study, all OUMS-27 cells were killed by ethanol *in vitro* using concentrations of 42.5%.

Our study used OUMS-27, a cartilage-forming chondrosarcoma-derived cell line.[16] While we acknowledge that all tests were performed in an *in vitro* environment, OUMS-27 may be regarded as the most appropriate cell line currently available given that it retains its cartilaginous phenotype *in vitro*, thereby simulating the clinical situation as closely as possible. The high number of dead cells at the start of the tests is comparable with that of other recorded experiments with this cell line, which is known to be quite fragile. Test III of experiment 2 (phenol *vs* OUMS-27) showed a curve, which, while starting later than the other four tests performed, displayed the same slope and also resulted in 100% cell death.

Normally, using flow cytometry, a forward scatter threshold is set in order to reduce the collection of system noise during measurement. Once a cell is prone to necrosis, it becomes permeable to propridium iodide, which can be readily measured by flow cytometry. However, when cells are exposed to a high concentration of phenol or ethanol, they disintegrate completely into small fragments, which generate a similar scatter. As a result, the final percentage of cells killed decreases by a few percent from 100% (Figures 3 and 4).

There have been a few reports[13,14] on the testing of the cytotoxic effects of phenol on cartilage-forming tumours. These studies, however, have focused on the necrotising effect of phenol, rather than impaired tumour cell viability. Lang et al.[14] and Lack et al.[15] concluded that while phenol might kill different benign and malignant cells it had no cytotoxic effect on cartilaginous tumours. They suggested that this was probably the result of the cartilaginous matrix.

In an investigation of the necrotising effect of phenol on vertebral bodies of fresh animal cadavers a defect was made in four vertebrae and phenol in different concentrations was applied to the defects for 30 to 120 seconds.[14] Necrosis was measured by determining the thickness of the cell layer demonstrating nuclear pyknosis or necrosis. The cellular effects on the bone-marrow cells were evaluated visually and the thickness of necrosis was measured microscopically. At concentrations of phenol of 10% to 25%, cells started to show aberrant nuclear structures. At concentrations of 50% to 75% there was a uniform zone of necrosis with a width of 630 to 747 µm. At a concentration of phenol of 90% less necrosis was identified when judged by the width of the zone of necrosis. In their discussion, the authors doubted whether a zone of necrosis of 0.7 mm to 0.8 mm was generally sufficient to prevent local recurrence.

In another study on the necrotising effect of a solution of phenol of 75% in 20% ethanol on normal tissue, which had been harvested during surgery or at post-mortem, cell death was also determined by measuring the thickness of the cell layers demonstrating nuclear pyknosis or necrosis using light microscopy, and taking ocular measurements in µm.[15] It was concluded that phenol induced varying extents of necrosis within different tissues, but that it did not have any apparent effect on cartilaginous tissue or cartilaginous tumours in any case.

By contrast, our study did not focus on the necrotising effect of phenol but instead investigated how phenol impaired tumour-cell viability. It showed that both phenol and ethanol had profound cytotoxic potential on *in vitro* chondrosarcoma cell lines. Furthermore, it also indicated that this potential had a concentration range, which could be applied in a clinical setting. On this basis we plan to perform an additional investigation into the effect of different concentrations of phenol and ethanol on chondrosarcoma pellets in which the matrix between the chondrosarcoma cells is present.

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4

Chapter 4

Phenol levels during intralesional curettage and local adjuvant treatment of benign and low-grade malignant bone tumours

Clinical Sarcoma Research, 2012, 2:10

S.H.M Verdegaal[a], J. den Hartigh[b], P.C.W. Hogendoorn[c], H.F.G. Brouwers[a] and A.H.M. Taminiau[a]

[a]Department of Orthopaedic Surgery
[b]Department of Clinical Pharmacy and Toxicology
[c]Department of Pathology
Leiden University Medical Center, Leiden, The Netherlands

Abstract

Background

Phenol is widely used for years as local adjuvant treatment for bone tumours. Despite its use for a long time, no information is available about the local concentration of phenol that is achieved in an individual patient, and the most sufficient and safe procedure to wash out the phenol after using it as local adjuvant.

Questions/purposes

1. What is the initial local concentration of phenol in the tissue of the cavity wall after the application of phenol?
2. How quickly is phenol 85% diluted by washing the bone cavity with ethanol 96% solution?
3. Is the degree and speed of dilution influenced by the size of the cavity?
4. How many times should the cavity be rinsed to obtain sufficient elimination of phenol?

Methods

A basic science study was performed at respectively 16 and 10 patients, treated by intralesional curettage and adjuvant therapy for low-grade central chondrosarcoma of bone.

Test 1: in 16 patients ten samples were collected of the mixture of phenol and ethanol from the bone cavity.

Test 2: in 10 patients, two biopsy samples were taken from the cavity wall in the bone during surgery.

Results

Phenol concentrations had wide variety in different patients, but all decreased by rinsing with ethanol.

Conclusions

Ethanol 96% is effective to wash out local applicated phenol, by rinsing the bone cavity six times. The local concentration of phenol diminishes to an acceptable concentration of 0.2%. This study provides new insights to safely further improve the surgical technique of intralesional treatment of bone tumours.

Background

In the surgical treatment of benign bone tumours, intralesional curettage has been performed throughout the past century. Over the recent years, its use has been extended to low-grade intramedullary malignant tumours in some instances. Unfortunately, this surgical technique has the risk of local recurrence from tumour cells that may be left behind. For this reason curettage was supplemented by the use of a local adjuvant, such as phenol, liquid nitrogen or bone cement (poly methyl methacrylate, PMMA). Various studies have demonstrated that by using adjuvant, the results of local therapy have been greatly improved.[1-4]

With regard to liquefied phenol, only a few documented studies have been published, despite of its routine use over a long period in orthopaedic practice. Previously, we were able to show that in case of low-grade chondrosarcoma, phenol is able to kill tumour cells *in vitro* already at a concentration of 3%.[5] In clinical settings, liquefied phenol is known to reduce local recurrence rates in different benign bone tumours such as aneurysmal bone cysts, chondroblastoma and giant cell tumours from 41% to 7-9%.[1,2] Liquefied phenol, containing 82.0 to 86.5% w/w phenol in water, is a colourless or faintly coloured liquid. It may be used as a preservative in pharmaceuticals and chemicals, but is also widely used in household products. Liquefied phenol is readily absorbed via inhalation, ingestion and dermal contact, causing both local and systemic toxicity. The elimination half-life ranges from 1 to 14 hours.[6] It is eliminated in the urine, mainly as sulfate and glucuronide conjugates. Liquefied phenol causes cell-wall disruption, precipitation, denaturation of proteins and coagulation necrosis. Aqueous solutions as dilute as 10% may be corrosive.

Clinical symptoms of phenol-ingestion may include local corrosion with pain, nausea, vomiting and diarrhea.[6] Systemic toxicity may consist of CNS depression, circulatory and respiratory failure, pulmonary edema, and hepatic and renal injury. Poisoning may occur from skin contact, especially from wounds. Applied to the skin, phenol causes blanching and corrosion in a concentration of 1-2%, depending on the exposure time.

In case of surgery, a bone-window is created in order to perform a subsequent curettage of the tumour. To ensure that the cavity that has been formed is microscopically tumour-free, the walls are cauterised with an 85% solution of liquefied phenol. Given that liquefied phenol has toxic and potentially carcinogenic properties when used systemically over a longer period, the cavity is then rinsed a number of times

with a 96% solution of ethanol. The cavity is filled with allograft bone-chips and the bone-window, which has also been rinsed with liquefied phenol and ethanol, is placed back in position. The above mentioned surgical technique has our preference given its advantage to induce minimal local damage and a biological reconstruction. Alternative products to fill the resultant defect after curettage are PMMA, auto graft bone chips or synthetic bone materials.
Regarding the surgical technique, some questions remain:
1. What is the initial local concentration of phenol in the tissue of the cavity wall after the application of liquefied phenol 85%?
2. How quickly is the liquefied phenol 85% diluted by washing the bone cavity with ethanol 96% solution?
3. Is the degree and speed of dilution influenced by the size of the cavity?
4. How many times should the cavity be rinsed to obtain sufficient elimination of phenol?
Answering these questions will further elucidate the safe practice of phenol-assisted, intralesional curettage of low grade malignant intramedullary bone tumours.

Patients and methods

Patients

16 patients (5 male, 11 female) with a median age of 48 (range 26-70) at time of surgery, were treated for grade I chondrosarcoma. The tumours are predominantly located in the proximal humerus (7/16, 44%) and the distal femur (5/16, 31%) (Table 1). The lesions were histologically classified by an experienced pathologist according to the recently published consensus criteria[7] and graded according to Evans.[8]

Clinical setting

Following a trial on three patients to test the feasibility of the study, 16 patients, suspected with grade I chondrosarcoma following X-ray and dynamic Gd-MRI, [9] were treated according to the surgical technique described above. Two tests were performed.

Table 1. Patient information, tumour volume and cavity surface

patient	age/gender	PA	location	tumour volume (cm3)	cavity surface (cm2)
1	41/M	CHS I	proximal humerus	27	73,4
2	39/M	CHS I	distal femur	48,1	74,2
3	40/F	CHS I	distal tibia	9,2	24,6
4	41/M	CHS I	proximal humerus	26,3	62,3
5	45/F	CHS I	proximal humerus	40,8	72,6
6	70/F	CHS I	distal femur	10,9	62,3
7	55/M	CHS I	distal femur	39,4	74,6
8	59/F	CHS I	proximal humerus	14,7	33,4
9	49/F	CHS II	proximal humerus	9,7	25,8
10	48/F	CHS I	distal femur	33	58,8
11	67/F	CHS I	distal tibia	5,2	16,8
12	44/F	CHS I	proximal humerus	25,8	49,4
13	33/M	CHS I	metacarpal V	4,4	15,3
14	50/F	CHS I	humerus	44	74,5
15	55/F	CHS I	proximal humerus	32	59,3
16	26/F	CHS I	distal femur	20	51,5

Test 1 was done on all 16 patients, test 2 on 10 patients. Before performing surgery including these tests, all patients were informed and gave their approval. Patient material was used in a coded fashion according to the local ethical regulation and in accordance to the national ethical guidelines (Dutch organisation of scientific societies FEDERA; 'Code for proper secondary use of human tissue' in the Netherlands).

Treatment protocol

Test 1

Depending on the size of the cavity, 2 to 4 ml of liquefied phenol 85% was applied with one or more small gauzes at the inner surface of the wall for three minutes after curettage of the bone tumour. Then the cavity was filled with an ethanol 96% solution. The volume of ethanol differed depending on the size of the cavity. Sub-

sequently, this mixture of phenol and ethanol was extracted by syringe and sealed immediately in a polypropylene container. This procedure was repeated ten times and labelled accordingly (sample numbers I to X).

Test 2

During surgery two biopsies were taken. The first biopsy (A) was taken after the cavity had been swabbed with the 85% phenol solution. A second biopsy (B) was taken after the cavity had been rinsed thoroughly ten times with ethanol, as described in Table 2.

These biopsies (A and B), plus the ten flush solutions (I to X) were then further investigated to determine the concentration of phenol.

Table 2. Concentrations of phenol in sixteen patients during washing out phenol with ethanol

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
I	929	2435	8032	3264	9661	13000	289	1300	1500	1500	1500	2299	20974	1239	373	160
II	449	900	2889	730	1780	563	193	787	734	1500	1478	816	13932	889	247	118
III	177	469	2169	293	1097	201	555	473	329	539	1199	523	4652	644	159	97
IV	92	313	1019	83	944	119	156	418	249	195	495	292	1739	465	82	66
V	84	185	797	106	632	162	167	313	150	160	185	261	1932	407	77	89
VI	69	189	522	73	312	126	146	289	127	87	249	223	1287	307	82	54
VII	65	128	400	49	168	101	98	191	97	126	157	160	805	214	65	64
VIII	65	146	381	72	227	72	114	126	97	116	217	140	900	215	65	39
IX	42	325	294	59	392	138	52	124	110	57	178	111	597	162	87	42
X	105	217	279	47	200	54	64	98	88	60	143	117	417	182	60	29

Characteristics of the 16 patients where the tests during surgery were performed. Depending on the location of the chondrosarcoma, the cavity surface varies.

The surface area of the cavity

It is necessary to approximate the shape of the cavity, because the actual surface area does not fit in any standard mathematical model and therefore cannot be measured with absolute precision. Therefore we projected a cylinder over the lesion using the largest diameter of the cartilaginous tumour of the long bones, measured in three dimensions on the pre-operative MRI. A standard formula was used to calculate the inner surface of this cylindrical curetted bone cavity: $2\ \pi r\ (r+h)$. R represents the average of half the width (mediolateral) and half the depth (anteroposterior), which often corresponds with the inside diameter of the bone. H is the length of the tumour taken in a craniocaudal direction.

