Individual

Clinical

Advanced decision-making and

Risk

Evaluation for Ewing sarcoma

Sarah Bosma

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Do no harm

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Summary and general discussion

CHAPTER 1

General introduction and outline of this thesis

A 12-year-old healthy boy presents at the emergency department with a non-traumatic 6-week history of pain in the right thigh and a swelling of the right thigh for the last 3 days. The pain is worse at night and during and after exercise. There is no pain or swelling in other locations and there are no complaints of night sweats, fever or weight loss. Physical examination of the right thigh shows a swelling of hard consistency and a diffuse edge over a length of about 20 cm and a width of about 10 cm. There is no redness or warmth of the skin. There is suspicion of a bone or soft-tissue sarcoma and given the patients' age a Ewing sarcoma is among the possibilities.

Background

Ewing sarcoma (ES), firstly discovered in 1921 by dr. James Ewing (1), is a highly aggressive primary sarcoma of the bone and soft-tissue with an undifferentiated small round cell phenotype. (2) ES is a rare disease with an incidence of 0.1/100.000 in Europe. (3) It mainly affects the paediatric and adolescent population, with a peak incidence in the second decade of life, and slight male dominance. (4)

Aetiology

Ewing sarcoma is characterized by the presence of a chromosomal translocation between the Ewing's sarcoma breakpoint region 1 gene (EWSR1) and various genes encoding for ES specific transcription factors. Approximately 85% of the patients present with a t(11;22)(q24;q12) translocation that leads to a fusion between EWSR1 on chromosome 22 and the Fried leukemia virus integration site 1 gene (FLI1) on chromosome 11. This results in an EWS-FLI1 fusion gene encoding a chimeric transcription factor (EWS-FLI1) that plays part in development and behavior of cells. The remaining 10-15% are characterized by alternate translocations resulting in the EWSR1 gene being fused with other transcription factors including ERG, ETV1, ETV4 or FEB or rarely by EWSR1 being replaced by another member of the TET family of transcription factors, FUS. (2, 5-7) The products resulting from these fusions all lead to the production of an oncogenic transcription factor that play part in development and behavior of cells.

Histogenesis and histology

The histogenetic origin of Ewing sarcoma has been debated over the years and remains controversial. The lack of genetic subtypes (approximately 85% harbor a t(11;22) rearrangement) suggest that ES is derived from a single cellular lineage. Both the neural crest stem cells (NCSC) and mesenchymal stem cells (MSC) have been proposed as origin. ES can express neural antigens, like gastrin-releasing peptide (a protein normally expressed by the brain and neuroendocrine cells) on its surface, can synthesize choline acetyltransferase and some tumors contain Homer-

Wright rosettes. The expression of immunohistochemical markers and the ultrastructural features in combination with the ability to differentiate along neural pathways in vitro suggest a neuroectodermal origin, with the neural crest as most likely progenitor. (8, 9) Other studies show that the expression of the ES fusion protein EWS-FLI1 blocks MSC differentiation and knockdown of EWS-FLI1 drives the ES transcriptome towards that of MSCs, suggesting a mesenchymal origin of Ewing sarcoma. (10, 11) An epithelial origin has also been suggested, since cell-cell adhesion molecules such as claudin 1 and tight junction protein ZO1 are expressed on ES cells. (12)

Ewing sarcoma is a small, round cell sarcoma. The cells can exhibit a variable degree of neural differentiation, although often subtle and only detected by immunohistochemical staining. ES is periodic acid-Schiff (PAS) positive (figure 1A) and a high nuclear to cytoplasmic ratio is generally present. The tumor cells frequently undergo necrosis and mitotic activity is usually low. No routinely used histochemical or immunohistochemical stain can positively distinguish ES from other undifferentiated small round cell tumors of childhood, but almost all ES cells express CD99 or MIC2 (figure 1B). CD99 is a cell surface glycoprotein (designated CD99, MIC2 surface antigen or p30/32MIC2), which is encoded by the CD99 (MIC2X) gene. It is a sensitive marker for ES but lacks specificity since it can also be positive in other tumors (lymphoblastic lymphoma, rhabdomyosarcoma, synovial sarcoma, mesenchymal chondrosarcoma, blastemal component of Wilms tumor) and normal tissues are also immunoreactive with anti-MIC2 antibodies. The nucleus of the tumor cells contains FLI-1, antibodies against FLI-1 are specific for ES. Based on the degree of neural differentiation, the tumor cells can also express neuron-specific enolase (NSE), synaptophysin, and S-100 protein. (13-16) For definitive diagnosis cytogenetic, by fluorescence in situ hybridization (FISH), or molecular genetic studies, by reverse transcription polymerase chain reaction (RT-PCR), looking for particular chromosomal translocations and/or their fusion transcripts are required.

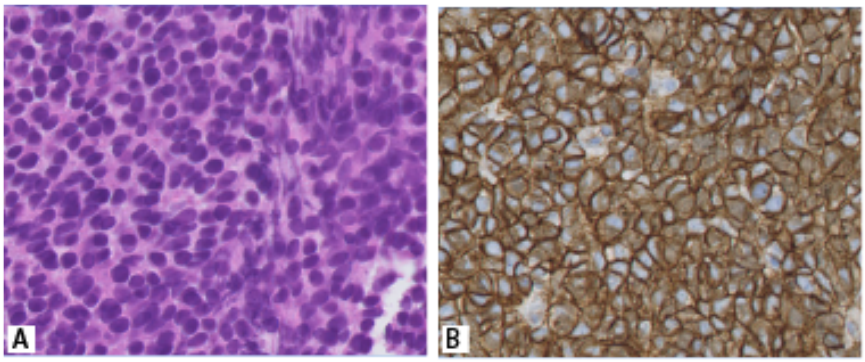

Figure 1 – Microscopic images of Ewing sarcoma
A) Small uniform cells with scanty cytoplasm and round hyperchromatic nuclei (HE x 400). B) Characteristic CD99 immunoreactivity of the cell membranes (HE x 400).

Clinical presentation

Patients with Ewing sarcoma usually present with locoregional pain, predominantly at night, for weeks to months. At the start the pain is often mistaken for growing pain or sport injuries (such as tendinitis and muscle pain). Other symptoms, like swelling and functional impairment vary, depending on the duration of the symptoms and tumor site. Functional impairment occurs if the tumor is located in or close to the joint, pleural involvement is possible when the tumor is located in the rib and muscle weakness or neurological pain can arise if the tumor is located in the spine. In case of pain without clear cause and symptoms lasting for more than one month further investigation is advised.

About 10 to 20% of patients with Ewing sarcoma have systemic symptoms like fever, weight loss, fatigue and anemia. Fever is usually caused by cytokines of tumor cells and it is a sigh of advanced disease. At the time of diagnosis 20-25% of the patients are diagnosed with metastatic disease. Metastasis occurs to the lungs (40%), to the bone/bone marrow (40%), a combination of bone with lung or other sites (brain, liver, lymph nodes) (10%). (17, 18)

Ewing sarcoma most often arises from the long bones of the extremity (predominantly the femur, but also tibia, fibula and humerus) and the pelvic areas. The spine, hands and feet can also become affected, but this happens considerably less often. EICESS trial (19) showed that about 50% of the ES tumors arise in the axial skeletal of which halve in the pelvic and 50% arise in the extremities, see also figure 2. This distribution varies with age, older patients (20-24 years old) tend to have more pelvic and axial tumors than children (0-9 years old). (20) A small proportion of ES occurs in the soft-tissue only, also known as extra-skeletal ES. This happens more frequently in older female patients at the extremity.

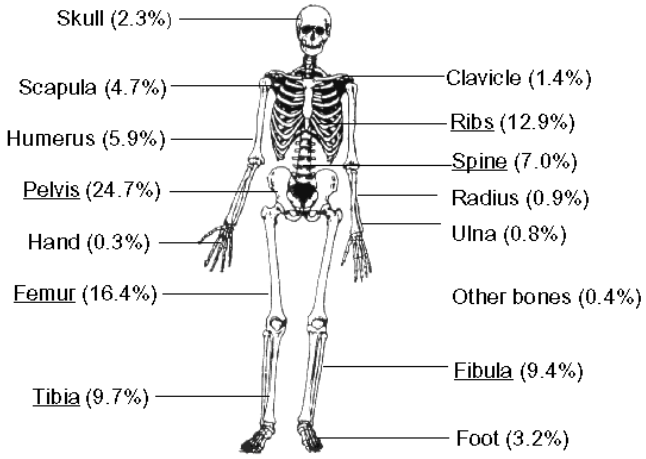

Figure 2 – Distribution of primary tumor sites in Ewing sarcoma (19)

Imaging, diagnosis and staging

Diagnostic work-up starts with the medical history, with a focus on characteristic symptoms such as duration, intensity and timing of pain. Physical examination consists of inspection and palpation of the tumor and organ function test to assess eligibility for systemic treatment. Laboratory test should include complete blood count, blood serum chemistry (lactate dehydrogenase (LHD) and alkaline phosphate (AP)), erythrocyte sedimentation rate (ESR) and coagulation test. (21)

A conventional radiograph in two planes is generally performed as first line imaging showing an aggressive periosteal reaction in the diaphysis or metaphysis of the bone. This periosteal reaction can present as a uniformly dense, single thin layer of new bone about 1-2 mm from the cortical surface (single layer periosteal reaction), but more often a multilayered or onion skin periosteal reaction with multiple concentric parallel layers of new bone adjacent to the cortex is seen. In aggressive bone lesions such as Ewing sarcoma the periosteum does not always have time to ossify during new bone formation (either in single layer or multilayer periosteal reaction) and only the edge of the raised periosteum is ossified. This phenomenon is called the Codman triangle. A hair-on-end periosteal reaction is also seen in Ewing sarcoma. This represents spicules of new bone formation along vascular channels and the fibrous bands that anchor tendons to bone and signifies a rapid underlying process that prevents formation of new bone under the raised periosteum. Additionally, a moth-eaten or permeative type of bone destruction is often observed. (21) Figure 3 shows some of the typical features seen on a radiograph of an Ewing sarcoma. If there is a suspicion of a malignant lesion based on conventional radiographs a magnetic resonance imaging (MRI) of the whole bone or compartment is advised to allow for more visualization of the extent and the periosteal reaction. (23) All patients with suspicion of a primary malignant bone tumor based on radiological assessment should be referred to a specialized bone sarcoma center for local staging followed by biopsy (if indicated) and the results should be discussed in a multidisciplinary setting. (22) A core-needle biopsy is carried out under imaging control and supervision of the oncologic surgeon, since the biopsy tract is considered contaminated and should be removed together with resection specimen. (21) The biopsy sample is subjected to cytogenetic (FISH) or molecular genetic studies (RT-PCR) looking for particular chromosomal translocations and/or their fusion transcripts to confirm diagnosis of ES. A bone marrow biopsy from the posterior iliac crest may be considered in the staging, but several studies underline that 18F-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET)/CT is a valuable method for metastatic bone marrow assessment. (23, 24) To evaluate the presence of metastasis and/or the response to treatment additional CT of the lungs to detect small lesions and whole body imaging is required. Whole-body MRI and FDG-PET/CT are increasingly used to replace bone scintigraphy, because of higher sensitivity. (23, 25-29) Finally, evaluation of renal, cardiac and auditory function is needed before the start of treatment, since chemotherapy can result in organ

dysfunction. For male patients in the reproductive age sperm storage is recommended and ovarian tissue sampling or cryopreservation for female patients. (21)