Sampling

The flush solutions, from test 1, and the bone biopsies, from test 2, were stored in a refrigerator until analysis. Flush solutions were analyzed as such, or after dilution (see phenol analysis). Bone biopsies were dispersed in 5.0 ml ethanol 96% v/v; an aliquot of this solution was analyzed.

Phenol analysis

The phenol concentration of the ethanolic flush solutions was determined by High Performance Liquid Chromatography (HPLC) with spectrophotometric detection. Briefly, chromatographic analysis of the samples (diluted with ethanol to fit within the concentration range of the calibration curve, if necessary) was performed on a silica reversed phase column (Nucleosil C18, 100 x 3 mm, 5 µm particle size) with a mobile phase consisting of 25 mM phosphate buffer containing 0,5 % triethylamine pH 3.0 and acetonitrile (83+17, v/v). The column flow rate was 0.4 ml/min and detection was performed at 212 nm. The injection volume was 10 µl. The phenol concentrations in the flush solutions were calculated from calibration graphs obtained by simultaneous analysis of phenol standard solutions in ethanol 96% v/v in the concentration range 30 – 1500 ppm phenol. Up to 500 ppm there was a linear relation between response and concentration; above 500 ppm a quadratic function had to be used. Inter-day reproducibility showed a coefficient of variation of 5.1% at a concentration level of 50 ppm and 10.6 % at 5 ppm (n=6). The lower limit of quantifation (LLOQ) was 5 ppm. Basic descriptive statistics were employed.

Results

Test 1

The phenol concentrations measured in the flush solutions ranged from 29 to 20974 ppm. Large inter-patient differences were observed. Figure 1 shows a graphical presentation of the decay in phenol concentration over time observed for each patient, expressed as a percentage of the concentration measured in the first flush sample. After washing the bone cavity five times with ethanol 96%, in most of the patients the phenol levels measured are <280 ppm.

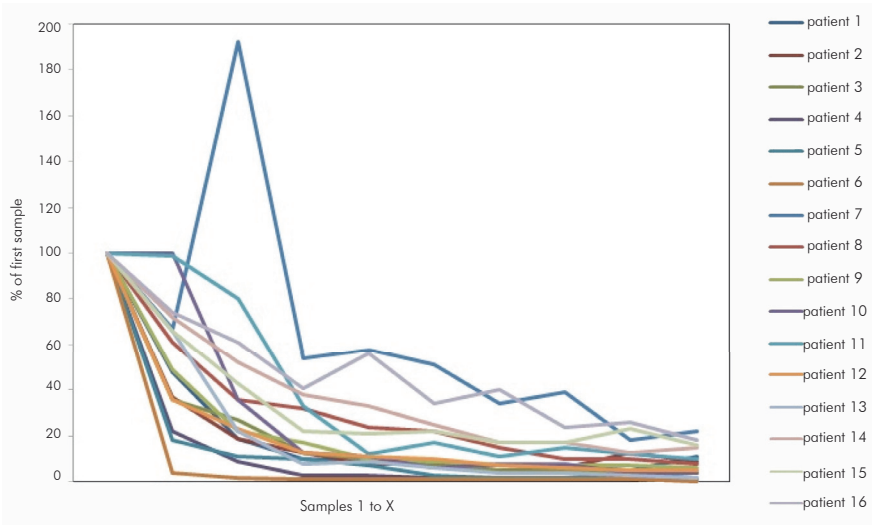

Figure 1.
Concentrations of phenol in ethanol. Results of ten samples of ethanol with phenol in 16 patients; samples II to X are reproduced as the fraction of the phenol concentration, measured in the first sample.

Patient number 7 has one very high concentration of phenol in sample III (Table 2). We can only explain this as a sample error, because from sample IV to X the concentration decreases.

Test 2

In Table 3 the phenol concentrations found in the biopsies, dispersed in 5 ml. ethanol, are presented. For all patients the phenol concentration before (A) and after flushing the cavity with ethanol 96% (B) is shown. Remarkable differences were measured, quantative as well as in reducing the concentration of phenol washed out by ethanol.

Tumour volume

Median volume of the cavity is 24.4 cm3 (range 4-48), and depends on the location of the tumour. The smallest volumes are measured in the distal tibia and fifth metacarpal bone.

No correlation is seen between the concentrations of phenol in ethanol in large or small cavities (Figure 2).

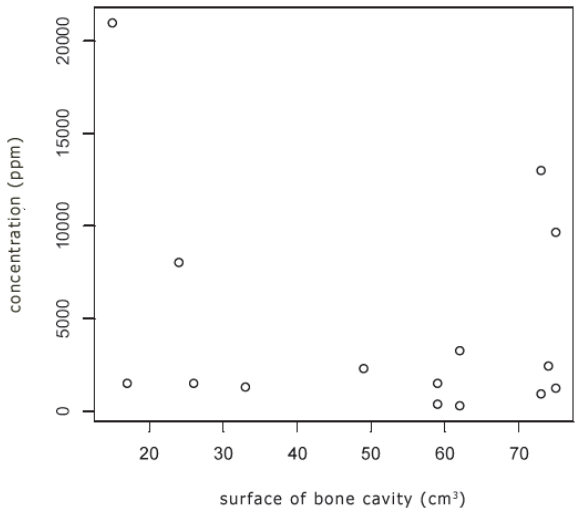

Figure 2.
Box Plot of concentration phenol and surface of bone cavity. Relation between concentrations of phenol measured in ethanol (ppm) and surface of the bone cavity. No correlation is seen between the concentrations of phenol in ethanol in large or small cavities.

Table 3. Concentrations of phenol solved in 5 ml. of ethanol during surgery

	8	9	10	11	12	13	14	15	16
biopsy A (ppm)	1598	888	168	479	1043	25	572	94	144
biopsy B (ppm)	408	418	39	106	636	17	587	11	18

Results of phenol samples 9 patients (8-16); first bone biopsy (A) was taken after application phenol 85%. After washing ten times with ethanol 85% the second biopsy (B) was performed. The concentrations of phenol (ppm) in this table are solved in 5 ml. of ethanol.

Discussion

The use of chemical cauterization for benign and low-grade malign bone tumours was first proposed by Bloodgood.[10] Although phenol is used for many years as adjuvant therapy in the intralesional treatment of benign and low-grade malignant bone tumours, no information is available about the local concentration of phenol in individuals, the most sufficient and safest procedure to wash out the phenol after

the local use.
Some surgeons have a dislike to the use of phenol as adjuvant, due to the possible predisposition to infection, the inhibition of the incorporation of grafts, systemic toxic effects on patients, and possible adverse effects on personnel in the operating theatre.
In our opinion, the highest estimated risk for using phenol is the people who work with it in the operating room. To prevent skin contact, using the routine measures such as by wearing proper protection to hands and face is sufficient. In case of skin contact, the skin should be irrigated with polyethylene glycol (PEG) 400 solution, isopropanol 70% or, if not available, with water.[10-14] During surgery, there is a risk of fire, with the evaporation of ethanol and simultaneous use of electric cauterization devices, as this can cause a spark. Therefore, we strongly recommend disconnecting the electric devices from the moment phenol and ethanol bottles are to be opened.
The two biopsies A and B were taken from the cavity wall before starting washing out and after rinsing ten times with ethanol 96%. In biopsy B, the quantity of phenol is diminished, as expected, but still there is quite a concentration left. This is due to the fact, that phenol is very lipophylic and therefore not easy to remove with ethanol 96%. The results also show remarkable differences in a quantative way. In these patients, tumour volume, differences in dilution by wound fluid and blood in the cavity, and differences in the ml. of phenol applicated explain these results. In patient 14, the concentration even increases a little bit after washing out with ethanol. In this patient, it seems that it is hard to wash out the local phenol from the cavity wall.
Concerning the initial local concentration of phenol in the cavity wall after application of phenol, two biopsies were curetted. Test 2 shows the concentrations in biopsy A and B. Starting with 85% of liquefied phenol, the mean initial local concentration of phenol measured was 557 ppm (range 25-1598, biopsy A, table 3). The measurements have a large variation.
Patient 13 suffered from a small chondrosarcoma of the metacarpal bone. The volume of the tumour was 4,4 cm^3. Biopsy A and B show 25 and 17 ppm phenol respectively. Nevertheless, the first sample in test 1 has a very high concentration of phenol in ethanol, 20974 ppm. The possible explanation of these controversial measurements is, that the location where the biopsy A and B were taken from the metacarpal bone can almost only be cortical border of the tumour. As cortical bone won't absorb phenol very well, the concentrations in biopsy A and B are low.
To investigate the effect of ethanol to dilute the applicated phenol, 10 samples in 16 patients were collected. In test 1, in all 16 cases the highest concentration of phenol

was measured in the first sample. However, the absolute values differ. Despite the fact that the same procedure was performed in all cases, no procedure is the same given the different volumes of the tumours varying from 4 to 48 cm^3. However, the absolute values differ. Despite the fact that the same procedure was performed in all cases, no procedure is the same given the different volumes of the tumours varying from 4 to 48 cm^3. In patient number seven, the IIIrd sample shows an increase of the concentration phenol. From the IVth sample, the line decrease further on sample II. The IIIrd sample must be considered to be a sample error. In these tests, the cavity was filled with ethanol ten times, and phenol concentrations were measured. After washing the cavity 6 times, in 10 of 16 patients the fraction of the initial concentration of phenol is <10%. The quantative concentration of phenol is <1300 ppm in all cases, which means an estimated concentration of phenol of <0,2%.

The degree of dilution is not influenced by the size of the cavity after curettage. However, it is remarkable that the highest concentrations in the first samples of test 1 all concerned small tumour volumes. This can be explained by the relatively small volume of ethanol where the phenol is diluted in.

We did not specifically study systemic effects of phenol exposure in our patients; however, on retrospective review, no adverse effects or secondary malignancies were noted.

In the few studies that described measurements of phenol, this was done by taking samples of urine, as liquefied phenol is eliminated by the kidneys. However, it is hard to define a 'thresh hold' for the toxic level.

This study shows that the adverse effects on the whole body due to the use of liquefied phenol as adjuvant in the intralesional curettage of benign and low-grade malignant bone tumours are reduced to safe concentrations by washing phenol out by ethanol. Phenol is a safe adjuvant when used in a proper way, taking the above mentioned statements into account. Washing the cavity six times with ethanol 96% will be sufficient to diminish the local concentration of liquefied phenol to an acceptable concentration of <0.2%.

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Authors' contributions

S.H.M.V. participated in the conception and design of the study, performed samples during surgery, performed analysis and interpretation of the data, and drafted the paper. J.d.H. participated in the conception and design of the study, the technical design of the phenol analysis including performing of the laboratory tests, and interpretation of the data. H.F.G.B. contributed in acquisition and interpretation of data, and performing the tables and figures. P.C.W.H. participated in the conception and design of the study, the analysis and interpretation of the data. A.H.M.T. participated in the conception and design of the study, performed the surgery and took samples during surgery and performed analysis and interpretation of the data. All authors read and approved the final manuscript.

Conflicts of interests

The authors declare that they have no competing interests.

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5

Chapter 5

Low-grade chondrosarcoma of long bones treated with intralesional curettage followed by application of phenol, ethanol and bone-grafting

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S.H.M. Verdegaal[a], H.F.G. Brouwers[a], E.W. van Zwet[b], P.C.W. Hogendoorn[c] and A.H.M. Taminiau[a]

[a]Department of Orthopaedic Surgery
[b]Department of Biostatistics
[c]Department of Pathology
Leiden University Medical Center, Leiden, The Netherlands

Background

A common treatment of low-grade cartilaginous lesions of bone is intralesional curettage with local adjuvant therapy. Because of the wide variety of different diagnoses and treatments, there is still a lack of knowledge about the effectiveness of the use of phenol as local adjuvant therapy in patients with grade I central chondrosarcoma of a long bone.

Methods

A retrospective study was done to assess the clinical and oncological outcomes after intralesional curettage, application of phenol and ethanol, and bone-grafting in 85 patients treated between 1994 and 2005. Inclusion criteria were histologically proven grade I central chondrosarcoma and location of the lesion in a long bone. The average age at surgery was 47.5 years (range, 15.6 to 72.3 years). The average duration of follow-up was 6.8 years (range, 0.2 to 14.1 years). Patients were evaluated periodically with conventional radiographs and Gadolinium-enhanced magnetic resonance imaging (Gd-MRI) scans. When a lesion was suspected on the basis of the MRI, the patient underwent repeat intervention. Depending on the size of the recurrent lesion, biopsy followed by radiofrequency ablation (lesions <10 mm) or curettage (lesions ≥10 mm) was performed.

Results

Of the 85 patients, eleven underwent repeat surgery because a lesion was suspected on the basis of the Gd-MRI studies during follow-up. Of these eleven, five had a histologically proven local recurrence (a recurrence rate of 5.9% [95% confidence interval, 0.9% to 10.9%]), and all were grade I chondrosarcomas. General complications consisted of one superficial infection, and two femoral fractures within six weeks after surgery.

Conclusions

This retrospective case series without controls has limitations, but the use of phenol as an adjuvant after intralesional curettage of low-grade chondrosarcoma of a long bone was safe and effective, with a recurrence rate of <6% at a mean of 6.8 years after treatment.

Level of Evidence

Therapeutic Level IV. See Instructions for Authors for a complete description of levels of evidence.

Malignant cartilaginous tumours are the second largest group of primary bone tumours.[1-4] Most arise *de novo*, although a small subset appears to be secondary to a pre-existing enchondroma.[2,3] Approximately 90% of chondrosarcomas are of the conventional type. These are subdivided into peripheral and central subtypes on the basis of their distinct oncogenic pathway.[3] Central chondrosarcomas constitute about 75% of all chondrosarcomas; the majority are low-grade.

The most important predictors of poor survival of patients with chondrosarcoma are a high histological grade and a patient age of more than fifty years.[5] Surgery is the primary treatment of cartilage tumours, with the extent of the resectional margins depending on the tumour grade and location.[6,7] Radiation therapy and chemotherapy have no substantial role in the treatment of chondrosarcomas.[4, 8-12]

Grade I tumours are characterized by a local destructive growth pattern and a tendency for local recurrence after surgery without adequate margins.[13] The clinical course cannot always be predicted on the basis of the histological grade alone.[1,13] Distant metastasis of low-grade chondrosarcoma is very rare (2% to 5%).[10,13-15] Five-year patient-survival rates of 85% to 90% have been described for grade I chondrosarcoma.[13-15]

The outcome of treatment of low-grade chondrosarcoma of long bones is good, but obtaining wide margins of resection can be associated with complications and morbidity. As a result, intralesional treatment has been used for low-grade chondrosarcoma. Different forms of adjuvant therapy to reduce the local recurrence rates have been reported.[16-19]

The use of polymethylmethacrylate (PMMA)[20] is based on the hypothesis that it kills the residual tumour cells by thermal heating of the bone cavity following curettage. The maximum peripheral extent of a thermal lesion induced by polymethylmethacrylate ranges from 2 to 5 mm in cancellous bone.[20,21] An advantage of using polymethylmethacrylate is the possibility of early weight-bearing.[21] Cryosurgery is performed with cycles of low temperature to induce tissue necrosis with the intent of ablation by freezing, holding of freeze, thawing, and repetition of this cycle.[17] The local extent of treatment with cryosurgery is at least 7 to 12 mm beyond the surgical margin.[22] The side effects of cryosurgery are nerve damage (temporarily), fractures, and infections.[18,23]

Application of 85% phenol as adjuvant therapy followed by washing of the cavity with 96% ethanol has been found to be effective treatment of chondrosarcoma-derived cell lines *in vitro*.[24] It is difficult to measure the depth of necrosis after application of phenol because phenol causes cell-wall disruption precipitation and coagulation necrosis. We are not aware of any clinical studies of the *in vivo* effect of

phenol as an adjuvant to curettage in the treatment of low-grade chondrosarcoma. The reported results of treatment of patients with chondrosarcoma are difficult to interpret because of differences in grading criteria, combining of axial and appendicular tumours, and mixing of treatments.[8,10,14,15,25] The goal of this study was to determine the clinical outcomes of patients with grade I chondrosarcomas of appendicular long bones, all of whom were treated, during one procedure, with intralesional curettage, followed by adjuvant therapy consisting of 85% phenol and 96% ethanol, followed by bone-grafting.

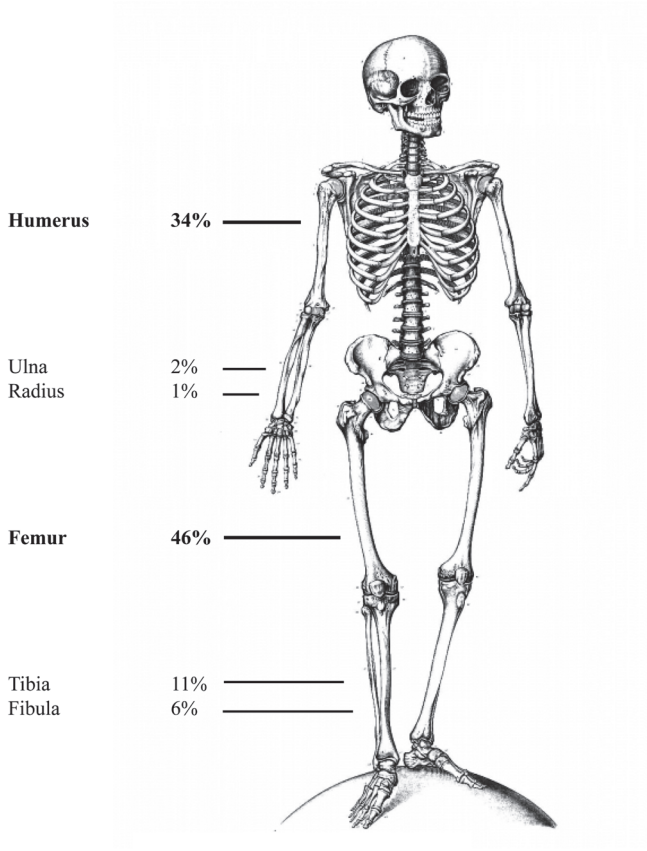


Figure 1.
Distribution of the eighty-five chondrosarcomas in the appendicular long bones. The proximal part of the humerus and the distal part of the femur were most frequently affected.

Materials and methods

We performed a retrospective study of 85 patients in whom a grade I central chondrosarcoma of a long bone had been treated in our hospital between 1994 and 2005. Patients from hospitals in the surrounding areas who were suspected of having chondrosarcoma were referred to our musculoskeletal oncology department. All patients underwent a preoperative gadolinium enhanced magnetic resonance imaging (Gd-MRI) scan prior to surgery.

The indication for surgery was the likely presence of low-grade chondrosarcoma[26,27] located in one of the long bones on the Gd-MRI scan. The average age at surgery was 47.5 years (range, 15.6 to 72.3 years). Surgery consisted of an oncologically safe biopsy, followed by intralesional curettage immediately or two weeks later.

There were 85 patients in this series. Frozen-section biopsy followed by curettage was performed during the same operation in 25 cases, whereas sixty patients had a two-stage procedure. In 13 of these 60 patients, the biopsy had already been done by the referring physician. All biopsy results were reviewed again by an experienced pathologist at our hospital (P.C.W.H.) who specializes in the pathology of bone and soft tissue tumours. The lesions were histologically classified according to the recently published consensus criteria[28] and graded according to the system described by Evans et al.[13] Patients were included in this study on the basis of a histological diagnosis of grade-I central chondrosarcoma located in a long bone (Figure 1).

The initial volume of the tumour was measured preoperatively with use of dynamic Gd-MRI scans. Due to the difficulty in measuring a three-dimensional structure on two-dimensional MRIs, all lesions were measured by projecting an imaginary cylinder. The average maximal radius r (anterior-posterior and medial-lateral) and maximal height of the tumour h (craniocaudal) were used as parameters to calculate the volume of a cylinder ($V = \pi r^2 h$). Depending on the largest diameters, different sequences of the MRIs were used.

Depending on the site of the tumour, patients received general or regional anesthesia for definitive treatment. Following preoperative identification of the precise location of the bone window on MRI, a small incision was made without the use of any Hohmann retractors to avoid possible tumour spill to other anatomical compartments. A window was thus created in the middle of the length of the tumour. The chondrosarcoma was removed macroscopically with use of small curets. No high-speed burr was used. The mechanical extension of the margin was determined by both the cortical border and the intramedullary canal. If there was doubt about whether all of the cartilage had been removed, fluoroscopy was used to detect any

calcified cartilage. A solution of 85% phenol (Liquid Phenol, Ph Ned Ed VI quality; BUFA bv, Pharmaceutical Products, Uitgeest, The Netherlands) was applied for a period of five minutes to the interior of the remaining bone cavity with a surgical swab. The phenol was subsequently rinsed with a 96% ethanol solution. Finally, the bone cavity was filled with deep-frozen, non irradiated allograft bone chips derived from donor femoral heads (Bio Implant Services, Leiden, The Netherlands). During surgery, the bone window was submerged in a phenol solution and then rinsed with ethanol. Following placement of the bone graft, the bone window was replaced.

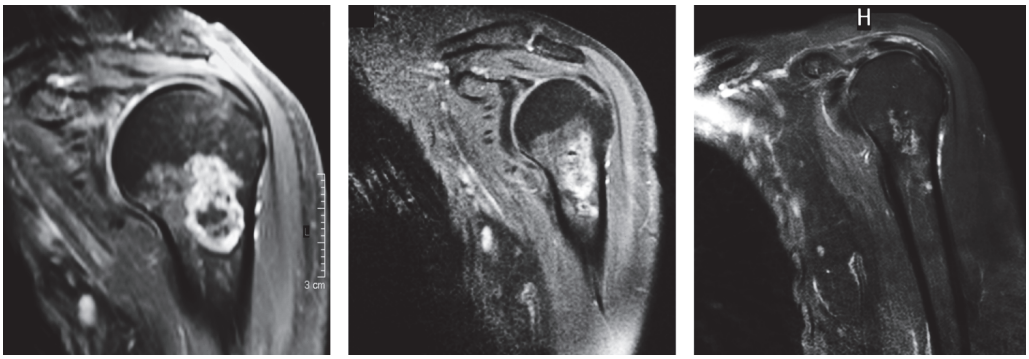
Prior to discharge, a postoperative radiograph was made for all patients to ensure that there were no postoperative complications. Additional radiographs were obtained six and twelve weeks after the procedure to establish the extent to which the patient could safely resume normal activities.

Patients were also scheduled for a dynamic Gd-MRI scan[27] six months after the procedure. The first scan was used as a baseline so that, with the following scans, a distinction could be made between the postoperative effects and the possible recurrence of chondrosarcoma. Dynamic Gd-MRI scans, in addition to radiographs, repeated six months later and then periodically (Figure 2). Patients were evaluated clinically on an annual basis. The average duration of follow-up was 6.8 years (range, 0.2 to 14.1 years).

In case a recurrence of the cartilaginous tumour was suspected on evaluation of the Gd-MRI scan, and the recurrent lesion was ≥10 mm, curettage, phenol application, and bone-grafting, as described for the index procedure, was repeated and the curetted tissue was evaluated histologically. With small lesions (<10 mm), a computed tomography-guided biopsy was performed and the specimens were evaluated histologically. In the same procedure, radiofrequency ablation was performed, with acceptance of overtreatment in the cases in which no recurrence of tumour would be diagnosed. Following these procedures, patients with recurrent chondrosarcomas were treated according to the postoperative protocols described above, including follow-up Gd-MRI scans.

Data analysis was performed with Excel (Microsoft, Redmond, Washington), SPSS (version 17.0 for Windows; SPSS, Chicago, Illinois), and R (version 2.10.0; R Foundation for Statistical Computing, Vienna, Austria).

We used the Kaplan-Meier product-limit estimator to analyze the survival rate, with recurrence as the end point. We treated patients who were lost to follow-up as censored at their last recorded visit. One patient died of an unrelated cause during the follow-up period, and we treated this death as a competing risk.



A B C

Figure 2. A series of T2-weighted Gd-MRI scans of a 62-year-old female patient with a grade I chondrosarcoma.

A Preoperatively, the typical ring-and-arc structure of a cartilage tumour is seen in the proximal part of the humerus.
B On the first postoperative MRI, acquired eight months postoperatively, postoperative edema persists.
C After seven years, the bone graft is incorporated, and no signs of tumour recurrence are seen.

Results

General

The average duration of follow-up for the patients was 6.8 years (range, 0.2 to 14.1 years), with five patients being followed for less than two years. One patient, followed for two months, came from abroad to have surgery in our clinic and returned to his home country after surgery. One female patient, 72 years of age at the time of surgery, lived at a substantial distance from the hospital; given her age, she was referred to a hospital near her home for follow-up. Three other patients were lost to follow-up. With use of the Kaplan-Meier estimator, the patients who were followed for a limited duration were censored at their last recorded visit.

Patients were admitted to the hospital for one to three days, depending on the site of the chondrosarcoma. Postoperative management depended on the tumour site and the size of the bone window. Patients with a chondrosarcoma in the upper extremity were managed with a sling for two to six weeks postoperatively. Following curettage in the lower extremities, patients were either non-weightbearing or partially weightbearing for six weeks and used crutches once they were mobile. None of the patients were treated with internal fixation, and casts were not necessary because of the less

invasive and limited nature of our surgical procedure compared with wide resection and reconstruction of the long bone.[29]

Tumour volume

The preoperative mean volume of the lesions, measured on MRI scans, was 23.7 cm^3 (range, 1 to 104 cm^3). The median volume was 18.8 cm^3 (Figure 3).