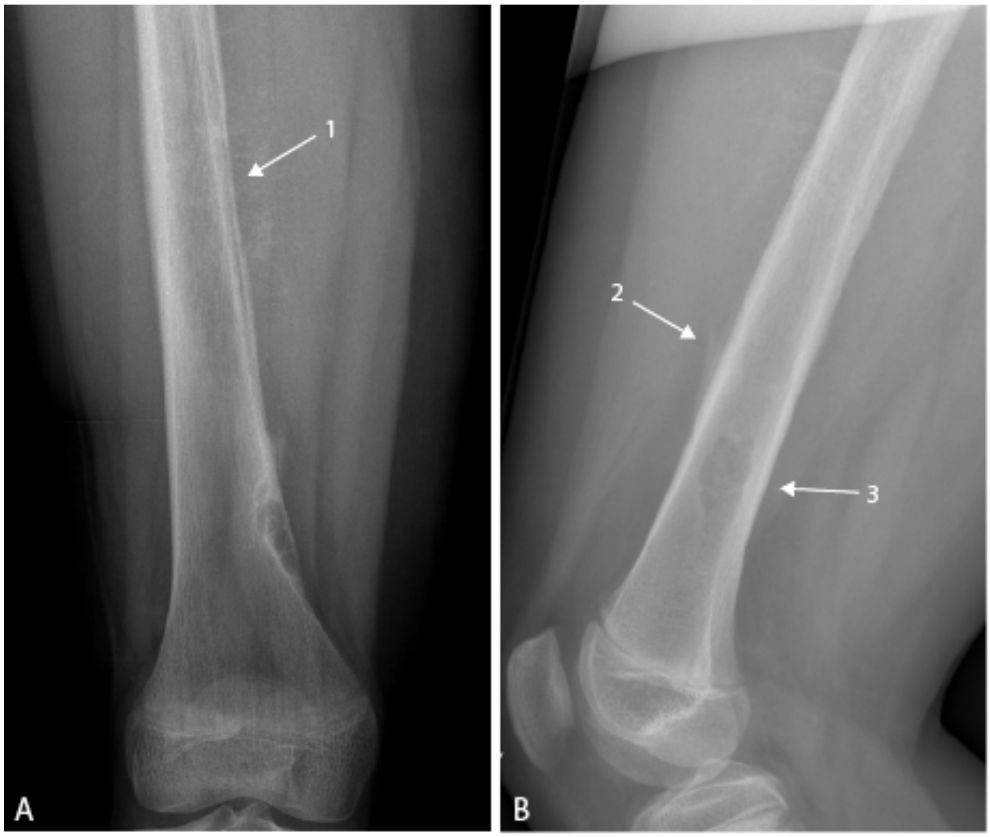

Figure 3 – Radiograph of Ewing sarcoma
A) Anterior-posterior image of the right femur showing widespread cortex destruction with a hair-on-end periosteal reaction (arrow 1). B) Lateral image of the right femur that shows a Codman triangle (arrow 2) and moth-eaten, permeative destruction of the bone (arrow 3).

An ultrasound is made that shows a soft tissue mass originating from the femur with a periosteal reaction. A conventional radiograph is made that shows widespread cortex destructions and aggressive periosteal reactions (figure 3). There is a high suspicion for a malignant bone sarcoma and the patient is referred to a bone sarcoma center for further evaluation and diagnosis. Laboratory tests results are as followed: white blood cell count 6.5 x109, LDH 351 U/L, C-reactive protein 40.5 mg/L, ESR 60 mm, AF 270 U/L. After local staging by MRI (figure 4A) a biopsy is performed which

shows small blue round cells (figure 1A) and strong CD99 positivity (figure 1B). Molecular studies show a t(11;22) rearrangement that confirms the diagnosis of Ewing sarcoma. A Bone marrow biopsy was performed that showed no morphological changes. Chest CT showed no sign of pulmonary metastasis (figure 5A) and whole body staging by FDG-PET/CT showed no metastasis (figure 5B). The disease extent was considered localized and treatment was started according to EWING 2008.

Multimodal treatment

Patients with Ewing sarcoma are evaluated in a multidisciplinary team (e.g. radiologist, chemotherapist, pathologist, surgical or orthopaedic oncologist, radiation oncologist). Standard treatment consists of chemotherapy followed by local control of the tumor, either surgery, radiotherapy or a combination of both, and adjuvant chemotherapy.

Chemotherapy

The introduction of chemotherapy and the work of cooperative study groups drastically improved the outcome and survival of Ewing sarcoma. In non-metastatic Ewing sarcoma 10-year overall survival is currently 65 to 70%. (19, 30) It all started with a single agent approach that rapidly evolved to multiagent chemotherapy and from adjuvant to neoadjuvant setting.(31-35) Current trials all employ 3 to 6 cycles of multidrug chemotherapy, followed by local therapy and another 6 to 10 cycles of multidrug chemotherapy with 2 to 3 week intervals. The total treatment duration is about 1 year. (21) Based on cooperative trials the most active chemotherapy agents include doxorubicin, cyclophosphamide, ifosfamide, vincristine, dactinomycin and etoposide (31-33, 36, 37) Almost all current protocols are based on a combination of five to six of these agents. An interval compressed chemotherapy with dose-dense regimens was associated with a positive outcome in pediatric (<18 years) patients with Ewing sarcoma. (38) High-dose chemotherapy with busulfan and melphalan (BuMel) in combination with stem cell rescue is only indicated for a selected group of localized Ewing sarcoma patients with a poor response to neo-adjuvant chemotherapy and/or a tumor volume of more than 200 ml. No benefits for patients presenting with pulmonary metastasis was shown. (34, 39)

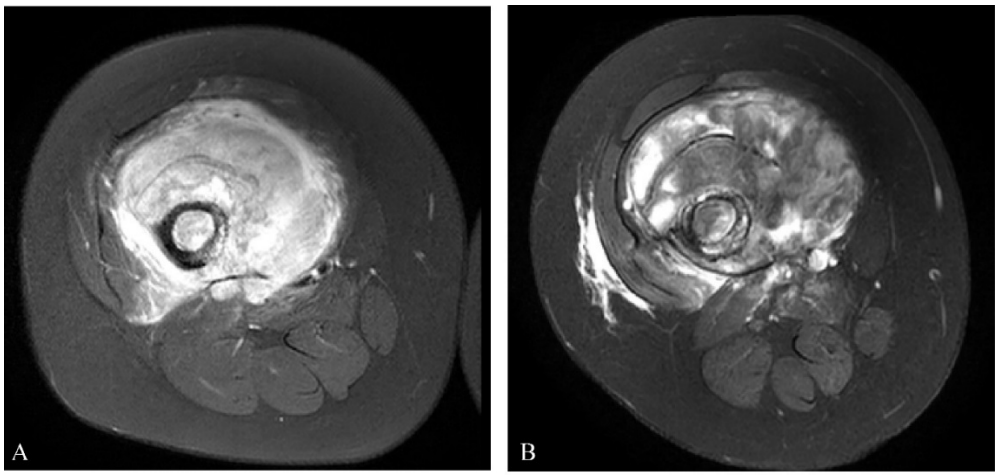

Figure 4 – Local imaging by MRI of Ewing sarcoma
A 12-year-old boy with Ewing sarcoma of the right thigh. Axial fat-suppressed T2-weigthed images at diagnosis (A) and after 6 cycles of VIDE chemotherapy (B) show a lesion in the right thigh with a circumferential soft-tissue mass that shows a high signal. After chemotherapy a volume decrease of the soft-tissue component is seen, but there is still a soft-tissue mass remaining.

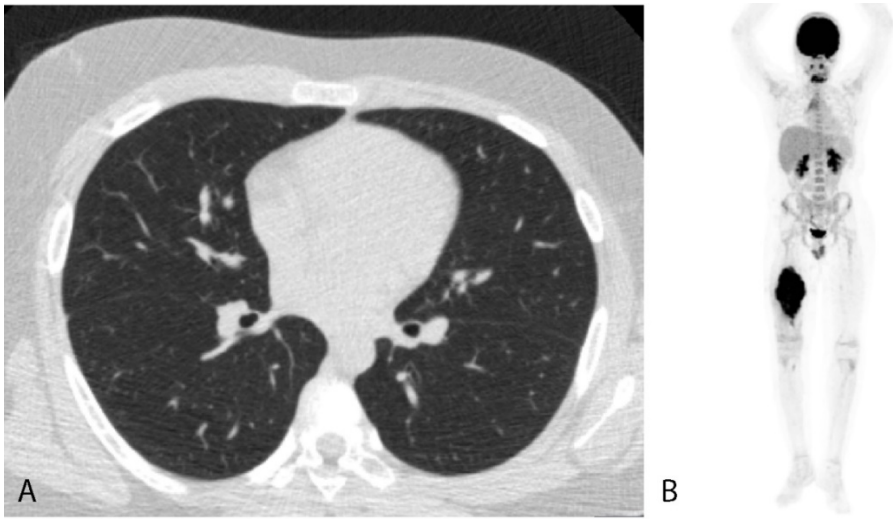

Figure 5 – Whole body staging in Ewing sarcoma
A 12-year-old boy with Ewing sarcoma of the right thigh. A) Chest-CT showing clear lung fields without nodules, consolidations or lymphadenopathy. There is no sign of pulmonary metastasis. B) Whole body FDG-PET/CT showing high FDG-uptake at the right thigh. There is no increased FDG-uptake elsewhere in the skeleton apart from physiologic uptake at the growth plates and hematopoietic bone marrow of the axial skeleton.

According to the EWING 2008 protocol six cycles of VIDE chemotherapy (vincristine, ifosfamide, doxorubicin, etoposide) were administrated. After the 6th cycle the response of the tumor to chemotherapy was assessed by MRI (figure 4B). There is a decrease in the volume of the soft-tissue component, however on dynamic MRI fast uptake of contrast is shown indicative for vital tumor cells. The radiological response is considered poor.

Local control measures

Chemotherapy alone can't eradicate Ewing sarcoma tumor cells and local therapy, either surgery, radiotherapy or both, is crucial for management and high cure rates. Ewing sarcoma is radiosensitive, but given the higher risk of local recurrence with radiotherapy as sole treatment of the primary tumor, complete surgical excision, where feasible, is preferred. Surgery involves excision of all tissue that was originally involved with tumor and resection of the post-chemotherapy volume is not recommended unless surgery is followed by radiotherapy. Radiotherapy as sole treatment is generally applied if complete surgical excision causes excessive morbidity. Radiotherapy doses range from 45 to 60 Gy, depending on location. Pre-operative radiotherapy could be used to further reduce the tumor and make surgery possible in cases where complete resection is not feasible after chemotherapy. Postoperative radiotherapy is indicated in case of inadequate surgical margins and poor histological response (defined as less than 90% necrosis). De dose of postoperative radiotherapy is also 45 to 60 Gy and depends on the margins, histological response and location. Intralesional surgery provides no benefit when compared to radiotherapy alone and should therefore be avoided. (36, 40-42) Complications of both surgery and radiotherapy are significant. Surgical resection could result in functional deficits and radiation carries long-term risks of secondary malignancy and bone growth disturbances in children. (40, 45, 46) Several retrospective, non-randomized trials have been performed to evaluate different local treatment approaches in ES, indicating that surgery with or without radiotherapy is better than radiotherapy alone. (36, 43, 44)

The results of the MRI are discussed in the multidisciplinary team. To further improve the response, reduce the remaining soft-tissue mass and make joint sparing surgery possible the patient is treated with preoperative radiotherapy of 52Gy. 7 weeks after the last radiation surgery is performed using surgical navigation. The femur is reconstructed with a 3D printed custom made endoprosthesis (figure 6). Histopathological examination shows wide tumor margins and a histological response of 90-99% necrosis. 2 years after surgery the patient is still alive without evidence of disease.