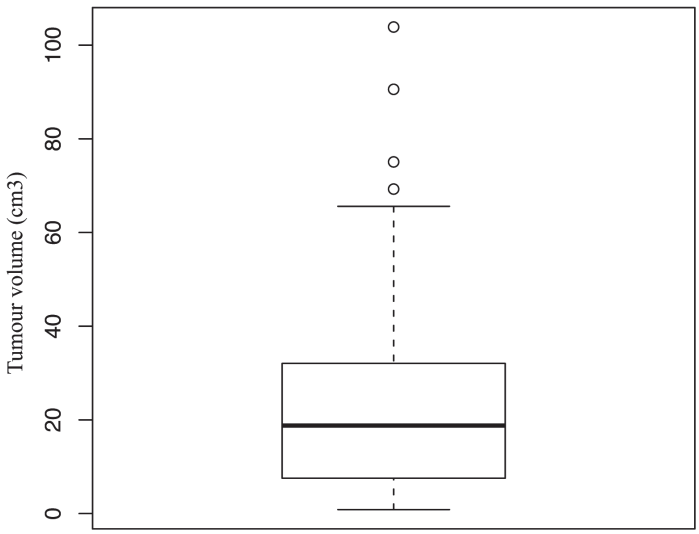


Figure 3.
Box plot for preoperative tumour volume, showing a median volume of 18.8 cm^3 (range, 1 to 104 cm^3) and a large spread of volumes forthe larger tumours above the median.

Histological findings

All patients were diagnosed with a grade I central chondrosarcoma, according to the recently published consensus criteria[28] and the system described by Evans et al.[13] (Figure 4).

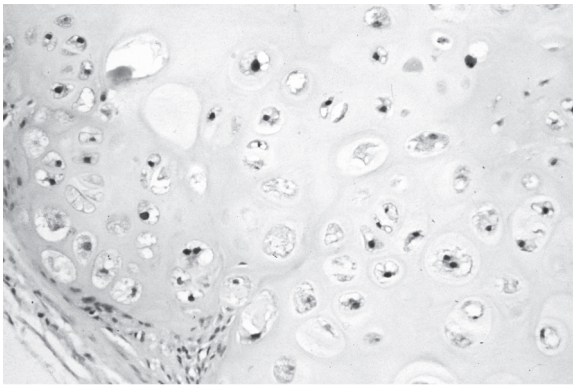


Figure 4.
Photomicrograph of a specimen of a grade-I chondrosarcoma displaying typical hypocellular, slightly pleomorphic chondrocytes (hematoxylin and eosin, magnification 200x)[28].

Comorbidity

Four (7%) of the 55 women had a history of breast cancer or developed this tumour during the period of follow-up of the cartilage tumour.

Complications

One patient developed a superficial wound infection postoperatively, which resolved with antibiotics. Two (5%) of the 39 patients treated for a tumour in the femur experienced a femoral fracture, which was likely due to the bone window, within six weeks after surgery. One of these patients was treated with open reduction and internal plate fixation, and the other was treated with an intramedullary nail. Gd-MRI performed five years after removal of the nail did not show any sign of tumour recurrence. Patients with chondrosarcoma of the femur appear to have a higher fracture risk, which can be addressed with a hip spica cast and non-weightbearing with two crutches. The prophylactic use of internal fixation should be avoided, to allow follow-up MRI and to avoid a second surgical procedure for removal of the metallic implants.

Repeat interventions

Remaining tumour was suspected in eleven patients on the basis of postoperative Gd-MRI scans. All of these patients underwent repeat procedures. Depending on the size of the lesion, biopsy followed by radiofrequency ablation (for tumours of <10 mm) or repeat curettage (for those of ≥10 mm) was performed. All tissue obtained with biopsy prior to radiofrequency ablation or with curettage was sent for histological analysis. These analyses showed recurrence of the grade-I chondrosar-

Table 1. Data on the patients with suspected local recurrence*

case	age (yrs)/ sex	diagnosis	tumour site	volume of initial tumour (cm^3)	treat-ment	time to suspected local recurrence (months)	historical findings	treatment of suspected local recurrence
1	52/F	CHS I	proximal humerus	5.7	CPEB	8	No recurrence	CPEB
2	38/F	CHS I	femur	48	CPEB	20	CHS I	RFA
3	15/F	CHS I	proximal humerus	45.7	CPEB	25	CHS I	CPEB
4	48/M	CHS I	proximal humeral	16	CPEB	32	No recurrence	CPEB
5	52/F	CHS I	proximal humerus	52	CPEB	34	CHS I	RFA
6	28/F	CHS I	femur	28	CPEB	40	No recurrence	CPEB
7	63/F	CHS I	proximal humeral	5.7	CPEB	63	No recurrence	RFA
8	32/F	CHS I	proximal humerus	8.1	CPEB	64	CHS I	RFA
9	52/F	CHS I	proximal humerus	48.1	CPEB	68	No recurrence	RFA
10	51/M	CHS I	distal femoral	36.2	CPEB	89	No recurrence	RFA
11	38/F	CHS I	proximal humerus	23.5	CPEB	91	CHS I	CPEB

CHS I = grade I chondrosarcoma
CPEB = curettage, 85% phenol, 96% ethanol, and bone-grafting
RFA = radiofrequency ablation
Eleven patients with a suspected lesion on Gd-MRI had repeat intervention. Five patients had a histologically proven recurrence of a grade I chondrosarcoma. No tumour progression was seen.

coma in three of the six patients who underwent radiofrequency ablation and no signs of recurrence in the other three. Of the five patients who underwent repeat curettage, two were found to have recurrence of the grade-I chondrosarcoma and three had no signs of recurrence (Table 1). The recurrence rate in this series was 5.9% (95% confidence interval [CI], 0.9% to 10.9%).
The serial Gd-MRI scans did not show any signs of tumour recurrence in the remaining 74 patients. With regard to the ability of the Gd-MRI to predict recurrence of chondrosarcoma in this series, the positive predictive value was 45% and the negative predictive value was 100% (Table 2).

Table 2. Suspected lesions versus histologically proven recurrence*

Suspected lesion on Gd-MRI	Histologically proven recurrence		
	Yes	No	Total
Yes	5	6	11
No	0	74	74
Total	5	80	85

*Eleven of the eighty-five patients had, on the postoperative Gd-MRI scans, a suspected lesion and underwent a second surgical procedure. Of these eleven patients, five had a recurrence proven by histological examination. Positive predictive value = 45%, negative predictive value = 100%, sensitivity = 100%, and specificity = 93%.

Survival

The survival rate, with histologically proven recurrence of grade-I chondrosarcoma as the end point, was 91.3% (95% CI, 84% to 99.4%) at a mean of 6.8 years after the first surgery (Figure 5).

Mortality

One patient died, due to an adenocarcinoma of the pancreas, during the follow-up period.

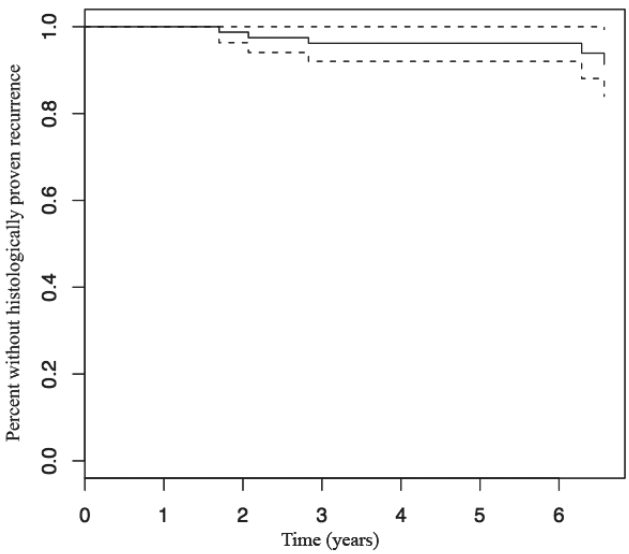


Figure 5.
Survival curve, showing five patients with a postoperative recurrence of a grade I chondrosarcoma as the event. The survival rate, with histologically proven recurrence of grade I chondrosarcoma as the end point, was 91.3% (95% CI, 84.4% to 99.4%) at a mean of 6.8 years after the first surgery.

Discussion

We describe a large group of patients with grade I central chondrosarcomas of the long bones who were treated with intralesional curettage followed by application of phenol and ethanol as adjuvant therapy and then by bone-grafting. The use of phenol as an adjuvant in the treatment of bone tumours has been described only in small patient series that have included a variety of tumours, both benign and malignant.[20-32] Although efficacy of this treatment has been proven *in vitro*[24], it has been difficult to convincingly demonstrate the efficacy of phenol as an adjuvant in humans.

This study is limited by its observational and retrospective design. We did not use a control group to compare the results. The ideal situation would be to perform a prospective, multicenter, randomized trial comparing phenol with cryotherapy or polymethylmethacrylate adjuvant treatment.

Four (7%) of the 55 women in our series had also been diagnosed with breast cancer in the past or during the follow-up period. Odink et al.[33] described an odds ratio of 7.62 to be diagnosed with both breast cancer and a cartilaginous tumour. Therefore,

physicians should be aware of this combination of diseases whenever either a cartilaginous tumour or breast cancer is diagnosed in a female patient.[33-35]

Performing follow-up only with radiographs to detect local recurrence overestimates the disease-free survival.[10,18] We therefore use Gd-MRI to follow our patients. On the first postoperative scan, it is sometimes difficult to distinguish between the normal postoperative appearance as a consequence of the use of bone grafts from femoral heads and the presence of postoperative edema at the surgical site.[36] A second scan is therefore often decisive because the postoperative changes are lessened and the lesion suspected of containing residue is still clearly enhanced within ten seconds on the dynamic series of the Gd-MRI scan.[27,37] In retrospect, the cause of the residual tumour in the two recurrent femoral cases in our series was surgical in nature. The residual tumour remained as a result of incomplete curettage, primarily as a consequence of a bone window that was too small or had been placed in a suboptimal location; this is particularly a risk for femoral diaphyseal lesions. No significant correlation was seen between preoperative tumour size and recurrence rates (Table I).

The distinction between benign and malignant cartilaginous tumours is often subject to discussion. To improve the reliability of the diagnosis of these lesions, Eefting et al. performed a study on interobserver variability.[28] With use of the recently proposed consensus criteria, 94.7% of their cases were diagnosed correctly (sensitivity, 95%; specificity, 95%). Eighteen pathologists from Europe and the United States participated in that study. In our study, all specimens were reviewed again by an experienced pathologist who is familiar with the above criteria.

In our series, recurrence was identified in five patients (5.9% [95% CI, 0.9% to 10.9%]). None of the recurrent tumours had a higher histological grade than the original tumour. However, eleven patients underwent repeat surgery because residual lesion was suspected. Regarding the use of Gd-MRI to predict recurrence of tumour, the positive predictive value was 45% and the negative predictive value was 100%. In this series, postoperative Gd-MRI overestimated the number of recurrent lesions. We started using this new method of treating low-grade central chondrosarcoma in 1994, at which time the technique was deemed controversial in Europe because of concerns about its oncological safety. In light of this, the threshold for surgery in the event of a suspected lesion on postoperative Gd-MRI was low. Throughout the past fifteen years, we have gained greater insight into the benign nature of these residuals or recurrent lesions and we now take a more conservative approach toward repeat surgery for small suspected lesions. Despite the large number of false-positive results in the past, Gd-MRI remains the most sensitive tool for

detection of small residual or recurrent lesions.
The use of phenol as an adjuvant as described in this study has potential advantages for the patients. In contrast to cryosurgery, where up to 14% of patients sustain a postoperative fracture,[18] there was no need for prophylactic implant placement to prevent fractures. Moreover, joints adjacent to the surgical site are not impaired by this procedure.

Source of funding

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6

Chapter 6

MR imaging of atypical cartilaginous tumour/grade I central chondrosarcoma after curettage and phenol application; recommendations for follow-up.

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S.H.M. Verdegaal[a], C.S.P. van Rijswijk[b], H.F. Brouwers[a], P.D.S. Dijkstra[a], M.A.J. van de Sande[a], P.C.W. Hogendoorn[c], A.H.M. Taminiau[a]

[a]Department of Orthopaedic Surgery
[b]Department of Radiology
[c]Department of Pathology
Leiden University Medical Center, Leiden, The Netherlands

Abstract

Postoperative characteristics of uncomplicated recovery after curettage for atypical cartilaginous tumour (ACT)/grade I central chondrosarcoma or the presence/development of residual or recurrence of tumour remain difficult. We reviewed 75 cases of patients, who were treated by intralesional curettage, phenol and donor bone grafting. The first postoperative Gd-enhanced MR imaging was performed within one year after surgery. With a minimum of two scans and a mean follow-up of 72 months (range 13-169 months), patients underwent a second intervention in case of suspected lesions. These were defined as fast enhancement of the lesion or nodules increasing in size in time.

The low threshold to plan a second intervention in case of suspicious lesions on the postoperative MR images can be explained due to the period (from 1994) the patients were treated for the cartilaginous lesions. Either radiofrequency ablation or curettage, phenol and bone grafting were used, depending on the size of the lesion (<10 mm or ≥10 mm). In 14 patients (18.6%) a second intervention was assessed after a mean period of 59 months (range 8-114), of whom six patients had histologically proven recurrence (8%). No upgrading in tumour grade was seen at time of recurrence. Based on the experiences in this study, we could outline a classification of four patients groups, with a different follow-up intensity and treatment. This resulted in a flow diagram for a proper and safe follow-up for this specific patient group.

Introduction

Primary central chondrosarcoma accounts for about 20% of malignant bone tumours, and is the third most common primary sarcoma after myeloma and osteosarcoma.[1] The tumour can develop in any bone that derived from enchondral ossification and most of them are diagnosed by coincidence following radiological survey for other reasons. The pelvis, femur and humerus are the most frequently affected bones, whereas the small bones of the hands and feet are rarely involved (1% of all chondrosarcomas).[1-3]

Patients with atypical cartilaginous tumour (ACT)/grade I central chondrosarcoma of the long bones have been treated for years in the recent past with wide resection and reconstruction. This kind of surgery is associated with proper local control, but

often impairs limb function due to the sacrifice of a significant segment of bone or joint. Nowadays, intralesional curettage in combination with at least one adjuvant (cryosurgery, phenol or polymethylmetacrylate (PMMA) and donor bone grafting (not in case of PMMA) is state of the art in the treatment of ACT/grade I central chondrosarcoma. The bone graft may contain some cartilage from the femoral head surface. This less invasive technique is associated with fewer postoperative complications and a reported local recurrence rate of 7.5%.[4-6] Application of phenol after the curettage was shown to be beneficial in reaching tumour control *in vitro* as well as *in vivo*.[7-8]

The sensitivity for diagnosis for static contrast enhanced MRI has been established and is not controversial in literature anymore.[9] Subsequently, the sensitivity for grading was found to be higher in a setting using dynamic contrast-enhanced imaging which is the recommended method of choice currently and is used in this study.[10-12]

One of the challenges is to differentiate normal postoperative changes from development of local recurrence in the area of the bone graft. However, there is limited literature on postoperative findings in patients after intralesional curettage of ACT/ grade I chondrosarcoma. The published data are hard to interpret due to small patient series and different case mixes (differences in location, tumour grade and the type of adjuvant used). In addition, the optimal frequency and timing of imaging during postoperative follow-up is yet unknown.

The purpose of this retrospective study is to identify MR imaging features of local residual or recurrent disease and normal postoperative changes after intralesional treatment of ACT/ grade I chondrosarcoma, and to design a flow chart for different treatment options in case of suspicious lesions on MR imaging.

Materials and methods

Study population

Between 1994 and 2005, 75 consecutive patients with a histologically proven ACT/ grade I central chondrosarcoma of long bones were treated at the Leiden University Medical Center.[6] Diagnosis was established by histological biopsy (45% one-stage, 55% two-stage). Histological criteria were the ones applied according to the 2013 WHO classifications and all histological diagnosis were reviewed.Diagnostic workup was according to the ESMO guidelines.[13-15] Pre-operatively, a Gd-MRI scan

was performed.
All patients underwent intralesional curettage followed by the use of phenol 85% and ethanol 96% to destroy any tumour cells remaining after curettage. Subsequently, the bone cavity was filled with donor bone chips obtained from donor femoral heads (Dutch Bone Bank Foundation, Leiden, The Netherlands). The study population consisted of 27 male and 48 female patients; mean age at surgery was 47.1 years (range 15-70 years). Lesions were located in the femur 37 (49%), humerus 24 (32%), tibia 6 (8%), fibula 5 (7%), ulna 2 (3%) and radius 1 (1%). All patients underwent conventional radiography (Table I) and at least two postoperative MR scans. Two independent observers retrospectively reviewed all conventional radiographs and MR imaging studies. In case of discrepancy the two observers re-evaluated the case in concert to see if they could reach a consensus view on the case. A second treatment was performed when local recurrence was suspected on MR imaging during follow-up. Local recurrences were treated by radiofrequency ablation (lesion size <10mm) or with repeated intralesional curettage, phenol and bone grafting (lesion size ≥10 mm).

Table 1. Overview of Imaging follow-up protocol

time, post-operative	patient history and physical examination	conventional radiography	MR imaging
before discharge		+	
6 weeks		+	
12 weeks		+	
6-12 months*			+
2 years#	+		+

* Introduced since 2003 as baseline MRI
\# After 2 years, follow-up continues annually or every two years.

Conventional radiography

Conventional radiographs were obtained in two directions. The images were reviewed in order to evaluate the consolidation of the bone window, the incorporation of the bone graft and signs suggestive for local recurrence or residual disease (e.g. increasing focal radiolucent areas or chondroid matrix mineralization).

MR imaging

The MR studies consisted of standard T1- and T2-weighted fat-suppressed MR images, dynamic contrast-enhanced imaging as well as static late contrast-enhanced T1-weighted MR images with fat suppression. All MR images were acquired on a Philips 0.5 (T5-II; Philips Medical Systems) or 1.5T (NT; Philips Medical Systems) MR system using a surface coil.
We assessed the appearance of any enhancement areas in the treated region within the medullary cavity and the consolidation of the bone window. In time, increasing, decreasing of the enhancement and decreasing of postoperative edema, or the occurrence of new lesions suspicious for recurrence of chondrosarcoma were scored.

Results

Patients group

All patients received at least two MR studies during follow-up (range 2-8) with a mean follow-up time of 70 months (range 8-169 months). A second treatment was performed due to radiological suspicion of residual or recurrent tumour in 14 patients. Eight out of 14 patients underwent curettage and five out of 14 patients underwent RF ablation. In one case, ten years after curettage of an ACT of the distal femur, this patient underwent surgery for total knee replacement. During surgery, a biopsy was taken. Histology of the tissue was not conclusive (Table 2, patient 2). Histological examination was performed on small needle biopsy taken during the RF ablation session or on curettage tissue material. Unfortunately, no histological biopsy was performed in two patients during RF ablation (Table 2, patient 4 and 7). Histological examination proved six recurrences and was negative for histological of recurrence in five patients.

Conventional radiography

Complete consolidation of the bone window was observed in all patients. Two patients suffered from a femoral fracture through the bone window, within six weeks after curettage. Gradual incorporation of the bone graft used for filling the medul-

Table 2.

patient	age at first surgery:	group:	location of the ACT:	volume of primary tumour cm^3:	months till second intervention:	type of intervention:	histology:	recurrence after second OR:	disease-free period after second intervention (months):
1	F, 28 yrs	II	femur, diafyse	28	40	curettage	neg	no	190
2	F, 61 yrs	II	femur, distal	11.9	114	TKA	nc*	no	30
3	F, 38 yrs	III	humerus, prox	23.5	79	curettage	pos	no	211
4	M, 51 yrs	III	femur, distal	36.2	90	rfa	nbt**	no	65
5	F, 53 yrs	III	humerus, prox	5.7	8	curettage	neg	no	258
6	F, 16 yrs	III	femur, prox	45.7	25	curettage	pos	no	150
7	F, 41 yrs	III	humerus, prox	5	107	rfa	nbt**	no	45
8	F, 64 yrs	III	humerus, prox	5.7	64	rfa	neg	no	unknown
9	M, 49 yrs	III	humerus, prox	16	32	curettage	neg	no	255
10	F, 39 yrs	III	femur	48	20	rfa	pos	no	182
11	F, 33 yrs	IV	humerus, prox	8.1	75	curettage	pos	yes	***
12	F, 53 yrs	IV	humerus, prox	48.1	68	rfa	neg	no	215
13	F, 53 yrs	IV	humerus, prox	52	34	curettage	pos	yes	****
14	F, 56 yrs	IV	tibia, prox	?	69	curettage	pos	no	116

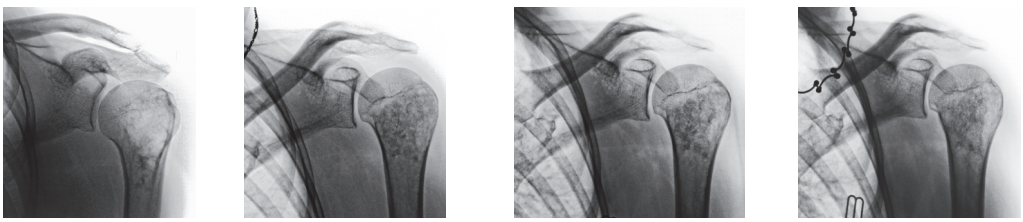
* nc: non conclusive

** nbt: no biopsy taken

*** new small lesion on Gd-MRI 2 years after curettage. New rfa performed 3.5 years later

**** one year and three years after rfa new lesions, resp re-curettage and rfa. Histology suspected for new ACT

lary bone cavity after curettage was seen in time in most patients (Figure 1). However, in some patients small radiolucent defects in the bone graft remained on serial follow-up radiographs, although not increasing in size. In our series, no patients showed any suspicion for local recurrence on conventional radiographs.



A B C D

Fig 1. Postoperative conventional radiographs.

A. Lobulated lesion in the proximal humerus meta-diaphysis of the left arm. The lesion is predominantly lytic with some chondroid mineralisation in the distal part. The cortex seems intact. Histological examination after curettage demonstrated ACT/ grade I central chondrosarcoma.
B. Two months after curettage shows the bone graft. No postoperative complications.
C. Six months after curettage shows early incorporation of the bone graft.
D. 24 months after curettage shows further incorporation of the bone graft.

MR imaging

The recognition of a number of characteristics on the postoperative images leading to false positive predictors of recurrence resulted in the formulation of 4 different imaging patterns (Table 3).
I. A small enhancing rim of granulation tissue surrounding the area of the bone graft. This area becomes smaller in time and the surrounding bone marrow edema diminishes in time. No residual or recurrent enhancing nodules suspicious for ACT/ chondrosarcoma grade I. II. Nodules are seen in or surrounding the bone graft. The granulation zone is not well defined. These nodules are diminishing in size during follow-up MR imaging. III. Nodules are seen in or surrounding the bone graft.
These nodules are stable or increase in size during follow-up MR imaging. IV. A small enhancing rim of granulation tissue surrounding the area of the bone graft is seen on the first post-operative MR images consistant with the normal aspects of the bone graft. Development however of new enhancing nodules in the treated region, which are suspicious for local recurrence during follow-up MR imaging.
Dovetailing these groups resulted into the proposed flow chart for follow-up and

treatment in Figure 6. Soft tissue edema around the bone window was frequently-seen on the first postoperative MR images, which however diminished in time on follow-up MR images (Figure 2). 40 of all patients (54%) demonstrated an imaging pattern consistent with group I (normal postoperative appearances)(Figure 3). 18 Patients (24%) demonstrated an imaging pattern consistent with group II. In this group one patient was treated with a second curettage that showed no signs of recurrence on histological examination. 13 Patients (17%) demonstrated an imaging pattern consistent with group III (Figure 4) and four patients (5%) demonstrated an imaging pattern consistent with group IV (Figure 5).

Table 3. MR imaging patterns	
group I	Normal aspect of the bone graft without suspicion for residual or recurrent tumour on all postoperative MR images
group II	Nodules within the granulation zone on postoperative MR imaging, diminishing in size during follow-up
group III	Nodules within the granulation zone on postoperative MR imaging, stable or increasing in size during follow-up or fast enhancement of the nodules
group IV	Normal aspect of the bone graft on postoperative MR images. Development of a new enhancing lesion suspicious for local recurrence during follow-up
*no signs of new tumour **not conclusive, or no biopsy performed (n=2)	

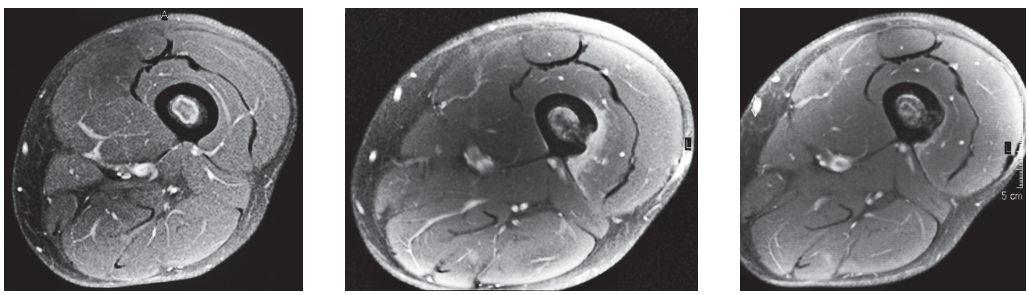


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Figure 2. Axial MR images of early postoperative changes. 31 year old male with an ACT/grade I central chondrosarcoma in the left distal femur. Axial T1-weighted MR images after intravenous contrast administration with fat-suppression through the same position on different time-points.