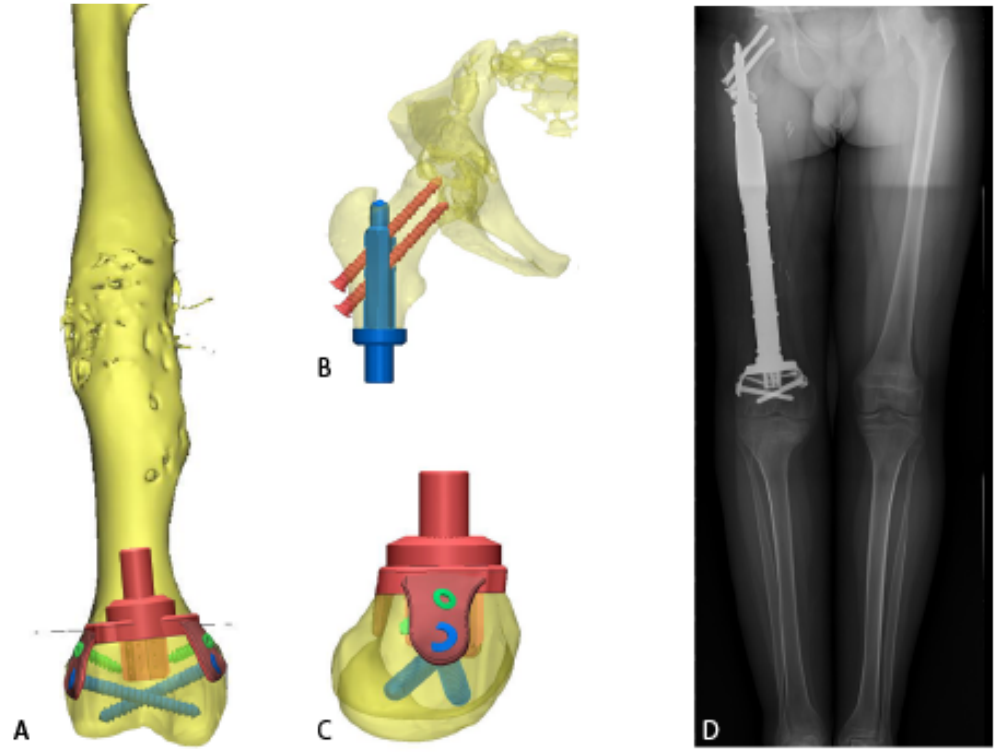

Figure 6 – Reconstruction of the femur with 3D printed custom made implant.
Hip- and knee-sparing custom made 3D printed endoprosthesis. A+C) Fitting of the knee-sparing computer-aided design (CAD) model on 3D reconstruction showing the planning of the screw placement. B) Fitting of the hip-sparing CAD model showing the planning of the screw placement. D) Anterior-posterior image of the hip- and knee-joint sparing custom made endoprosthesis used for reconstruction of the femur.

Metastatic Ewing sarcoma

In non-metastatic Ewing sarcoma 10-year overall survival is currently 55 to 65%, but survival in metastatic Ewing sarcoma is still dismal. (37, 47, 48) In case of extra-pulmonary metastasis, survival is worse compared to patients that present with lung metastasis alone (<20% for extrapulmonary metastasis versus 30-40% for patients with solitary pulmonary metastasis). (19, 47, 49, 50) The treatment approach for patients that present with metastatic disease follows the same principle as that of patients that present with localized disease. Achieving local control in all metastatic sites has been reported to improve clinical outcome. In patients that present with lung metastasis, whole-lung irradiation might improve survival. (51) The role of surgical resection of residual lung metastasis is less defined. The chemotherapy is similar to that for localized disease, but response is generally less durable. There is no clear evidence for high-dose chemotherapy in metastatic disease, but protocols differ among centers and countries. There are no randomized studies to provide the

evidence. An IESS study showed no benefit from the addition of IE to standard regimen VDCD for patients with metastatic disease. (52) In another intergroup study increasing the dose intensity did not improve outcome compared to standard dose intensity and increased toxicity and risk of secondary malignancies without improving EFS or OS. (53)

Recurrent Ewing sarcoma

In primary non-metastatic disease 30-40% of patients experience recurrence, in metastatic disease this number increases to 60-80%. Relapse is mostly systemic (71-73%), followed by combined (12-18%) and local (11-15%) relapse. (54, 55) 5-year post-relapse survival is poor, 15-25%, with local recurrence faring better than systemic. (54, 56, 57) Even though recurrent ES is almost always fatal, further responses to chemotherapy often happen and are valuable for survival prolongation. Fast relapse, within 2 years, is associated with worse survival. (54) Treatment in case of relapse is not standardized and depends on many factors such as site of relapse, prior treatment and the patients perspective. Among the possible options for chemotherapeutic treatment are: alkylating agents (cyclophosphamide and high dose ifosfamide) in combination with topoisomerase inhibitors (etoposide and topotecan), irinotecan with temozolomide or gemcitabine and docetaxel, high dose ifosfamide or carboplatin with etoposide. Doxorubicin is often no longer feasible due to previous achieved maximum cumulative doses. (58, 59) The role of surgery and radiotherapy is less defined. If prior treatment did not include surgery, resection or amputation is possible. Radiotherapy is generally only administrated in a palliative setting. (60)

Aim of this thesis

The aim of this thesis is to provide individual clinically advanced and response adaptive treatment strategies for Ewing sarcoma. As a result of collaborating trials survival of ES drastically improved from approximately a 10% 5-year overall survival (OS) with radiotherapy alone in the 1970s to almost 70% 5-year OS in patients with localized disease. However, local recurrence, distant metastasis and poor survival in patients with metastatic Ewing sarcoma, with a 5-year overall survival of 20-35%, still remain of great concern. Many trails have been performed to reveal prognostic factors of Ewing sarcoma. Assessment of the complexity of these prognostic factors is important in predicting the effect of treatment on the course of the disease for each patient and tumor specifically. Up until today such a prognostic model for Ewing sarcoma has not yet been identified and validated. Prediction models can assist in stratifying treatment according to the individual patients' risk profile, before, but also during treatment. As demonstrated in the case presented above, there are several multidisciplinary decision points during Ewing sarcoma treatment where new information comes available. For example, after the 5^{th} or 6^{th} induction chemotherapy

cycle, where decisions for local treatment need to be made, or after surgery where surgical margins and histological response influence the choices for adjuvant treatment. Currently, it is unclear how these risk factors that come available during treatment affect survival. Development of risk- and response adaptive treatment strategies could assist patients and their multidisciplinary teams in their shared decision making. Apart from the importance of accurate survival estimation, accurate staging is also of great importance for individual treatment strategies. Detection of all metastatic lesions in patients with oligometastatic disease has become relevant, as a curative rather than a palliative treatment objective and achieving local control at these sites has been reported to improve clinical outcome. The best staging modality needs yet to be identified. Also, treatment of Ewing sarcoma is multimodal and surgery, if feasible, is crucial for curative management. However, accurate detection and localization of tumor boundaries, especially in anatomical complex locations such as the pelvic is challenging. Inadequate surgical margins lead to a higher risk of local recurrence which has major impact on oncological outcome. Developments in intra-operative imaging, like CT-based navigation systems and near infrared (NIR) fluorescence guided surgery (FGS) make accurate defining and localization of surgical margins possible. They represent a whole new field of precision medicine. As shown in figure 6, CT-based navigation systems provide new treatment options for patients, thereby improving function outcome and healthcare quality. The indications, benefits for the patient ad implementation in Ewing sarcoma treatment are not yet clearly established.

Outline of this thesis

The first part of this thesis focusses of survival prediction. In **chapter 2** we performed a systematic review on the current known prognostic factors for overall survival and event-free survival. The aim of this systematic review is to provide an overview of prognostic factors for survival that can be used in the development of prediction models and clinical trial design. **Chapter 3** reports the first prediction model we developed. This is an easy-to-use model that predicts overall survival from the date of diagnosis and after surgery. Furthermore, it evaluates if and how survival changes during the course of treatment as more information comes available. In **chapter 4** a multistate model was developed to further assess the effect of known risk factors on local recurrence, distant metastasis and death, considering patient- and tumor characteristics and local treatment modality. To provide a more in-depth analysis of disease evolution in Ewing sarcoma.

The second part of this thesis focusses on pre-operative and intra-operative imaging techniques. In **chapter 5** we retrospectively compared the diagnostic yield of ^{18}F-FDG PET-CT to whole-body MRI for detection of skeletal metastasis in Ewing sarcoma. Since, accurate detection and localization of all metastases in Ewing

sarcoma is very important because treatment of all these sites potentially provides a curative approach.

About 25% of the Ewing sarcomas arise from the pelvic. Pelvic and sacral bone sarcoma resections are challenging due to anatomical and surgical complexity. Computer assisted surgery could assist in achieving higher surgical accuracy. In **chapter 6** we therefore compared the accuracy in terms of surgical margin achieved of navigated pelvic and sacral primary bone sarcoma resections to non-navigated resections. However, surgical navigation is CT-based and only guides the osteotomy. Ewing sarcoma generally presents with a large soft tissue mass. Intra-operative distinction between healthy and tumorous tissue is of paramount importance but challenging, especially after chemotherapy. Near infrared (NIR) fluorescence guided surgery (FGS) is able to facilitate determination of tumor boundaries intra-operatively. **Chapter 7** provides an overview of possible tumor-specific biomarkers in Ewing sarcoma suitable for NIR FGS in Ewing sarcoma.

In **chapter 8** the main results of the studies in this thesis are summarized. **Chapter 9** discusses the outcomes of the previous chapters, places them into a clinical context and concludes with future perspectives and implication for research. A summary of this thesis in Dutch is presented in **chapter 10**.

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PART I

Individual survival prediction

CHAPTER 2

Prognostic factors for survival in Ewing sarcoma: a systematic review

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Abstract

Development of a prognostic model for survival can assist in stratifying treatment according to the individual patients' risk, leading to risk- and response adaptive treatment strategies which allow for early decision making. The aim of this systematic review is to provide an overview of prognostic factors for overall survival (OS) and event-free survival (EFS) in Ewing sarcoma to be used in the development of prediction models and clinical trial design. A literature search was performed using Pubmed, Embase, Web of Science, Academic search premier and Cochrane databases. Studies were eligible if: 1) Sample size ≥100; 2) Follow-up ≥2 years or dead within 2 years; 3) Recruitment after 1975; 4) Outcome measure OS or EFS; 5) Multivariate analysis to assess the effect of prognostic factors on survival outcomes; 6) Study published in English. In case studies were derived from the same database the most all-embracing was selected. Study selection and quality assessment was performed by two reviewers independently. For each risk factor a level of evidence synthesis was performed. Kappa-statistic was used to determine inter-observer agreement. A total of 149 full-text articles were found, 21 eligible for inclusion. 24 prognostic factors were investigated, 14 relevant for this review. Prognostic factors associated with survival include metastasis at diagnosis, large tumors (volume ≥ 200 ml or largest diameter ≥ 8 cm), primary tumors located in the axial skeleton, especially pelvic and a histological response of less than 100%. These factors should be included as risk factors in the development of prediction models for ES.

Introduction

Ewing sarcoma (ES), first described in 1921 by James Ewing (1), is a small, round cell sarcoma that shows pathognomonic molecular findings and varying degrees of neural differentiation. (2) It is the second most frequent primary malignant bone sarcoma in children and young adults, showing a peak incidence in the second decade of life. As seen in many pediatric tumors there is a slight male dominance. (3-5) Caucasians are affected more than Asians and the negroid race, among whom the disease is rare. (6, 7) ES tends to arise from the diaphysis of long bones of the extremities (predominantly the femur) and the pelvic area with early involvement of the surrounding soft tissue. The soft tissue mass is usually large, circumferential about the involved bone and might even exceed the intraosseous component in size. (2, 8) Treatment of Ewing's sarcoma is multimodal, consisting of chemotherapy, surgery and/or radiotherapy. Improvement in survival outcomes is the result of collaborating trials; overall survival (OS) improved from approximately 10% at 5 years with radiotherapy alone to 55 to 65% in patients with localized disease, probably due to a multimodality approach. (6-11) At the time of diagnosis about 20 to 25% patients present with metastatic disease. Metastasis usually occurs to the lungs (70 to 80%) and to the bone (40 to 45%). Despite current aggressive cytotoxic treatment regimens the 5-year OS of patients with metastatic ES ranges from 20 to 35%. (6-11) Even in primary non-metastatic disease 30 to 40% of patients experience recurrence, either local, distant or combined, during follow-up. Survival after recurrence is poor, with 5-year post-relapse survival varying from 15 to 25%, local recurrence doing better than distant recurrence. (12-15)

Personalized medicine is becoming more and more important, especially in cancer treatment in order to avoid under-treatment of high-risk patients or over-treatment in low-risk patients or in patients for whom treatment is expected to have limited benefit. Many trials have been performed to study prognostic factors of Ewing sarcoma in order to define risk groups that need tailored treatment. Development of a prognostic model for survival can assist in stratifying treatment according to an individual patients' risk profile, so that risk- and response adaptive treatment strategies can be developed to allow early decision making and shared decision making. Until today such a prognostic model for Ewing sarcoma has not yet been developed and validated.

The aim of this systematic review is to provide an overview of prognostic factors for survival in Ewing sarcoma in order to develop prediction models for survival.

Methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. (16) The review protocol for this

study was prospectively registered at PROSPERO[1] (registration number CRD42017080534). Due to the presence of heterogeneity in treatment modalities among studies only a systematic review is performed.