A. The lesion demonstrates typical peripheral guirlande-like enhancement.
B. The patient has been treated by curettage through a lateral approach. There is marked enhancement surrounding the bone window six months after treatment. The bone window is partial consolidated and demonstrates reactive periosteal and soft tissue edema.
C. Complete consolidation and incorporation of the bone graft 24 months after surgery.

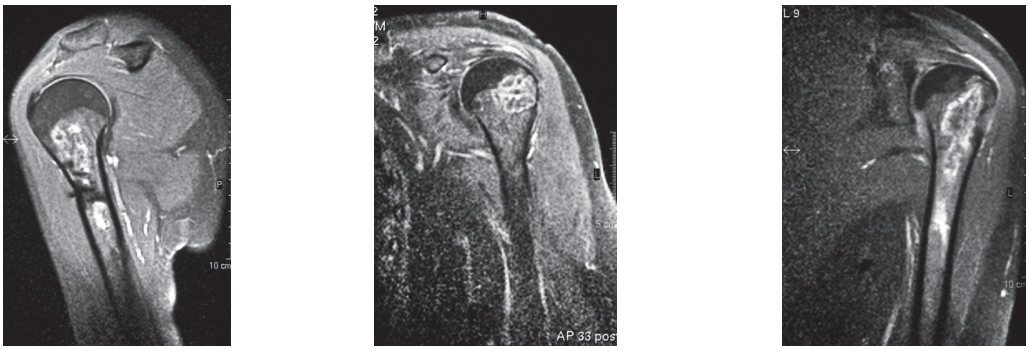

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Figure 3. Illustration of group I: residual nodules, which diminish in time. Female, 46 years, with ACT/grade I central chondrosarcoma of left proximal humerus.

A. Coronal T1-weighted MR imaging after Gadolinium with fat suppression after diagnosis; ring-and-arc enhancement of a cartilage tumour.
B. Six months after curettage shows the bone graft surrounded by an enhancing zone of granulation tissue with some soft tissue edema at the bone window. Still areas of hyper-intensity remain.
C. Incorporation of the bone graft, decreasing in size. No separate cartilaginous nodules suspicious for local recurrence. Areas still enhance using Gd, but decrease in size.

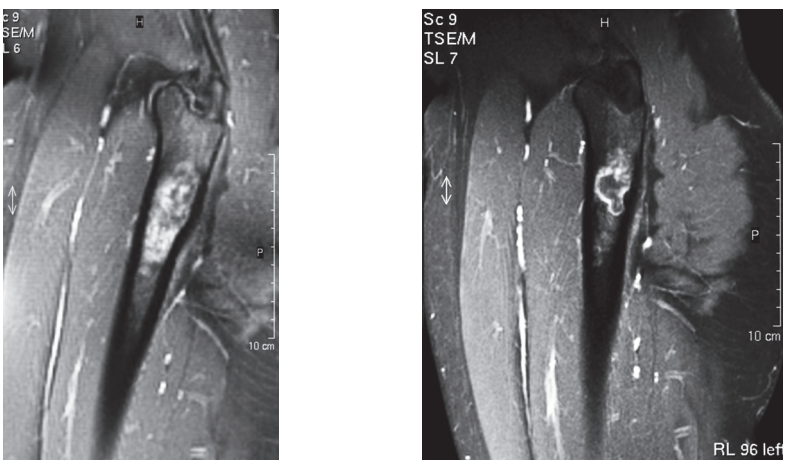

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Figure 4. Illustration of group III. 15 years-old female with ACT/grade I central chondrosarcoma in the proximal femur. Sagittal T1-weighted MR images after intravenous contrast administration with fat-suppression.

A. First post-operative MR examination is performed 7 months after curettage. The marrow cavity in the proximal femur demonstrates marked and inhomogeneous enhancement which could represent extensive granulation tissue, however small residual nodules cannot be excluded.
B. Six month after previous MR examination demonstrates a well-circumscribed lesion with a typical guirlande-like enhancement pattern consistent with a recurrence. The enhancing granulation zone/tissue is markedly reduced. Repeated curettage was performed and histological examination showed ACT/grade I central chondrosarcoma.

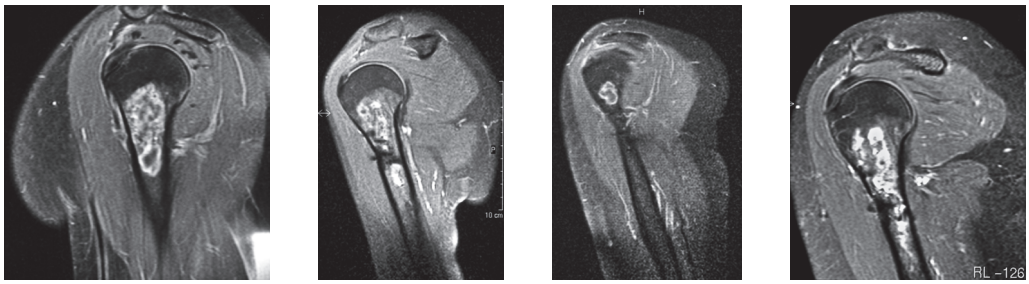

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Figure 5. MR imaging pattern of group IV with a local recurrence 61 months after initial surgery. 39 year old female with ACT/grade I central chondrosarcoma in the right proximal humerus. Oblique T1-weighted MR images after intravenous contrast administration with fat-suppression.

A. Preoperative MR image demonstrates the typical enhancement pattern consistent with a cartilage tumour.
B. Postoperative MR image, 12 months after treatment demonstrates inhomogeneous enhancement of the marrow cavity.
C. Postoperative MR image, 3 years after treatment demonstrates further resorption or incorporation of the bone graft mimicking small cartilage nodules.
D. Postoperative MR image, 5 years after treatment demonstrates a well-circumscribed lesion with a typical guirlande-like enhancement pattern consistent with a local recurrence. Histological examination confirmed ACT/ grade I central chondrosarcoma.

Discussion

Follow-up of patients undergoing intralesional surgery for a low-grade or locally aggressive tumour like ACT/grade I central chondrosarcoma is important. The chance the lesion will develop in grade II or III chondrosarcoma, and the small risk for dedifferentiation of the tumour justifies the follow-up on these patients group. We defined four groups of imaging features and designed a flow-chart for recommendation of postoperative follow- up imaging.

Despite the fact that recently the underlying molecular genetic defects responsible for the genesis of central cartilaginous tumours were untangled, systematic treatment targeting this pathway is still lacking, leaving surgery the only treatment option.[16-18] Intralesional curettage followed by local adjuvant is currently the accepted treatment for ACT/grade I central chondrosarcoma of long bones. However, long-term follow-up data on imaging characteristics and recurrence data are lacking in the current literature. In our retrospective study, we included a large number of patients all treated with intralesional curettage with regular MR imaging follow-up. Since our first patients have been treated in 1994, the threshold for a second treatment has been low because of limited knowledge about recurrence frequency

and clinical behaviour in that time period. With our current knowledge, we would-not have treated the patient in imaging pattern group II (Table 2, patient 1). MR imaging showed an ill-defined granulation zone containing some nodules. These nodules decreased slightly in size on follow-up MR imaging but under the suspicion of local residual disease, patient underwent repeated curettage with no signs of malignancy on histological examination.

Conventional radiographs in the post-operative period have proved to be important to detect early complications linked to the surgery such as fissures, fractures or large residual calcifications.

However, in our study group we didn't detect any of the local recurrences on conventional radiographs.

The number of planned re-interventions in this study is 13 (17%). One patient had a second biopsy during a total knee arthroplasty (Table 2, patient 2). However, only in six patients (8%) the histological confirmation of residual or recurrent ACT/chondrosarcoma grade I could be made (three patients of MR pattern group III and three patients of group IV). Unfortunately, in two patients in the RFA-group no biopsy was taken. No upgrading or dedifferentiation of the cartilaginous tumour was seen at recurrence. In five patients, there was no sign of tumour recurrence or the volume of tissue was too small to make a definite diagnosis. Over the past years, improvements in biopsy needle design, sampling technique and expertise of radiologists have developed in concert with oncological and quality control guidelines which emphasize the need for adequate biopsy prior to percutaneous radiofrequency ablation in the same session.[15]

On postoperative MR imaging we carefully evaluated the treatment area. In all patients, we observed bone graft material demonstrating signal intensities more or less comparable with normal bone marrow surrounded by a zone of variable thickness. This zone showed predominantly high signal intensity on T2-weighted fat suppressed images and demonstrated enhancement on the late static contrast-enhanced MR images consisting of granulation tissue as a fibrovascular reaction of the host to the allograft bone chips in combination with the necrotizing effect of the applied phenol. On the first postoperative MR images (about six months after surgery) it can, therefore, be difficult to recognize small residual nodules or foci of remnant chondroid tumour because they demonstrate similar imaging features as granulation tissue. However, in time, the granulation tissue zone will become smaller and well-demarcated. If the enhancing nodules persist (MR imaging pattern group III) or if new enhancing nodules develop (MR imaging pattern group IV) than local recurrence of residual tumour need to be considered. Depending on the

size, the location and patient-related factors a wait-and-see policy can be considered or a second treatment can be performed (RF ablation or intralesional curettage). Moreover, the bone graft used for filling the defect in the marrow cavity after curettage may contain cartilage chips of the surface of the bone graft e.g. femoral head. This may lead to a false-positive interpretation of residual foci of chondroid tissue, however in time the foci will not increase.

The dynamic contrast-enhanced MR images may be potentially very helpful in differentiating recurrent disease from granulation tissue on follow-up MR imaging. However, in our patients, the diagnosis of recurrent disease was suggested (Group III and IV) based on the morphological criteria as described in Table 3. In our patients, the dynamic contrast-enhanced sequence was of poor quality or not performed in the correct area of interest as MR examinations performed on the 0.5 T MR systems could cover only two slices through the postoperative area.

Based on the recurrences we have seen and the MR imaging patterns we recommend a work up as detailed in Figure 6. We are convinced that curettage combined with adjuvant and bone grafting is a safe and patient friendly procedure for patients with central ACT/chondrosarcoma grade I. When follow-up is performed according to the proposed schedule eventual local recurrences are detected accurately and in a safe time window.

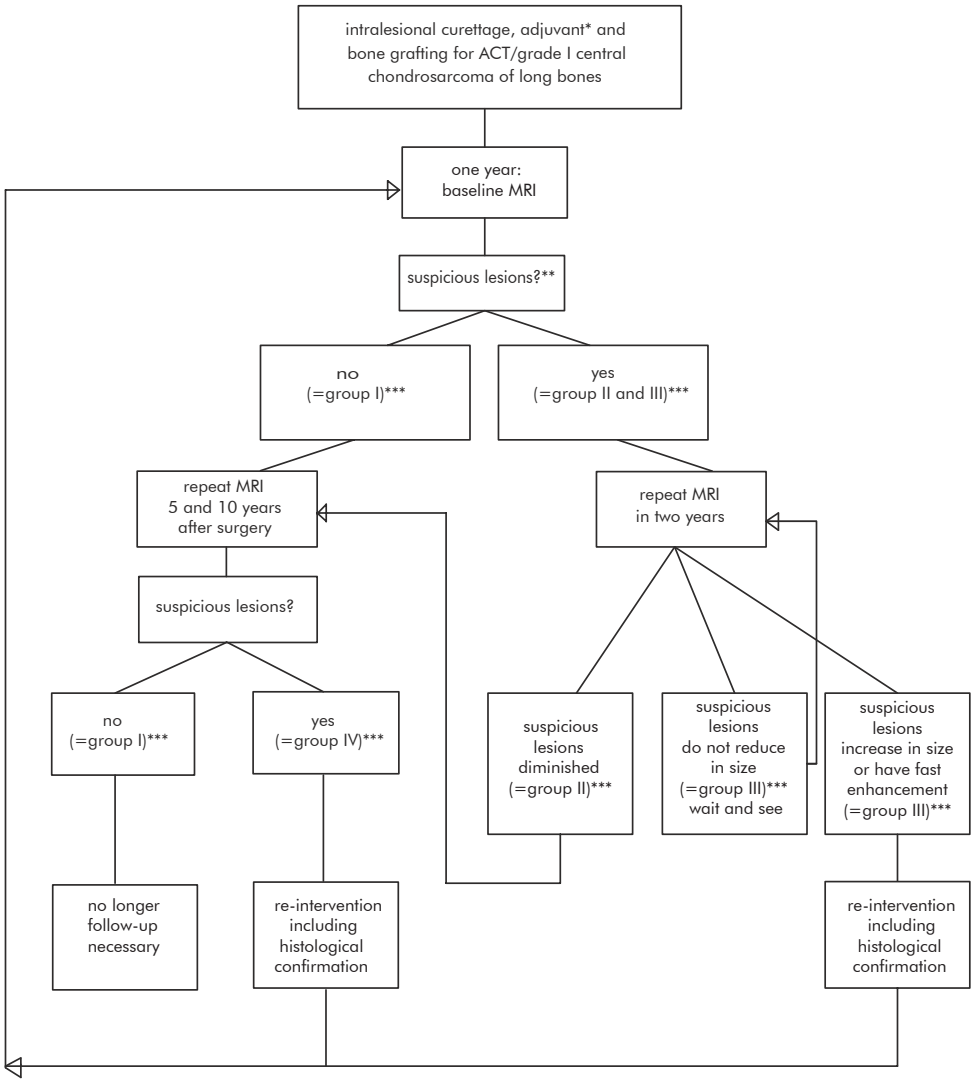

Figure 6: Recommendation for follow-up of patients after intralesional treatment for ACT/grade I central chondrosarcoma of the long bones.