Search strategy

Search strategies were run in the following databases in October 2017: PubMed MEDLINE, Embase, Cochrane Library, Web of Science and Academic Search Premier. Search strategies for all databases were adapted from the PubMed MEDLINE strategy. The search strategy specified keywords related to "Ewing sarcoma", "survival", "prognostic factors" and abbreviations thereof. The complete search strategies for each database are available in supplementary file 1. The results of all searches were combined and duplicates were removed.

Eligibility criteria

Clinical trials (phase I, II and III), prospective and retrospective cohort studies were all considered for inclusion in this review. Case reports and other type of publications including reviews, viewpoints or conference reports were excluded. Studies were eligible for inclusion if the following criteria were met: (1) Sample size of at least 100 patients with Ewing sarcoma eligible for analysis; (2) Follow-up of at least 2 years or patient died within 2 years; (3) Recruitment period started after 1975 to assure appropriate imaging and diagnosis; (4) Outcome measure is overall survival or event-free survival; (5) A multivariate analysis was employed to assess the effect of prognostic factors on survival; (6) The study is published in the English language. If studies were derived from the same database the most all-embracing study was selected. Separately published subgroup analyses of the same trail or performed in the same dataset were not included in this systematic review. The eligibility of the studies was assessed by two independent review authors (SB and OA). Disagreements were solved during a consensus meeting. In case of persisting disagreements a third reviewer (PDSD) was consulted.

Risk of bias

The Quality In Prognosis Studies (QUIPS) tool developed by Hayden et al. (17) was used to assess the risk of bias. The QUIPS tool uses six domains to evaluate the validity and bias in studies of prognostic factors: study participation, study attrition, prognostic factor measurement, outcome measurement, confounding and analysis. The six domains of bias were scored as "high" (3 points), "moderate" (2 points) or "low" (1 point). The total score for each study ranges from 6 to 18 points, to distinguish high risk of bias studies from low risk of bias studies the cut-off was set at a maximum of 50% (≤9 points). Risk of bias was scored by two review authors (SB and OA) independently. Disagreements were resolved during a consensus

[1] http://www.crd.york.ac.uk/prospero

meeting. If disagreements persisted a third reviewer (PDSD) made a final decision about the risk of bias. Methodological quality of the included studies was assessed according to the grading of recommendation, assessment, development and evaluation (GRADE) approach. (18)

Data extraction

The following data was extracted from the included studies: study design, database/trial , study population, sample size, treatment (chemotherapy regimen, local treatment modality), recruitment period (years), median follow-up (years), prognostic factors investigated, outcome measure and results. For the level of evidence synthesis the risk factors age, size, volume, serum LDH level and histological response were combined regardless of differences in the cut-off points used.

Data analysis

Due to the presence of heterogeneity among treatments a meta-analysis is not performed, instead a level of evidence synthesis was conducted for each prognostic factor. If the results of at least 75% of the studies analyzing the effect of a specific prognostic factor point in the same direction the findings were considered consistent. Level of evidence is defined as “strong” if there are consistent findings (≥ 75%) in multiple high-quality cohorts. If the results in ≥ 67% multiple high-quality cohorts go in the same direction the level of evidence is defined as being “moderate”. When a prognostic factor is only investigated in a single high-quality cohort or shows consistent findings (≥75%) in one or more low-quality cohorts the level of evidence is considered “limited”. If the results show inconsistent findings, meaning that the results point in different directions, the level of evidence is considered “inconclusive”, irrespective of study quality. In case of multiple high-quality cohorts only the high-quality cohorts are used to define the level of evidence.

Statistical analysis

Inter-observer agreement for the risk of bias assessment was determined by the kappa-statistic. (19) All analyses were performed using SPSS 23.0, Armonk NY, IBM Corp.

Results

Study selection

The initial search strategy identified 3716 records (Pubmed *n* = 1543; Embase *n* = 1247; Web of Science *n* = 834; Cochrane library *n* = 62; Academic Search Premier *n* = 30). After removal of 1842 duplicates, 1874 records were available for screening (fig1 flow-chart). After screening of titles and abstracts, 149 full-text articles were obtained, 128 did not meet the eligibility criteria: 45 studies were derived from the

same database; 31 studies did not report a multivariate analysis; 20 studies investigated another outcome, 19 studies did not focus solely on Ewing sarcoma; 7 studies had missing information on the recruitment period and/or follow-up and of 6 studies the full-text article was not available. In total 21 studies (20-40) were included (Figure 1). The reviewers initially disagreed on 21 inclusions during the selection process. Consensus was reached for all studies.

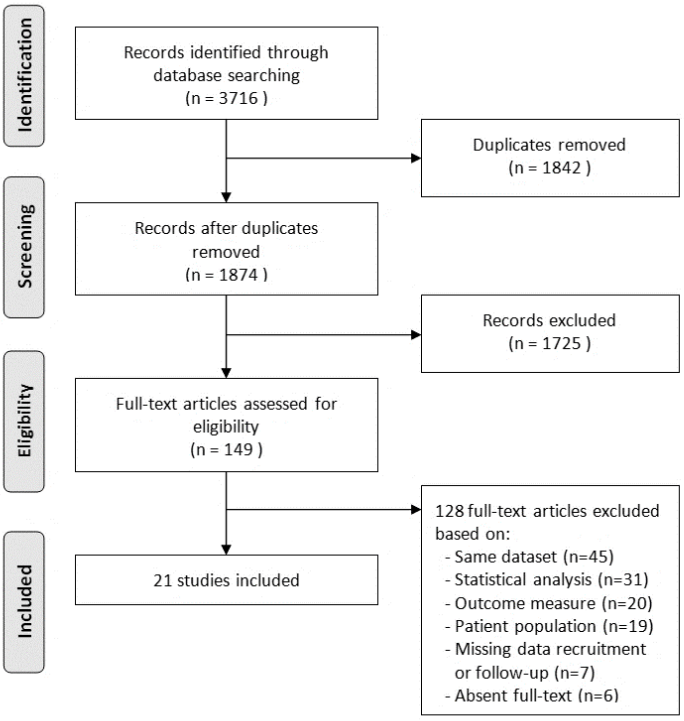

Figure 1 – Flowchart of the study selection process

Study characteristics

The characteristics of the 21 included studies are presented in Table 1. In five studies the results were based on prospectively collected data, in the other 15 studies the results were based on retrospectively collected data. In all cohorts patients were treated with neo-adjuvant chemotherapy followed by local treatment, surgery and/or radiotherapy of the primary tumor and adjuvant chemotherapy. The chemotherapy regimens used vary among the studies, but in all cohorts a polychemotherapy regimen was used. The follow-up duration was reported in 16 studies and ranged from 2 to 12 years. All included studies reported the recruitment period, duration ranged from 3 to 37 years.

Risk of bias

Agreement on the risk of bias score was obtained for 17 out of the 21 included studies (81%). For the remaining 4 studies consensus was reached. A substantial inter-observer agreement was obtained for the overall risk of bias score (kappa 0.76). The domains prognostic factor measurement and statistical analysis showed the lowest level of agreement (kappa 0.46 and 0.31 respectively). The domains study attrition and study confounding showed the highest level of agreement (kappa 0.72 and 0.79 respectively). The complete results of the risk of bias score and inter-observer agreement are available in supplementary file 2.

Level of evidence for prognostic factors

24 prognostic factors were distinguished in the 21 included studies. Several studies investigated prognostic factors specific to the received treatment of the patients in the cohort, such as type of chemotherapy protocol (number of drugs, type of drugs, intensity, dose, etcetera) or treatment era. These prognostic factors were not considered relevant for the purpose of this study and are therefore excluded. Ten prognostic factors were only investigated once: socio-economic status (SES) (27), erythrocyte sedimentation rate (ESR) (22), white blood cell (WBC) count, hemoglobin level, albumin level, duration of symptoms, the presence of systemic symptoms (41), the presence of fever (28), hepatoma-derived growth factor (HDFG) and p53 expression. (32) Since these prognostic factors were only investigated in a single study, the level of evidence could by definition never exceed the level of “limited” and they are therefore excluded. Among the remaining 14 prognostic factors, 13 for overall survival and 13 for event-free survival are detailed in Table 2 and 3 respectively.

ID	Author, year, country	Database	Study design	n	Study population	Local treatment	Period of recruitment	FU[a] (years)	Outcome measure[b]	Risk of bias[c]
1	Fizazi, 1998, France (20)	Hospital database, multicenter	R	182	LOC + MET	S 47% RT 81%	1982 - 1992	5.5	OS	Low
2	Cotterill, 2000, United Kingdom (21)	MRC/UKC-CSG: ET-1, ET-2 CESS-group: CESS-81. CESS-86	P	796	LOC	S 35% RT 40% S+RT 25%	1978 - 1993	6.6	EFS	Low
3	Oberlin, 2001, France (22)	EW88	P	141	LOC	S 37% RT 26% S+RT 37%	1988 - 1991	8.5	EFS	Low
4	Jenkin, 2002, Saudi Arabia (23)	Hospital database,	R	163	LOC	S 18% RT 67% S+RT 12%	1975 - 1998	3.9	OS	Low
5	Bacci, 2006, Italy (24)	Hospital database,	R	512	LOC + MET	S 38% RT 35% S+RT 27%	1979 - 1999	12	EFS	Low
6	Obata, 2007, Japan (25)	Hospital database, multicenter	R	243	LOC + MET	S 36% RT 24% S+RT 40%	1981 - 2003	5.5	EFS	Low
7	Rodriquez-Galindo, 2007, USA (26)	Hospital database,	R	222	LOC + MET	S 20% RT 55% S+RT 25%	1979 - 2004	11.7	OS EFS	Low
8	Lee, 2010, USA (27)	California Cancer Registry (CCR)	R	725	LOC + MET	S 55% RT 53%	1989 - 2007	NR	OS	Low
9	Gaspar, 2012, France (28)	EW93	P	214	LOC	S 48% RT 14% S+RT 38%	1993 - 1999	8	EFS	Low
10	Drabko, 2012, Poland (29)	Hospital database, multicenter	R	119	LOC + MET	S±RT 89% RT 11%	1999 - 2006	4.5	OS EFS	High
11	Arpaci, 2013, Turkey (30)	Hospital database, multicenter	R	114	LOC + MET	S 31% RT 18% S+RT 41%	2001 - 2010	2	OS EFS	Low
12	Koohbanani, 2013, USA (31)	Hospital database,	R	135	LOC + MET	RT 42% S 50%	1987- 2011	3.4	OS	Low

ID	Author, year, country	Database	Study design	n	Study population	Local treatment	Period of recruitment	FU[a] (years)	Outcome measure[b]	Risk of bias[c]
13	Yang, 2014, China (32)	Hospital database,	R	108	LOC + MET	S 75%	1990 - 2010	NR	OS	High
14	Biswas, 2015, India (33)	Hospital database,	R	224	LOC	S 27% RT 45% S+RT 28%	2003 - 2010	3.4	OS EFS	Low
15	Brunetto, 2015, Brazil (34)	SOBOPE – EWING1	P	175	LOC + MET	S 49% RT 28% S+RT 23%	2003 - 2010	4.4	OS EFS	High
16	Marina, 2015, USA (35)	Children's Oncology Group (INT-0091, INT-0154 and AEWS0031)	R	1444	LOC	NR	1988 - 2005	NR	EFS	Low
17	Albergo, 2016, UK (36)	Hospital database,	R	293	LOC	S±RT100%	1980 - 2012	9.1	OS EFS	Low
18	Foulon, 2016, France (37)	E.U.R.O-EWING 99	P	599	LOC	S 76% S+RT 24%	1999 - 2009	6.2	EFS	Low
19	Friedman, 2017, USA (38)	Hospital database,	R	300	LOC + MET	S 42% RT 22% S+RT35%	1975 - 2012	7.8	OS	Low
20	Miller, 2017, USA (39)	National Cancer Data Base	R	1031	LOC + MET	S 46% RT 33% S+RT 21%	1998 - 2012	NR	OS	Low
21	Verma, 2017, USA (40)	Surveillance, Epidemiology and End Results (SEER)	R	1870	LOC + MET	S 52% RT 23% S+RT 25%	1983 - 2013	NR	OS	Low

Table 1 – Characteristics of the 21 included studies

FU = follow-up; NR = not reported; R = retrospective; P = prospective; LOC = localized Ewing Sarcoma; MET = metastatic Ewing Sarcoma; OS = overall survival; EFS = event-free survival; S = surgery; RT = radiotherapy.

a. *Median follow-up in years.*
b. *Outcome measures overall survival (OS) and event-free survival (EFS) are computed from the date of diagnosis or first day of treatment.*
c. *Risk of bias was assessed using the QUIPS tool. (17) Studies were scored based on six domains, if a study scored ≤9 points the risk of bias was considered low.*

Prognostic factors

The presence of metastasis at diagnosis, tumor size and site of the primary tumor were strongly associated with overall survival (OS). Prognostic factors that were commonly studied and did not have significant independent prognostic influence on OS include gender, serum LDH level, tumor origin and radiological response. The level of evidence for an association with OS for age, local treatment modality, race/ethnicity, site of metastatic lesions, histological response and surgical margins was inconclusive, meaning that the results from several high quality cohorts give contradictory results.

The presence of metastasis at diagnosis, tumor volume and histological response were strongly associated with EFS. Prognostic factors that were commonly studied and did not have significant association on EFS are gender, tumor origin, radiological response and the site of the metastatic lesions. The level of evidence for age, location of the primary tumor, tumor size, serum LDH level and local treatment modality in association with EFS was inconclusive.

Metastasis at diagnosis

The presence of metastasis at diagnosis was found to be independent and significantly associated with poorer OS in seven out of eight (88%) high-quality cohorts, hazard ratios (HR) varied from 2.4 to 4.4. All high-quality cohorts (25, 26, 30) and one low quality cohort (34) found a clear association with poorer EFS, HR 1.5 to 2.2.

Tumor size

Seven high-quality cohorts (100%) evaluated the effect of tumor size on OS. Six studies found that a diameter of 8 cm or more was associated with a poorer OS (HR 1.5 to 2.5), the other study found that a tumor size of 10 cm or more was associated with a worse OS (HR 1.84; 95%CI 1.22-2.78;p=0.04). Two studies, using 5 cm and 8 cm as cut-off points found no clear association between tumor size and OS.

A tumor size of 8 cm or more was also associated with poorer EFS in four out of six (67%) high-quality cohorts (26, 33, 35, 36), HR 1.8 to 2.9. One study using 10 cm as cut-off point could not find a clear association.

Tumor volume

Five studies measured tumor volume of which four (80%) found that larger volumes are associated with poorer EFS. One study (21) found that patients with a tumor volume of 100 ml or more have poorer EFS (p=0.001, HR not given). Another study (24) found similar results, with a HR of 2.2 (95%CI 1.4-3.3; p<0.001) for a tumor volume of 150 ml or more. Two other studies (28, 37) found that patients with a tumor volume of 200 ml or more have a poorer EFS, RR 1.8 (95%CI 1.2-2.7; p=0.01) and HR 1.8 (95%CI 1.1-3.0; p<0.001) respectively.

Location of the primary tumor

Eight high-quality cohorts evaluated the effect of the primary tumor location on OS, six (75%) found significant results. Three compared extremity versus axial (including pelvic) location of which two (36, 40) found that patients with a tumor in the axial skeleton have poorer OS, HR 1.98 (p=0.038) and HR 1.3; 95% CI 1.0-1.5; p=0.021, respectively. Two studies (33, 39) compared tumors located in the pelvic or spine with all other locations and found similar results with a HR of 2.7 (95%CI 1.3-5.7; p=0.009) and HR 1.1 (95%CI 0.9-1.4) for tumors located in the pelvic or spine. One study (20) comparing pelvic versus non-pelvic locations found that patients with pelvic tumors have a higher risk of death, RR 1.9 (95%CI 1.3-2.9; p=0.0025). Another high-quality cohort (27) comparing a pelvic versus non-pelvic location could not confirm these results. The last study (23) compared tumors located in the proximal extremity or axial skeleton with all other locations and found that primary tumors in the proximal extremity or axial skeleton have poorer OS (p=0.02; HR not given).

Eleven high-quality cohorts evaluated the effect of the location of the primary tumor on EFS, six studies found a positive association. Five studies compared an extremity versus axial location of the primary tumor. Two (25, 36) found that patients with a tumor in the axial skeleton have a poorer EFS, RR 1.2 (95%CI 1.0-1.4; p=0.004) and HR 1.9 (p=0.02) respectively. Two studies (21, 35) found that patients with a pelvic primary tumor have a poorer EFS, HR 1.4 (p=0.003) and HR 1.3 (95%CI 1.1-1.7; p=0.009) respectively. One (26) other high-quality cohort could not find the same association. One study (28) showed that patients with a primary tumor located in the trunk or proximal extremity have a poorer EFS, HR 1.7 (95%CI 1.0-2.9; p=0.04). A study (37) found a HR of 2.1 (95%CI 1.1.-3.7) for a tumor in the axial skeleton, HR 2.3 (1.1-4.4) for a pelvic location and HR 3.5 (95%CI 1.3-9.5) for tumors in the sacrum or vertebrae compared to an extremity location.

Prognostic factor	Measure (good / poor survival)	Association	No association	Level of evidence
Metastasis at diagnosis	No / yes	**1, 7, 8, 11,** 13, 15**, 19, 20, 21**	10, **12**	S - 88%
Size	<10cm / ≥10cm <8cm / ≥8cm <5cm / ≥5cm	**1** **7, 8, 11, 14, 17, 20**	 15 13	S – 100%
Primary tumor site	Extremity / axial (incl. pelvis) Other / pelvic + spine Non-pelvic / pelvic Other	**17, 21** **14, 20** **1** **4**	10, **11**, 13 **8,** 15	S - 75%
Gender	Female / male	**20, 21**	**1, 4, 7, 8,** 10, **11, 12,** 13, **14,** 15**, 17, 19**	S - 82%
LDH	N / ≥ 2x N N / ≥ 1,5x N		**11, 14** 15	S - 100%
Origin	Soft-tissue / bone		13, **14, 19**	S - 100%
Age	<14y / ≥14y <15y / ≥15y <16y / ≥16y <18y / ≥18y Other	**4,** 10 **8, 20, 21** **12, 19**	**7** **14,** 15 **17** **1, 11,** 13	I - 55%
Local treatment modality	Surgery / no surgery Surgery ± RT / RT only RT / no RT Post-op RT / pre-op RT	**8,** 10, 13, **21** **14, 20** **8**	**11, 12,** 15 **19, 21** **21**	I - 50%
Race/ethnicity	White / non-white White / Hispanic	**19** **8, 12**	**20, 21**	I - 60%
Site of metastatic lesions	Lung only / other Lung / lung combined / other	**19**	**7,** 10, 15	I - 50%
Histological response (% necrosis)	100% / 99-50% / 0-50% ≥95% / <95% ≥90% / <90%	**17**	 **1,** 15 10, 13,	I - 50%
Surgical margin	Negative / positive	**20**	**11**	I - 50%
Radiological response	CR + PR / SD + PD		**11,** 15	L - 100%

Table 2 – Level of evidence for investigated prognostic factors for overall survival.

Abbreviations: S = strong; M = moderate; I = inconclusive; L = limited; N = normal level; RT = radiotherapy; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease.

The numbers refer to the study ID as presented in Table 1. Studies with a low risk of bias are presented in bold.

Histological response

The effect of histological response on OS was evaluated in five studies of which 2 high-quality cohorts. One study (36) found that patients with 100% necrosis have the best OS, compared to patients with 0-50% necrosis, HR 6.9 (p<0.001, 95%CI not given), and patients with 50-99% necrosis, HR 3.3 (p<0.001, 95%CI not given). The other high quality cohort (20), using 95% necrosis as cut-off point, found no clear association between histological response and OS. Three low quality cohorts using 95% necrosis and 90% necrosis as cut-off point could also find no clear association. Five high-quality studies investigated the effect of histological response on EFS of which four (80%) found a positive association. One study (22) found a HR of 5 (95%CI 2.5–10; p<0.001) for patients with less than 95% necrosis. Another study (24) found a HR of 5.1 (95%CI 2.9-9) for patients with Picci grade I and a HR of 2.4 (95%CI 1.2-4.6) for patients with Picci grade II. A French study (28) found a RR of 2.3 (95%CI 1.4-3.8; p<0.001) for patients with less than 90% necrosis and Albergo et al. (36) showed that patients with 100% necrosis have the best EFS, with a HR of 4.4 (p<0.001, 95%CI not given) for patients with 0-50% necrosis and a HR of 2.4 (p<0.001, 95%CI not given) for 50-99% necrosis.

Surgical margins

Two high-quality studies evaluated the effect of surgical margins on OS. In one study (39) patients with marginal or intralesional surgical margins have a HR of 1.6 (95%CI 1.1-2.5). The other study also evaluating marginal or intralesional margins versus radical margins did not find the same association. (30)

A significant association between intralesional or marginal (positive) surgical margins and poor EFS was found in two (24, 30) out of three (67%) high-quality cohorts, HR 1.3 ((5%CI 1.0-1.7; p=0.044) and p<0.001 (HR not given), leading to a moderate level of evidence.

Prognostic factor	Measure (good / poor survival)	Association	No association	Level of evidence
Metastasis at diagnosis	No / yes	**6, 7, 11, 15**	10	S – 100%
Volume	<100ml / ≥100ml <150ml / ≥150ml <200ml / ≥200ml	**2** **5** **9, 18**	 **3**	S – 80%
Histological response (% necrosis)	100% / 99-50% / 0-50% 100% / 90-99% / <90% ≥95% / <95% ≥90% / <90% Other	**17** **3** **9** **5**	 **18** 15 10 	S – 80%
Gender	Female / male		**2, 3, 5, 6, 7, 9,** 10 **11, 14,** 15**, 16, 17, 18**	S – 100%
Radiological response	CR / PR / SD / PD CR + PR / SD +PD CR / other		**11, 18** **3, 9,** 15 **6**	S – 100%
Origin	Soft-tissue / bone		**14, 18**	S – 100%
Surgical margin	Negative / positive	**5, 11**	**18**	M – 67%
Site of metastatic lesions	Lung only / other Lung only / lung combined / other		**7,** 10 15	M – 100%
Age	<14y / ≥14y <15y / ≥15y <16y / ≥16y <20y / ≥20y Other	**5,** 10 **2** **6** **16**	**7, 18** **3, 9, 14,** 15 **17** **3** **11**	I – 64%
Location primary tumor	Extremity / axial Other / pelvic + spine Non-pelvic / pelvic Distal / proximal /other Extremity / pelvic / sacrum + spine / other axial	**6, 17** **2, 16** **9** **18**	**3, 5,** 10, **11, 14** **7,** 15	I – 55%
Size	<8cm / ≥8cm <10cm / ≥10cm	**7, 14, 16, 17**	**3, 11,** 15 **6**	I – 57%
LDH	N / ≥ 2x N N / ≥ 1,5x N <500 U/L / ≥500 U/L	**2, 5**	**11, 14,** 15 **3**	I – 60%

Prognostic factor	Measure (good / poor survival)	Association	No association	Level of evidence
Local treatment modality	Surgery / no surgery	**9**	10, **11,** 15	
	Surgery / surgery + RT / RT	**5**	**7** **14**	I – 50%
	Surgery ± RT / RT only	**18**		
	No PORT / PORT			

Table 3 - Level of evidence for investigated prognostic factors for event-free survival.
Abbreviations: S = strong; M = moderate; I = inconclusive; L = limited; N = normal level; RT = radiotherapy; PORT = post-operative radiotherapy; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. The numbers refer to the study ID as presented in Table 1. Studies with a low risk of bias are presented in bold.