* as adjuvant, Radio Frequent Ablation, phenol or cryotherapy can be used

** enhancement of nodules within the granulation zone

*** group I: Normal aspect of the bone graft without suspicion for residual or recurrent tumour on all post operative MR images

group II: Nodules within the granulation zone on postoperative MR imaging, diminishing in size during follow-up

group III: Nodules within the granulation zone on postoperative MR imaging, stable or increasing in size during follow-up or fast enhancement of the nodules

group IV: Normal aspect of the bone graft on postoperative MR images. Development of a new enhancing lesion suspicious for local recurrence during follow-up

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7

Chapter 7

Summary, discussion and concluding remarks

This thesis presents the clinical characteristics and prognostic factors in patients with low-grade cartilaginous tumours diagnosed in the context of Ollier disease and Maffucci syndrome (**chapter 2**). And beyond, we focus on therapeutic options concerning the cytotoxic (**chapter 3**) and pharmacological (**chapter 4**) effects of phenol, and the clinical use of phenol as adjuvant therapy following intralesional curettage of an atypical cartilaginous tumour (ACT)/ low-grade central chondrosarcoma of long bones (**chapters 5 and 6**).

In **chapter 2**, a European retrospective multicenter study was performed to gain more insight in the presentation and characteristics of enchondromas in patients with Ollier disease and Maffucci syndrome. Furthermore, we tried to estimate the cumulative risk of secondary transformation of enchondroma over a lifetime looking for variables significantly associated with outcome and mortality. Data were collected by members of the European Musculoskeletal Oncology Society (EMSOS), a multidisciplinary society with special interest in bone and soft tissue tumours. 161 Patients were included. The overall incidence for developing chondrosarcoma was 40%, but may increase when considered as a lifelong risk due to age-dependency. Patients with enchondromas located in long bones or axial skeleton, especially the pelvis, have seriously increased risk of developing chondrosarcoma. Based upon this study, they are identified as the population that needs regular screening on early signs of malignant transformation.

Several studies were performed alongside to investigate phenol, an etching acid, that already was used for a long period in the surgical treatment of bone tumours. In **chapter 3**, the cytotoxicity of phenol on different cell-lines was described. Due to the fact that ethanol is standardly used during surgery to rinse phenol after application at the bone, ethanol was also assessed to sort out it's separate potential cytotoxic effect.
Two different cell-lines were used for these *in vitro* experiments. First SiHa, a cervical carcinoma cell line was used as a non-specific control. Secondly, we used OUMS-27, a human chondrosarcoma-derived cartilage producing cell line. End point of the test was cell death, measured by flow cytometry.
A first set of experiments on the SiHa cell line was used to detect the effective range of concentrations of phenol (0-10.63%) and ethanol (0-96%). In the second set, 14 different concentrations of phenol were tested in a range from 0.2% up to 1.5%. In ethanol, the second test was performed with 14 different concentrations from 10% up to 45%. All cells were killed with 1.5% phenol and 42.5% of ethanol. The

experiments were repeated four times. All five results were comparable.
In these experiments, we showed that both phenol and ethanol have the ability to kill chondrosarcoma cells *in vitro*. By using flow cytometry, we did not focus on the necrotising effect, but instead investigated how phenol and ethanol impair tumour-cell viability.

In the past, various studies demonstrated that by using an adjuvant in the intralesional treatment of bone tumours, the results of local treatment were greatly improved. Despite the use of phenol for a long time as adjuvant during surgery, hardly no studies were performed on details of local achieved concentrations of phenol. Subsequently, the number of times you need to rinse the bone cavity with ethanol to dilute phenol to an acceptable concentration. In **chapter 4**, we present two studies. In the first study (test 1, n=16), we collected 10 samples (I to X) of a mixture of phenol/ethanol solution, while rinsing the applicated phenol with ethanol 96%. In the second study (test 2, n=10), we collected 10 samples from the bone cavity with a mixture of phenol/ethanol solutions. Biopsy A was taken after applicating phenol 85% on the inner surface of the cavity wall, biopsy B was taken after rinsing the cavity ten times with ethanol 96%.
The phenol concentration of the ethanolic flush solution was determined by High Performance Liquid Chromatography (HPLC) with spectophotometric detection. The studies showed that rinsing the cavity five times already gives a phenol concentration <280 ppm, which is concidered to be a safe dose. The volume of the cavity had no correlation with the measured concentrations of phenol.
This study shows that the potential adverse effects of phenol in the treatment of patients can be reduced by using ethanol to wash out phenol to safe concentrations.

In **chapter 5**, a retrospective analysis was presented of 85 patients who had been treated in a specialized musculoskeletal oncology department for an ACT/grade I central chondrosarcoma of a long bone.
The indication for surgery was the likely presence of an ACT/grade I central chondrosarcoma on the Gd-MRI scans, located in one of the long bones. Surgery was performed by intralesional curettage, application of phenol, rinsing with ethanol, and bone-grafting. Patients were scheduled for a dynamic Gd-MRI scan six to twelve months after the procedure and than biannually.
The average duration of follow-up for the patients was 6.8 years (range, 0.2 to 14.1 years). Five patients had a follow-up of less than two years. Eleven patients underwent repeat intervention due to a suspicious lesion on the Gd-MRI studies during

follow-up. Depending on the size of the lesion, treatment by radiofrequency ablation or re-curettage was performed. Of these eleven, five had a histologically proven local recurrence (5.9% [95% CI 0.9-10.9%]).
The use of phenol as an adjuvant as described in this study has potential advantages for the patients. In contrast to other adjuvants, adjacent joints are not impaired by this procedure. Due to the fact that we did not need to plate long bones to prevent fractures, Gd-MRI scans could be used in the follow-up of these patients group.

Finally, in **chapter 6**, a study was presented concerning postoperative features on Gd-MRI after curettage and application of phenol in ACT/grade I central chondrosarcoma in long bones. Intralesional curettage with the use of a local adjuvant is state of the art in the therapy for ACT/grade I central chondrosarcoma nowadays. The role of preoperative conventional radiographs and magnetic resonance (MR) imaging is well established. However, there is still limited literature on the postoperative findings on MR imaging. There are only small series available, containing a large variety of diagnosis, surgical approach and use of different adjuvant therapies. Purpose of this retrospective study was to identify features on MR imaging of local residual or recurrent disease, besides normal postoperative changes after surgery.
Between 1994 and 2005, we included 75 patients with histologically proven ACT/grade I central chondrosarcoma of long bones. Preoperative plain radiographs in two directions and a Gadolinium-MR imaging were performed. Postoperative plain radiographs were performed before discharge, at 6 weeks and at one year. Gd-MR imaging was performed 6-12 months after surgery, and subsequently biannually.
Concerning plain radiographs, complete consolidation of the bone window was observed in all patients. Two patients suffered from a femoral fracture within 6 weeks of surgery. In our series, no patients showed any abnormalities, suspected for local recurrence.
On the basis of the results of the postoperative Gd-MR images of 75 patients, we could identify four groups, describing the increasing risk for recurrence of the tumour. Group 1 showed normal postoperative changes without any suspicion for residual or recurrent tumour on all postoperative MR images (54%). Group 2 showed nodules within the granulation zone, diminishing in size during follow-up (24%). Group 3 showed nodules, which size stayed stable or increased in time (17%). Group 4 first showed normal aspect of the granulation zone, but in time MR imaging showed the development of a new Gd-enhancing lesion suspicious for local recurrence during follow-up (5%).
A second operation was performed in 14 patients due to radiological suspicion of

residual or recurrent tumour on Gd-MR imaging. Depending on the size of the lesion, radiofrequency ablation (<10 mm) or curettage (>=10mm) was performed. Histological examination proved eight local recurrences.
Due to new insights during this study in combination with the recent experiences in the treatment of this patients group, this resulted in a flow-chart for the follow-up of patients suffering from ACT/grade I central chondrosarcoma of long bones (see figure 6, page 101).

General discussion

Ollier disease and Maffucci syndrome are rare disorders, with great impact on a patient's life. Besides the discomfort due to limb lenght discrepancies and deformities of hands and/or feet, the uncertainty of the fact that each of the dozens of enchondroma can one day transform to a chondrosarcoma, is hard to live with, not only for the (young) patient, but also for their parents and relatives.
Therefore, we performed a study to get more insight in the clinical behaviour of enchondromas in these two disorders.
Disadvantages are that the study is retrospective in design, and the patients are not followed in a cohort until death. Also, there is a selection bias due to the fact that our data were mainly collected from referral centers for musculoskeletal oncology. This may have lead to overestimation of the percentage of developing chondrosarcoma grade I-III.
Nevertheless, the study we performed and presented in chapter two contributes to the understanding of the clinical behaviour of enchondroma in patients suffering from Ollier disease or Maffucci syndrome.
To estimate the cumulative probability of secondary transformation over a lifetime, it is important to distinguish the distrubution patters of enchondroma. When enchondroma are found in the axial skeleton or long bones, (group II and III), the risk for developing chondrosarcoma is 44-50%. The odds ratio associated with secondary chondrosarcoma of the pelvis was 3.8 (95% CI 1.8-8.0, p=.001).
On plain radiographs and MR images enchondromas in Ollier disease and Maffucci syndrome usually show a more agressive picture than in solitary ACT/grade I chondrosarcoma. In general they still might be benign and therefore markers as growth and progression in time should be investigated before a biopsy is planned, to prevent misleading outcomes of the biopsy.
Due to the fact that both Ollier disease and Maffucci syndrome are associated with

a variety of other malignant tumours in the brain and abdomen, a cerebral or abdominal CT-scan should be performed with minimal neurological complaints or abdominal symptoms.
Throughout this thesis, one should read the different studies concerning phenol being aware of new insights in time, in which also the general accepted terminology changed (WHO 2012).
The treatment of ACT/grade I central chondrosarcoma has remarkably changed in the last three decades. Where till 1990 the common therapy was wide resection, therapy changed towards intralesional curettage followed by adjuvant therapy, or even watchfull waiting. The small chance of dedifferentiation of the lesions remains a concern.
This change in the approach towards the extend of surgical resections were made, was mainly based on two developments. The first was a better understanding of the behaviour of low-grade chondrosarcoma, regarding to the work done studying molecular genetics and histopathology. Clinical studies proved that no patients died as a result of ACT/grade I central chondrosarcoma of long bones, treated by intralesional curettage and the use of an adjuvant. The second was the upcoming role of Gd-MRI. Thanks to the use of Gd-MRI in different benign and malignant bone tumours, the positive predictive value for identifying malignant transformation increased in time.
This thesis contributed to the scientific foundation of the clinical use of phenol. Studies in the past always had discussions about the cell killing potentials of phenol, where endpoint for effectiveness of adjuvant was the measurable depht of necrosis. Due to the fact that phenol is very corrosive, the cells vanish. By using flow cytometry, cell pyknosis was proven in very low concentrations of phenol. Also, the separate and own effect of ethanol was proven in this thesis.
The clinical study in chapter 5 should also be judged in the timeframe. When we started to include the patients in 1994, many countries in Europe still treated this patients group with wide resections and reconstructions. This is why, already in case of little suspicion on residual or recurrent chondrosarcoma, we performed a new procedure.
Nowadays, in the Leiden University Medical Center, curettage, phenol cauterization and bone grafting is indicated in case the cartilage lesion is > five centimeter, in case of endosteal scalloping, enhancement of the lesion within 10 seconds on Gd-MR imaging or age of onset is > 50 years.
The patient is involved in the decision whether to plan an intervention or to wait and see, in the knowledge that the patient stays in the follow-up with periodically

planned MRI scans.
In case the MRI shows edema around the lesion, periostal edema or periostal reaction, a biopsy will be planned, to be sure no grade II chondrosarcoma is developing.
The developments in the wider use of radiofrequency ablation (RFA) are promising. Dierselhuis and Jutte recently published a small series of patients with enchondroma and ACT's < 3 cm's who were first treated with radiofrequency ablation, and had curettage afterwards to judge the percentage of necrosis of the lesion.
In the near future, studies should be performed on uni-probe and/or multi-probe radiofrequency ablation in one to three sessions in daycare, followed by MR imaging, not only in small lesions, but also in ACT/grade I chondrosarcoma <5 cm.
The future is to develop a prediction model, in which an individual risk model can be designed for each patient according to the growth or progression of a lesion suspected for enchondroma/ACT. In the next decades, this model will give more insight in the natural behaviour of these premalignant lesions, and lead to evidence based on shared decision making. It will create the optimal balance between safety, uncertainty and surgical risks you will or will not expose the patient to.