Local treatment modality
Eight high-quality studies investigated the effect of local treatment modality on OS. Two studies (27, 40) found that patients who have surgery for local treatment have a better OS compared to patients who don't undergo surgery, HR 0.7 (95%CI 0.5-0.9; p=0.002) and HR 0.6 (95%CI 0.5–0.7; p<0.001) respectively. These results were however not confirmed in two other studies (30, 31). Two studies (33, 39) found that patients only treated with radiotherapy (RT) for local treatment have poorer OS compared to patient treated with surgery with or without RT, HR 2.5 (95%CI 1.2-5.2; p=0.01) and HR 2.1 (95%CI 1.6-2.8) respectively. One study (27) specifically evaluated the use of RT and found that patients who receive RT have a better OS compared to patients who don't receive RT, HR 0.8 (95%CI 0.6-0.99; p=0.04). Two other studies (38, 40) also investigating RT versus no RT could not identify a clear association between the use of RT and OS.
Eight studies investigated the effect of local treatment modality on EFS. 3 high-quality cohorts found an positive association. One (24) found a HR of 1.6 (95%CI 1.1-2.5; p=0.015) for patients treated with radiotherapy (RT) only as local control. The other study (28) found that patients who did not undergo surgery for local control have a worse EFS, HR 2.2 (95%CI 1.4-3.6; p<0.001). Three other high-quality cohorts (26, 30, 33) did not find that patients who have RT only as local control measure have poorer EFS. One study (37) showed a better EFS for patients who have post-operative RT, HR 0.4 (95%CI 0.2-0.9) compared to no post-operative RT.

Age
The effect of age on OS was evaluated in eleven high-quality cohorts; six (55%) studies found that older age is associated with poorer OS. Three studies (27, 39, 40)

found that patients 18 years or older have poorer OS, HR 1.6 (95%CI 1.2-2.2; p<0.001), HR 1.9 (95%CI 1.5-2.4) and HR 1.9 (95%CI 1.6-2.2; p<0.001) respectively. Two studies used 14 years as a cut-off point of which one (23) found that patients 14 years or older have poorer OS (p=0.02; HR not given). Two other studies (31, 38) found that older patients have poorer OS, HR 1.03/years (p=0.036) and HR 2.8 (95%CI 1.3-5.6; p=0.005) for 10-18 years, HR 3.0 (95%CI 1.4-6.4; p=0.004) for 20-29 years, HR 4.5 (95%CI 2.0-10.6; p<0.001) for 30-39 years. The four remaining studies, using 15, 16, 26 and 30 years of age as cut-off points could not find an clear association between older age and OS.
Eleven high-quality cohorts evaluated the effect of age on EFS, in four cohorts a positive association between older age and EFS was found, HR 2.0 (95%CI 1.3-3.2; p=0.003) for patients 14 years or older (24), HR 1.6 (p<0.001) for patients 15 years or older (21), RR 1.2 (95%CI 1.0-1.5; p=0.004) for patients 16 years or older and RR 1.2 (95%CI 1.0-1.6; p<0.001) for patients 10-18 years and RR 2.1 (95%CI 1.6-2.9; p<0.001) for age above 18 years. (35) Other high-quality cohorts, two investigating 14 years, three 15 years, one 16 years, one 20 years and one 26 years as cut-off point could not find a clear association between older age and EFS.

Race / ethnicity
Three out of the five high-quality cohorts that investigated the effect of ethnicity on OS found a positive association. Two (27, 31) compared Hispanic to white and other ethnicities and found a HR of 1.3 (95%CI 1.0-1.8; p=0.04) and HR 1.9 (p<0.001, 95%CI not given) respectively for Hispanics. Three studies compared white to non-white race, one of these (38) found a HR of 2.1 (95%CI 1.3-3.3; p=0.002) for non-white race, the other two could not find a clear association between ethnicity and OS. (39, 40).

Site of metastatic lesions
The site of metastatic lesions as a prognostic factor for OS was evaluated in two high-quality cohorts. One study (38) found a HR of 3.2 (95%CI 2.0-5.2; p<0.001) for patients with only lung metastasis and a HR of 5.2 (95%CI 3.2-8.5; p<0.001) for patients with extrapulmonary metastasis. The other study and two low quality cohorts did not detect an association.

Serum LDH level
Two out of five high quality cohorts found that a serum LDH level two times the normal level is associated with poorer EFS, HR 4.2 (95%CI 2.7-6.5; p<0.001) (24) and p=0.03 (HR not given). (21) Two other high-quality cohorts (30, 33) did not find that serum LDH is associated with poorer EFS.

Discussion
The aim of this systematic review was to provide an overview of prognostic factors for survival in Ewing sarcoma in order to help guide development of prediction models and further studies.

The most significant prognostic factor influencing survival is the presence of metastasis at diagnosis. Other factors that consistently independent influence survival are: tumor size and volume, histological response and location of the primary tumor.

Large tumors were found to have an independent prognostic effect on survival. A tumor volume of 200 ml or more shows poorer EFS as well as a tumor diameter of 8 cm. Tumor dimensions can easily be recalculated into volume as shown by Göbel et al. (42). Ewing sarcoma arises from the long and flat bones and presents with a varying degree of soft-tissue component. (2, 8) Therefor volume is a more appropriate and accurate way of measuring tumor size, since the largest diameter could easily overestimate the size in the long bones and underestimate the size in case of an ellipse or round shaped tumor. With imaging modalities available nowadays, volume calculations can easily be made.

The prognostic significance of tumor location was commonly studied among the included studies, but evaluated by different means (extremity versus axial, pelvic versus non-pelvic etcetera). Overall tumors located in the axial skeleton, more specifically the pelvis were found to have poor overall survival and tumors located in the extremity, especially the distal extremity show better survival. A clear association with event-free survival (EFS) could not be found.

Histological response is used to tailor treatment in European trials for Ewing sarcoma (43) and considered of high prognostic value. The results from this systematic review show a tendency that necrosis of at least 90% improves EFS, evidence for a clear association with OS is however less consistent. Different cut-off points and different methods for evaluating and defining good histological response might explain this. Albergo et al. (36) found that patients with 100% necrosis of their tumor after neoadjuvant therapy have better survival over patients with viable tumor cells left, even if it is just 1%. The results from this review support this, with studies evaluating 90% and 95% cut-off points for good responders showing inconsistent results.

Concerning the primary tumor resection, the data presented here show moderate evidence that obtaining negative, disease free tumor margins is of prognostic significance for EFS. There are 3 studies showing that achieving positive margins is not protective for survival and two studies not confirming this. Heterogeneity among centers in defining and evaluating surgical margins and the use of post-operative radiotherapy in case of inadequate margins might explain these somewhat inconsistent results.

Association between risk factors as age, race/ethnicity, LDH, site of the metastasis lesions, local treatment modality and survival are not consistent. Age was evaluated in almost all studies, showing that older age is associated with a poorer survival. The best cut-off point (14 or 18 years) needs to be further evaluated, since strong evidence for a specific cut-off point is lacking. Results suggest that white patients have better survival than other ethnicities. With only a few studies evaluating this, the evidence is limited. The same accounts for the serum LDH level and site of the metastatic lesions. Only two studies found that an elevated LDH leads to poorer

EFS, no studies found any association with OS. Only a single study found that patients with only lung metastasis have a better OS compared to other metastatic sites.

Local treatment modality in relation with survival was evaluated by multiple studies which showed inconsistent results. The existing evidence available is based on retrospective, non-randomized trails. If surgery with or without radiotherapy is better than radiotherapy alone is still under debate. Many of these studies are affected by a selection bias, where radiotherapy is only indicated in specific groups of patients, for instance patients with less favorable prognostic factors. A recent systematic review by Werier et al. (44) on optimal local treatment strategies for localized Ewing sarcoma found that either surgery alone (if negative margins can be achieved) or RT alone are reasonable treatment options. The optimal local treatment should be decided by considering patient characteristics, side effects and patient preference. In order to assess the effect of local treatment on survival, randomized trials aimed at comparing surgery, radiotherapy and a combination of both or prospective comparative studies are needed.

Several limitations were observed despite the strict eligibility criteria for this study. Treatment of patients is heterogeneous among studies. Although all patients were treated with neo-adjuvant therapy, followed by local treatment of the primary tumor and adjuvant chemotherapy. The type of chemotherapy was not consistent among the studies. Chemotherapy agents, doses and combinations changed and differ among countries. There has been a major progress in improving chemotherapy protocols over the last decades and therefore improvement in survival. Presence of heterogeneity among treatment increases the risk of bias and therefor the quality of the results presented here. Also, several different cut-off points were used for the evaluation of age, size, volume, location of the primary tumor and histological response.

Conclusion

The presence of metastasis at diagnosis, large tumors (volume ≥ 200 ml or largest diameter ≥ 8 cm), primary tumors located in the axial skeleton, especially pelvic, and a histological response of less than 100% are strongly associated with poorer survival in Ewing sarcoma (ES). These factors should be included as risk factors in the development of prediction models for overall survival and event-free survival in ES. Insight about the effect of surgical margins and local treatment modality requires further investigation.

Supplementary file 1

Searches used in each database.

Database	Search strategy	Citations
PubMed	(("Sarcoma, Ewing"[Mesh] OR "Ewing sarcoma"[tw] OR "Ewing's Sarcoma"[tw] OR "Ewings Sarcoma"[tw] OR "Ewing's Tumor"[tw] OR "Ewings Tumor"[tw] OR "Ewing Tumor"[tw] OR "Ewing's Tumour"[tw] OR "Ewings Tumour"[tw] OR "Ewing Tumour"[tw] OR "Ewing sarcomas"[tw] OR "Ewing's Sarcomas"[tw] OR "Ewings Sarcomas"[tw] OR "Ewing's Tumors"[tw] OR "Ewings Tumors"[tw] OR "Ewing Tumors"[tw] OR "Ewing's Tumours"[tw] OR "Ewings Tumours"[tw] OR "Ewing Tumours"[tw] OR "Ewing-like sarcoma"[tw] OR "Ewing-like sarcomas"[tw] OR (ewing*[tw] NOT (ewingi*[tw] OR ewing test*[tw] OR ewingell*[tw]))) AND ("Survival"[Mesh] OR "Mortality"[Mesh] OR "mortality"[Subheading] OR "Survival Analysis"[Mesh] OR "Survival Rate"[Mesh] OR "survival"[tw] OR surviv*[tw] OR "Neoplasm Recurrence, Local"[Mesh] OR "Recurrence"[Mesh] OR "recurrence"[tw] OR recurr*[tw] OR recrudescen*[tw] OR relaps*[tw]) AND ("Prognosis"[Mesh:noexp] OR "prognosis"[tw] OR prognos*[tw] OR "prognostic factor"[tw] OR "prognostic factors"[tw] OR "Disease-Free Survival"[Mesh] OR "Disease-Free Survival"[tw] OR "Event-Free Survival"[tw] OR "Treatment Outcome"[tw] OR "outcome"[tw] OR "outcomes"[tw]) NOT ("Case Reports"[ptyp] OR "case report"[ti]) NOT ("Animals"[mesh] NOT "Humans"[mesh]))	1.543
EMBASE	((*"Ewing Sarcoma"/ OR "Ewing sarcoma".ti,ab OR "Ewing's Sarcoma".ti,ab OR "Ewings Sarcoma".ti,ab OR "Ewing's Tumor".ti,ab OR "Ewings Tumor".ti,ab OR "Ewing Tumor".ti,ab OR "Ewing's Tumour".ti,ab OR "Ewings Tumour".ti,ab OR "Ewing Tumour".ti,ab OR "Ewing sarcomas".ti,ab OR "Ewing's Sarcomas".ti,ab OR "Ewings Sarcomas".ti,ab OR "Ewing's Tumors".ti,ab OR "Ewings Tumors".ti,ab OR "Ewing Tumors".ti,ab OR "Ewing's Tumours".ti,ab OR "Ewings Tumours".ti,ab OR "Ewing Tumours".ti,ab OR "Ewing-like sarcoma".ti,ab OR "Ewing-like sarcomas".ti,ab) AND (exp *"Survival"/ OR exp *"Mortality"/ OR *"Survival Analysis"/ OR "survival".ti,ab OR surviv*.ti,ab OR *"Cancer Recurrence"/ OR *"Tumor Recurrence"/ OR *"Recurrent Disease"/ OR "recurrence".ti,ab OR recurr*.ti,ab OR recrudescen*.ti,ab OR relaps*.ti,ab) AND (exp *"Prognosis"/ OR "prognosis".ti,ab OR prognos*.ti,ab OR "prognostic factor".ti,ab OR "prognostic factors".ti,ab OR *"Disease-Free Survival"/ OR "Disease-Free Survival".ti,ab OR "Event-Free Survival".ti,ab OR "Treatment Outcome".ti,ab OR "outcome".ti,ab OR "outcomes".ti,ab) NOT ("Case Report".pt OR "case report".ti) AND exp "Humans"/) NOT (conference review or conference abstract).pt	1.247