8

Chapter 8

Nederlandse samenvatting

Dit proefschrift beschrijft in **hoofdstuk 2** de klinische kenmerken en prognostische factoren van laaggradige chondrosarcomen bij patiënten met de ziekte van Ollier en het syndroom van Maffucci. De overige hoofdstukken in dit proefschrift bevatten studies over de cytotoxische (**hoofdstuk 3**) en pharmacologische eigenschappen (**hoofdstuk 4**) van phenol, en de klinische toepassing hiervan als adjuvante behandeling na curettage van aspecifieke cartilagineuze tumoren (ACT's)/laaggradige centrale chondrosarcomen van de lange pijpbeenderen (**hoofdstukken 5 en 6**).
In **hoofdstuk 2** werd een Europese retrospectieve multicenter studie uitgevoerd om meer inzicht te krijgen in de karakteristieken en het gedrag van enchondromen bij patiënten met de ziekte van Olllier en het Maffucci syndroom. Het cumulatieve risico op secundaire transformatie naar een chondrosarcoom werd berekend en variabelen die invloed hebben op de mortaliteit werden geïdentificeerd.
De gegevens werden verzameld door leden van de Europese Musculoskeletale Oncology Society (EMSOS), een multidisciplinaire groep van medisch specialisten met speciale belangstelling voor bot- en weke delen tumoren.
In deze studie was de totale incidentie voor het ontwikkelen van een secundair chondrosarcoom 40%, maar dit percentage kan nog toenemen als dit wordt afgezet tegen het levenslange risico.
Concluderend hebben patiënten met enchondromen in de lange beenderen of het axiale skelet, en in het bijzonder het bekken, een sterk verhoogd risico op het ontwikkelen van een secundair chondrosarcoom. Geadviseerd wordt om deze groep regelmatig te screenen op vroege tekenen van maligne transformatie.
Vervolgens werd een aantal studies gedaan naar phenol, een etsend zuur, dat al langere tijd werd gebruikt bij de operatieve behandeling van bottumoren. In **hoofdstuk 3** werd gekeken naar de cytotoxiciteit van phenol op verschillende cellijnen.
Vanwege het feit dat ethanol standaard gebruikt wordt om phenol na applicatie in de botholte weg te spoelen (verdunnen) tijdens chirurgie, zijn aparte series van ethanol getest om het eigen celdodend effect aan te tonen dan wel uit te sluiten.
In de *in vitro* experimenten maakten we gebruik van twee soorten cellijnen. Als eerste SiHa, een cervixcarcinoom-cellijn. Als tweede OUMS-27, een humane kraakbeenproducerende chondrosarcoom-cellijn. Eindpunt van de test was celdood en werd gemeten middels flowcytometrie.
Een eerste reeks experimenten op de SiHa cellijn werd gebruikt om het effectieve bereik van de concentratie van phenol (0-10.63%) en ethanol (0-96%) te onderzoeken. In de tweede set werden 14 verschillende concentraties van phenol getest oplopend van 0.2% tot 1.5%. Met ethanol werd de tweede proef uitgevoerd met 14 verschillende concentraties oplopend van 10% tot 45%.

Alle cellen werden gedood bij concentraties van 1.5% phenol en 42.5% ethanol. De experimenten werden vier keer herhaald. Alle vijf resultaten waren vergelijkbaar. In deze reeks experimenten toonden we aan dat zowel phenol als ethanol de potentie hebben om *in vitro* chondrosarcoom-cellen te doden. Door het gebruik van flowcytometrie hebben we gekeken naar de apoptose en hiermee het effect van phenol en ethanol op de overleving van cellen.
In het verleden hebben verschillende studies aangetoond dat door toepassing van een adjuvans in de intralesionale behandeling van bottumoren, de resultaten van lokale behandeling sterk verbeterd zijn. Ondanks het feit dat phenol al lange tijd gebruikt wordt als adjuvans tijdens de operatie, waren er bijna geen studies bekend over de lokale concentratie van phenol die in de curettageholte bereikt wordt. Tevens was onbekend hoe frequent de holte vervolgens met ethanol gespoeld moet worden om de phenol te verdunnen tot een veilige rest-concentratie. In **hoofdstuk 4** werden twee fundamentele studies opgezet. In de eerste studie (test 1, n=16) werd de holte, nadat phenol was aangebracht en 2-4 minuten had ingewerkt, tien keer gespoeld met ethanol. Van elke spoeling werd een monster verzameld (I tot X). De concentratie phenol in de spoeloplossing werd bepaald door middel van hogedrukvloeistofchromatografie (HPLC) met spectofotometrie.
In test 2 werd een eerste biopt (A) genomen na aanbrengen van phenol 85% op het binnenoppervlak van de botholte. Een tweede biopt (B) werd genomen nadat de holte tien keer gespoeld was met ethanol 96%.
Het weergeven van de resultaten als de fractie van de concentratie phenol, gemeten in het eerste monster, toonde dat na vijf keer spoelen van de holte de phenolconcentratie onder de 280 ppm was, hetgeen beschouwd wordt als veilige concentratie. Het volume van de holte had geen correlatie met de gemeten concentraties van phenol. Deze studie toonde aan dat de mogelijke schadelijke effecten van phenol afnemen tot een veilige concentratie, door gebruik van ethanol om phenol uit te wassen.
Vervolgens werd in **hoofdstuk 5** in een retrospectieve studie een analyse gedaan naar 85 patiënten die waren behandeld in een gespecialiseerd oncologisch orthopaedisch centrum, in verband met een atypische cartilagineuze tumor ACT/centraal chondrosarcoom graad I van een van de pijpbeenderen.
De indicatie voor operatie was de verdenking op een ACT/graad I centraal chondrosarcoom gelegen in een van de lange pijpbeenderen op een Gd-MRI-scan. De operatie bestond uit intralesionale curettage, applicatie van phenol, uitwassen met ethanol, en opvullen van de holte met botchips. Patiënten werden gepland voor een dynamische Gd-MRI-scan zes tot twaalf maanden na de procedure en daarna tweejaarlijks.

De gemiddelde duur van de follow-up van de patiënten was 6,8 jaar (range, 0,2-14,1 jaar). Vijf patiënten hadden een follow-up van minder dan twee jaar. Elf patiënten kregen tijdens de follow-up een tweede interventie, omdat een verdachte laesie werd gezien op de Gd-MRI studies tijdens de follow-up. Afhankelijk van de grootte van de laesie werd radio frequente ablatie of re-curettage toegepast. Van deze elf patiënten hadden vijf een histologisch bewezen lokaal recidief (5,9% [95% CI 0,9-10,9%]).
Concluderend biedt gebruik van phenol als adjuvans zoals in dit onderzoek potentiële voordelen voor de patiënt. In tegenstelling tot andere adjuvante middelen, werden aangrenzende gewrichten niet aangetast door deze procedure. Vanwege het feit dat preventief geen platen hoeven te worden aangebracht om fracturen te voorkomen kan de Gd-MRI scan worden gebruikt bij de follow-up van deze groep patiënten.
Tot slot werd in **hoofdstuk 6** een studie gepresenteerd naar de postoperatieve kenmerken op rontgenfoto's en MRI-scans van ACT/centraal chondrosarcoom graad I in de lange pijpbeenderen. Tegenwoordig is intralesionale curettage met gebruik van een adjuvans de gouden standaard in de behandeling van atypische kraakbeentumoren/centraal chondrosarcoom graad I. De rol van pre-operatieve conventionele röntgenfoto's en gebruik van Gadolinium-MRI scan (Gd-MRI) is onomstreden. Er is echter nog beperkte literatuur over de postoperatieve bevindingen. Tot nu toe zijn het met name kleine series die een grote verscheidenheid aan diagnostiek, chirurgische benadering en het gebruik van het type adjuvante therapie beschrijven.
Het doel van deze retrospectieve studie was om op de MRI-kenmerken te definieren die tot de normale postoperatieve bevindingen horen, dan wel mogelijk een voorspelling kunnen geven over het al dan niet detecteren van een lokaal residu of recidief.
Tussen 1994 en 2005 werden 75 patiënten geïncludeerd met een histologisch bewezen atypisch cartilagineuze tumor ACT/centraal chondrosarcoom graad I van de lange pijpbeenderen. Preoperatieve rontgenfoto's werden uitgevoerd in twee richtingen, en een Gadolinium-MRI scan (Gd-MRI).
Postoperatieve rontgenfoto's werden gemaakt voor ontslag, na zes weken en een jaar na operatie. Een eerste Gd-MRI werd 6-12 maanden na operatie gemaakt, daarna twee-jaarlijks.
Met betrekking tot de röntgenfoto's werd volledige consolidatie van het botluik gezien in alle patiënten. In onze serie is op de röntgenfoto nooit een verdenking op lokaal residu danwel recidief geweest.
Aan de hand van de postoperatieve Gd-MRI beelden van 75 patiënten konden vier

verschillende groepen worden onderscheiden, op basis van het toenemende risico op residu/recidief tumor. Groep 1 toonde normale postoperatieve veranderingen zonder enige verdenking op residu/recidief op alle postoperatieve MRI beelden (54%). Groep 2 toonde nodules in de granulatie zone, welke afnamen in grootte in de tijd (24%). Groep 3 toonde nodules, welke qua grootte gelijk bleven of toenamen in de tijd (17%). Groep 4 toonde aanvankelijk een normaal aspect van de granulatie zone, maar in de tijd verscheen een nieuwe laesie, verdacht voor lokaal recidief (5%).
Een tweede operatie werd uitgevoerd bij 14 patiënten, volgend op radiologische verdenking van residu/recidief op Gd-MRI. Afhankelijk van de grootte van de laesie, werd radio frequente ablatie (<10 mm) of opnieuw curettage (> = 10 mm) verricht. Histologisch onderzoek toonde bij 8 patiënten opnieuw ACT/chondrosarcoom graad I. Met de opgedane kennis in dit onderzoek in combinatie met ervaringen in de kliniek is een stroomdiagram ontworpen voor de follow-up van deze patiëntengroep.

9

Chapter 9

List of publications

Publications

MR imaging of atypical cartilaginous tumour/grade I central chondrosarc after curettage and phenol application; recommendations for follow-up.
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Chapter 10

Curriculum Vitae

Curriculum Vitae

Suzan Verdegaal was born on December 20, 1971 in Leiden, The Netherlands. In 1990 she graduated from the VWO-B program at the Agnes College in Leiden and started her medical study at the University of Leiden. In 1994, she followed a course in physical antropology (Prof. dr. G.J.R. Maat). In 1996 she followed a traineeship in orthopaedic surgery at the St. Michael's Hospital in Toronto, Canada (Head: Dr. J.P Waddell) and registered as a Medical Doctor in 1998.
In July 1999, her surgical training started at the Department of General Surgery of the Bronovo Hospital in The Hague (Head: Dr. A.B.B. van Rijn). The training for orthopaedic surgery started in 2001 in the Leiden University Medical Center (Head: Prof. dr. P.M. Rozing). Here she started the first studies for this thesis (Promotores: Prof. dr. A.H.M. Taminiau and Prof. dr. P.C.W. Hogendoorn). Part of the orthopaedic training was performed at the Department of Orthopaedic Surgery of the Rode Kruis Hospital in The Hague (Head: Dr. C.F.A. Bos) and the Rijnland Hospital in Leiderdorp (Head: Dr. E.J. van Langelaan). During her residency she was president of the Dutch Orthopaedic Resident Society (DORS or VOCA), and member of the Concilium Orthopaedicum. In 2004, with Denise Eygendaal and Annechien Beumer, she founded the Dutch Orthopaedic Ladies Society.
Since October 2005 she is working as a consultant orthopaedic surgeon at the Department of Orthopaedic Surgery of the Alrijne Hospital (former Rijnland Hospital) in Leiderdorp, with special interest in hip surgery and hip revisions. She works in assocation with Cornelis Visser, Sebastiaan Jansen, Saskia Wiersma, Rachid Mahdad, Maarten Kroon, Joris Jansen, Eline Zwitser, Wilbert van Laar, Bernard Mullers and Hans Schüller.
She lives in Koudekerk aan de Rijn with her six-year-old son Lucas.