Web of Science	((TI=("Ewing Sarcoma" OR "Ewing sarcoma" OR "Ewing's Sarcoma" OR "Ewings Sarcoma" OR "Ewing's Tumor" OR "Ewings Tumor" OR "Ewing Tumor" OR "Ewing's Tumour" OR "Ewings Tumour" OR "Ewing Tumour" OR "Ewing sarcomas" OR "Ewing's Sarcomas" OR "Ewings Sarcomas" OR "Ewing's Tumors" OR "Ewings Tumors" OR "Ewing Tumors" OR "Ewing's Tumours" OR "Ewings Tumours" OR "Ewing Tumours" OR "Ewing-like sarcoma" OR "Ewing-like sarcomas") AND TS=("Survival" OR "Mortality" OR "Survival Analysis" OR "survival" OR surviv* OR "Cancer Recurrence" OR "Tumor Recurrence" OR "Recurrent Disease" OR "recurrence" OR recurr* OR recrudescen* OR relaps*) AND TS=("Prognosis" OR "prognosis" OR prognos* OR "prognostic factor" OR "prognostic factors" OR "Disease-Free Survival" OR "Disease-Free Survival" OR "Event-Free Survival" OR "Treatment Outcome" OR "outcome" OR "outcomes")) OR (TS=("Ewing Sarcoma" OR "Ewing sarcoma" OR "Ewing's Sarcoma" OR "Ewings Sarcoma" OR "Ewing's Tumor" OR "Ewings Tumor" OR "Ewing Tumor" OR "Ewing's Tumour" OR "Ewings Tumour" OR "Ewing Tumour" OR "Ewing sarcomas" OR "Ewing's Sarcomas" OR "Ewings Sarcomas" OR "Ewing's Tumors" OR "Ewings Tumors" OR "Ewing Tumors" OR "Ewing's Tumours" OR "Ewings Tumours" OR "Ewing Tumours" OR "Ewing-like sarcoma" OR "Ewing-like sarcomas") AND TI=("Survival" OR "Mortality" OR "Survival Analysis" OR "survival" OR surviv* OR "Cancer Recurrence" OR "Tumor Recurrence" OR "Recurrent Disease" OR "recurrence" OR recurr* OR recrudescen* OR relaps*) AND TS=("Prognosis" OR "prognosis" OR prognos* OR "prognostic factor" OR "prognostic factors" OR "Disease-Free Survival" OR "Disease-Free Survival" OR "Event-Free Survival" OR "Treatment Outcome" OR "outcome" OR "outcomes")) OR (TS=("Ewing Sarcoma" OR "Ewing sarcoma" OR "Ewing's Sarcoma" OR "Ewings Sarcoma" OR "Ewing's Tumor" OR "Ewings Tumor" OR "Ewing Tumor" OR "Ewing's Tumour" OR "Ewings Tumour" OR "Ewing Tumour" OR "Ewing sarcomas" OR "Ewing's Sarcomas" OR "Ewings Sarcomas" OR "Ewing's Tumors" OR "Ewings Tumors" OR "Ewing Tumors" OR "Ewing's Tumours" OR "Ewings Tumours" OR "Ewing Tumours" OR "Ewing-like sarcoma" OR "Ewing-like sarcomas") AND TS=("Survival" OR "Mortality" OR "Survival Analysis" OR "survival" OR surviv* OR "Cancer Recurrence" OR "Tumor Recurrence" OR "Recurrent Disease" OR "recurrence" OR recurr* OR recrudescen* OR relaps*) AND TI=("Prognosis" OR "prognosis" OR prognos* OR "prognostic factor" OR "prognostic factors" OR "Disease-Free Survival" OR "Disease-Free Survival" OR "Event-Free Survival" OR "Treatment Outcome" OR "outcome" OR "outcomes"))) NOT TI=("Case Report") NOT ti=(veterinary OR rabbit OR rabbits OR animal OR animals OR mouse OR mice OR rodent OR rodents OR rat OR rats OR pig OR pigs OR porcine OR horse* OR equine OR cow OR cows OR bovine OR goat OR goats OR sheep OR ovine OR canine OR dog OR dogs OR feline OR cat OR cats)	834

Cochrane	(("Ewing Sarcoma" OR "Ewing sarcoma" OR "Ewing's Sarcoma" OR "Ewings Sarcoma" OR "Ewing's Tumor" OR "Ewings Tumor" OR "Ewing Tumor" OR "Ewing's Tumour" OR "Ewings Tumour" OR "Ewing Tumour" OR "Ewing sarcomas" OR "Ewing's Sarcomas" OR "Ewings Sarcomas" OR "Ewing's Tumors" OR "Ewings Tumors" OR "Ewing Tumors" OR "Ewing's Tumours" OR "Ewings Tumours" OR "Ewing Tumours" OR "Ewing-like sarcoma" OR "Ewing-like sarcomas") AND ("Survival" OR "Mortality" OR "Survival Analysis" OR "survival" OR surviv* OR "Cancer Recurrence" OR "Tumor Recurrence" OR "Recurrent Disease" OR "recurrence" OR recurr* OR recrudescen* OR relaps*) AND ("Prognosis" OR "prognosis" OR prognos* OR "prognostic factor" OR "prognostic factors" OR "Disease-Free Survival" OR "Disease-Free Survival" OR "Event-Free Survival" OR "Treatment Outcome" OR "outcome" OR "outcomes"))	62
Academic Search Premier	(("Ewing Sarcoma" OR "Ewing sarcoma" OR "Ewing's Sarcoma" OR "Ewings Sarcoma" OR "Ewing's Tumor" OR "Ewings Tumor" OR "Ewing Tumor" OR "Ewing's Tumour" OR "Ewings Tumour" OR "Ewing Tumour" OR "Ewing sarcomas" OR "Ewing's Sarcomas" OR "Ewings Sarcomas" OR "Ewing's Tumors" OR "Ewings Tumors" OR "Ewing Tumors" OR "Ewing's Tumours" OR "Ewings Tumours" OR "Ewing Tumours" OR "Ewing-like sarcoma" OR "Ewing-like sarcomas") AND ("Survival" OR "Mortality" OR "Survival Analysis" OR "survival" OR surviv* OR "Cancer Recurrence" OR "Tumor Recurrence" OR "Recurrent Disease" OR "recurrence" OR recurr* OR recrudescen* OR relaps*) AND ("Prognosis" OR "prognosis" OR prognos* OR "prognostic factor" OR "prognostic factors" OR "Disease-Free Survival" OR "Disease-Free Survival" OR "Event-Free Survival" OR "Treatment Outcome" OR "outcome" OR "outcomes"))	30

Supplementary file 2

	Reviewer 1 - SB							Reviewer 2 - OA							
Study ID	Participation	Attrition	PF measure	Outcome	Confounding	Statistics	Score	Participation	Attrition	PF measure	Outcome	Confounding	Statistics	Score	Final score
1	1	2	1	1	1	1	7	7	1	2	1	1	1	1	7
2	1	1	1	1	2	2	8	8	1	1	2	1	2	1	8
3	1	2	1	1	2	1	8	8	1	2	1	1	2	1	8
4	1	2	2	1	1	2	9	9	1	2	2	1	2	1	9
5	2	1	1	1	1	1	7	7	2	1	1	1	1	1	7
6	1	2	1	2	2	1	9	9	1	2	1	2	2	1	9
7	1	1	1	2	2	1	8	8	2	1	1	1	2	1	8
8	2	2	1	1	2	2	10	9	2	1	1	1	2	2	9
9	2	1	1	1	2	1	8	11	1	2	2	2	2	2	9
10	2	1	1	2	2	2	10	10	1	1	2	2	2	2	10
11	2	1	1	1	2	1	8	8	2	1	1	1	2	1	8
12	1	1	2	1	2	1	8	7	1	1	1	1	2	1	8
13	1	2	2	2	2	2	11	11	1	2	2	2	2	2	11
14	1	1	1	2	2	1	8	8	1	1	1	2	2	1	8
15	1	1	2	2	2	2	10	10	1	2	2	2	1	2	10
16	2	2	1	2	1	2	10	9	2	2	2	1	1	1	9
17	1	1	1	1	1	1	6	6	1	1	1	1	1	1	6
18	1	1	2	2	2	1	9	9	1	1	2	2	2	1	9
19	1	1	1	1	1	1	6	6	1	1	1	1	1	1	6
20	2	2	2	1	1	1	9	9	2	2	2	1	1	1	9
21	1	2	1	1	1	1	7	7	1	2	1	1	1	1	7

Table 1 – Risk of bias scores for included studies
Abbreviations: PF = prognostic factor.

Domain	Kappa	Interpretation
Overall score	0.76	Substantial
Study participation	0.67	Substantial
Study attrition	0.72	Substantial
Prognostic factor measurement	0.46	Moderate
Outcome measurement	0.63	Substantial
Study confounding	0.79	Substantial
Statistical analysis and reporting	0.31	Fair

Table 2 – Inter-observer agreement

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CHAPTER 3

Easy-to-use clinical tool for survival estimation in Ewing sarcoma at diagnosis and after surgery

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Abstract

Accurate survival estimations in Ewing sarcoma are necessary to develop risk- and response adaptive treatment strategies allowing for early decision-making. We aim to develop an easy-to-use survival estimation tool from diagnosis and surgery.
A retrospective study of 1314 Ewing sarcoma patients was performed. Associations between prognostic variables at diagnosis/surgery and overall survival (OS), were investigated using Kaplan-Meier and multivariate Cox models. Predictive accuracy was evaluated by cross-validation and Harrell C-statistics.
Median follow-up was 7.9 years (95%CI 7.6-8.3). Independent prognostic factors at diagnosis were age, volume, primary tumor localization and disease extent. 5 risk categories (A-E) were identified with 5-year OS of 88%(86-94), 69%(64-74), 57%(50-64), 51%(42-60) and 28%(22-34) respectively. Harrell C-statistic was 0.70. Independent prognostic factors from surgery were age, volume, disease extent and histological response. In categories A-B, 5y OS increased to 92%(87-97) and 79%(71-87) respectively for 100% necrosis and decreased to 76% (67-85) and 62%(55-69) respectively for <100% necrosis. In categories C-E, 5y OS increased to 65%(55-75), 65%(52-78) and 52%(38-66) respectively for ≥90% necrosis and decreased to 38%(22-54), 11%(0-26) and 7%(0-19) respectively for <90% necrosis.
We present an easy-to-use survival estimation tool from diagnosis in Ewing sarcoma based on age, volume, primary tumor localization and disease extent. Histological response is a strong additional prognostic factor for OS.

Introduction

Ewing sarcoma (EwS) is an aggressive bone and soft-tissue tumor predominantly affecting children and young adults. (1) Management rapidly evolved over the last decades, leading to a multimodality approach consisting of chemotherapy, surgery and/or radiotherapy that has become the standard of care. As a result of collaborating trials overall survival (OS) improved drastically, with 10-year OS rates of 55-65% for localized disease. Survival in metastatic disease, present in 20-25% of the patients and usually affecting the lungs (70-80%) and bone/bone marrow (40-45%), is still dismal with 5-year OS varying from 20-35%. (2-5) In primary non-metastatic disease 30-40% of patients experience recurrence, in metastatic disease this number increases to 60-80%. Relapse is mostly systemic (71-73%), followed by combined (12-18%) and local (11-15%) relapse. (6, 7) 5-year post-relapse survival is poor, 15-25%, with local recurrence faring better than systemic. (6, 8, 9)

Personalized medicine encompasses tailoring of treatment based on individual patient characteristics, needs and preferences to improve outcome. Accurate estimations of survival according to the individual patient's risk profile at different time points are necessary to offer EwS patients the most appropriate treatment, balancing survival and prognosis with toxicity and quality of life. Especially in this young patient population, this balance is essential in our aim to provide the best possible care. Correct survival estimations are difficult and patients and physicians tend to be overoptimistic. (10) Better selection of risk groups and thereby adjusted treatment allows for early decision making, will help improve future outcomes and assists in clinical trial design.

Many studies evaluated the influence of various risk factors on survival in EwS. Only three (9, 11, 12) described combining these prognostic factors into risk groups. All three models present shortcomings. They are based on small homogeneous cohorts, that are either not validated or did not include all relevant variables in the model. Keeping these shortcomings in mind, our aim was to develop an easy-to-use survival estimation tool for EwS. Objectives are to: 1) Identify prognostic factors for overall survival from diagnosis and surgery; 2) Develop an accurate baseline prognostic model; 3) Validate the models' predictive accuracy; 4) Develop a second prognostic model from surgery.

Methods

This study was reviewed and approved by the Ethical Committee of the Leiden University Medical Center and granted a waiver for informed consent.

Study population

A retrospective analysis of patients (randomized and non-randomized) from the EURO-E.W.I.N.G 99 trial database was performed. As detailed in Figure 1, from 1480 available patients, 166 were excluded due to missing data. Thus, 1314 patients were eligible for analysis at diagnosis. Following induction chemotherapy 982

patients underwent surgery of the primary tumor, 190 were excluded due to missing data, resulting in 792 patients eligible for analysis at surgery. All patients were treated according to the protocol with the aim to administer six cycles of VIDE (vincristine, ifosfamide, doxorubic, etoposide) induction chemotherapy followed by local treatment of the primary tumor. The choice of local treatment, surgery, radiotherapy or both, was left to discretion of the multidisciplinary team. After local treatment patients received maintenance therapy.

Measures

For accurate risk group stratification large representative and contemporary datasets that closely reflect the target population are needed to enhance the relevance, reproducibility and generalizability of the model. (13-17) Cohorts often contain more variables than can reasonably be used for prediction. Therefore, the most predictive and sensible predictors should be selected. In order to provide all relevant risk factors for such a prognostic model a systematic review (18) on the current known prognostic factors for overall survival (OS) and event-free survival (EFS) was performed. Based on this systematic review we selected the most predictive and sensible predictors to be included in the univariate analysis. Prognostic factors and outcome were collected prospectively. Patient characteristics included gender and age. Tumor characteristics included location, type, volume at diagnosis, skip lesions, disease extent and number of metastatic lesions. Histological response (percentage necrosis) and resection margins were assessed on the surgical specimen by local pathologists.

Statistical analysis

The outcome of interest was overall survival (OS) measured from date of diagnosis or date of surgery, until last day of follow-up or date of death. Prognostic factors were evaluated using univariate Cox regression analyses; significant prognostic factors were subsequently included into a multivariate Cox model.

Significant risk factors at diagnosis from the corresponding multivariate Cox model were used to build a stratification scheme of prognostic groups. Prognostic groups were narrowed down into risk categories based on clinical expertise. Another set of risk categories was obtained from the same multivariate Cox model based on predicted survival; a leave-one-out cross-validation framework was used to form cross-validated risk categories on predicted 5-year survival probability (19). The prognostic value of the clinical risk categories was assessed by comparison with cross-validated risk categories. Details on cross-validation methodology and risk category classification are provided in supplementary file 1. Correspondence of clinical and cross-validated risk categories was evaluated using precision and recall (supplementary file 1). Discriminative ability of both stratification schemes was assessed using Harrell's C-index. (20) Observed survival probabilities of clinical risk categories and corresponding cross-validated counterparts were compared by Kaplan-Meier estimators.

Significant risk factors at surgery from the corresponding univariate analysis were used to build a second multivariate Cox model. Associations were considered significant at a rejection level of 5%. All analyses were performed using SPSS version 23.0, R version 3.4.3, and Python 3.6.5.

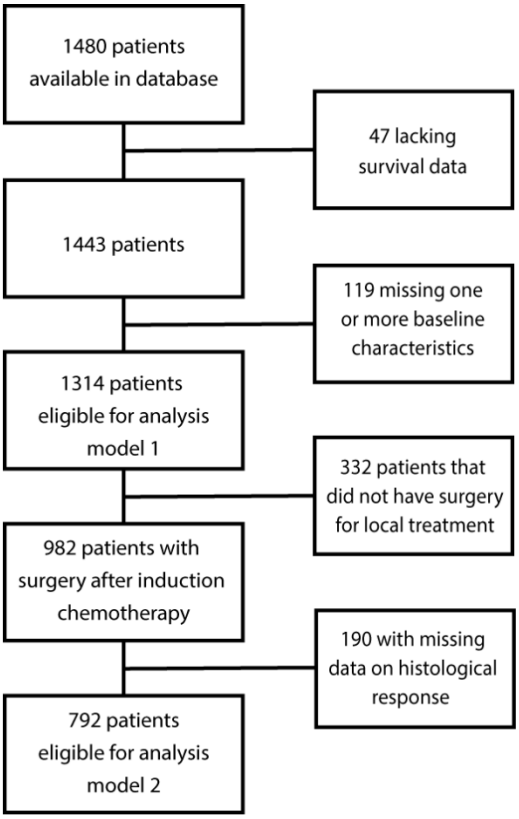

Figure 1 – Flowchart inclusion

Results

Baseline characteristics and treatment details of the 1314 patients at diagnosis are presented in Table 1. Median follow-up, assessed by reversed Kaplan-Meier method (21), was 7.9 years (95% confidence interval (CI) 7.6-8.3 years); 531 patients died. Localized disease was present in 916 (69.7%), pulmonary metastasis alone in 182 (13.9%) and extrapulmonary metastasis with or without additional pulmonary metastasis in 216 (16.4%) patients. The 5-year OS was 73% (95%CI, 70-76%), 53% (95%CI, 45-60%) and 28% (95%CI, 22-34%) respectively.

Prognostic factors at diagnosis

Univariate and multivariate Cox proportional hazard models were estimated to investigate the effect of risk factors on OS. Results are shown in Table 2. Univariate

analysis showed that age, volume, primary tumor localization, skip lesions, disease extent and number of metastatic lesions are significantly associated with OS. In multivariate analysis age ≥16 years (HR 1.36; 95%CI 1.15-1.62); p<0.001) volume ≥200 ml (HR 1.50; 95%CI 1.25-1.79;p<0.001), pelvic location (HR 1.34; 95%CI 1.07-1.67; p=0.015), pulmonary metastasis only (HR 1.79; 95%CI 1.42-2.27; p<0.001), extrapulmonary metastasis with or without pulmonary metastasis (HR 3.72; 95%CI 3.02-4.56; p<0.001) and ≥2 metastatic lesions (HR 2.80; 95%CI 2.33-3.36; p<0.001) remained significant for OS.

Baseline prognostic model

Based on the independent prognostic factors at diagnosis (age, volume, location and disease extent), 13 prognostic groups were created and 5 clinically relevant categories (A-E) were estimated. Table 3 provides a detailed description of the prognostic groups and corresponding OS at 3 and 5 years. The 5-year OS for categories A-E was 88% (95%CI 86-94), 69% (95%CI 64-74), 57% (95%CI 50-64), 51% (95%CI 42-60) and 28% (95%CI 22-34) respectively. Figure 2 presents a flowchart to stratify patients at diagnosis. Age only showed strong impact on survival in the first two prognostic groups. In the other prognostic groups survival was similar for patients aged younger than 16 and patients aged 16 and above. Age is therefor only included in the stratification scheme for the first two prognostic groups.

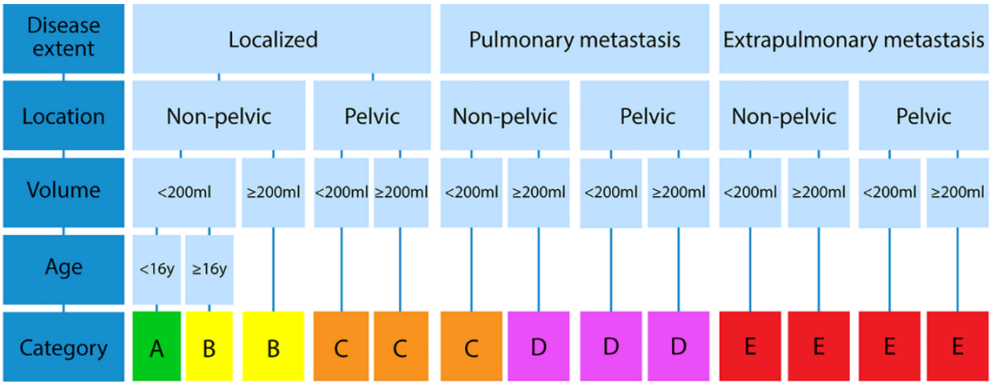

Figure 2 – Flowchart for stratification of Ewing sarcoma patients at diagnosis.

Harrell's C-statistic was 0.70. Discriminatory ability was further evaluated using cross validation. Detailed comparisons of OS in the clinical and cross-validated risk categories at 2, 3 and 5 years are presented in Table 4. Survival probabilities do not show any difference between clinical and cross-validated risk categories. The overall agreement is very good (precision 90.26%; recall 89.57%). Figure 3 illustrates the models' discrimination ability visualized by the spread of Kaplan-Meier estimates.

Characteristic	N (%)
Total	1314
Gender	
Male	792 (60.3)
Female	522 (39.7)
Age (mean, years +SD)	16,8 (9.9)
Origin	
Osseous	1107 (84.2)
Extra-osseous	207 (15.8)
Primary tumor localization	
Extremity	499 (38.0)
Upper	108 (8.2)
Lower	391 (29.8)
Axial	815 (62.0)
Pelvic	312 (23.7)
Other	503 (38.3)
Volume at diagnosis	
<200 ml	740 (56.3)
≥200 ml	574 (43.7)
Skip lesions at diagnosis	63 (4.8)
Disease extent	
Localized	916 (69.7)
Pulmonary metastasis	182 (13.9)
Extrapulmonary metastasis	216 (16.4)
Number of metastatic lesions	
One	43 (3.3)
≥2	355 (27.0)
Local treatment modality	
Surgery	550 (41.9)
Radiotherapy	193 (14.7)
Surgery + radiotherapy	432 (32.9)
Pre-operative radiotherapy	47 (3.6)
Post-operative radiotherapy	385 (29.3)
Unknown	139 (10.5)

Table 1 – Patient demographics at diagnosis
SD = standard deviation.
Continuous variables are presented by means along with corresponding standard deviation between brackets, categorical variables as a number with the percentage between brackets.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Gender				
Female	1			
Male	1.12 (0.94-1.34)	0.195		
Age				
<16 years	1		1	
≥16 years	1.53 (1.29-1.82)	**<0.001**	1.36 (1.15-1.62)	**<0.001**
Origin				
Osseous	1.13 (0.89-1.45)	0.313		
Extra-osseous	1			
Volume				
<200 ml	1		1	
≥200 ml	1.96 (1.65-2.33)	**<0.001**	1.50 (1.25–1.79)	**<0.001**
Location				
Extremity	1		1	
Axial (excl pelvic)	1.17 (0.95-1.43).	0.148	1.16 (0.94-1.44)	0.178
Pelvic	1.9 (1.54-2.35)	**<0.001**	1.34 (1.07-1.67)	**0.015**
Skiplesions at diagnosis				
No	1		1	
Yes	1.56 (1.10-2.22)	**0.013**	1.11 (0.76-1.60)	0.595
Disease extent				
Localized	1		1	
Pulmonary metastasis	2.05 (1.63-2.58)	**<0.001**	1.79 (1.42-2.27)	**<0.001**
Extrapulmonary metastasis	4.33 (3.56-5.28)	**<0.001**	3.72 (3.02-4.58)	**<0.001**
Number of metastatic lesions				
None	1		1	
One	1.71 (1.1-2.66).	**<0.001**	1.54 (0.98-2.40)	0.059
≥2	3.25 (2.73-3.87)	**<0.001**	2.80 (2.33-3.36)	**<0.001**

Table 2 – *Hazard ratio (HR) with corresponding 95% confidence interval (CI) from univariate and multivariate analysis at time of diagnosis (n=1314)*

Prognostic group	Disease extent	Location	Volume	Age	N	Overall survival (95%CI) 3 years	Overall survival (95%CI) 5 years	category
1	Localized	Non-pelvic	<200 ml	<16	296	90% (86-94)	88% (84-92)	A
2	Localized	Non-pelvic	<200 ml	≥16	207	80% (75-85)	71% (64-78)	B
3	Localized	Non-pelvic	≥200 ml		243	75% (70-80)	67% (61-73)	B
4	Localized	Pelvic	<200 ml		78	74% (64-84)	62% (50-74)	C
5	Localized	Pelvic	≥200 ml		92	67% (57-77)	53% (43-63)	C
6	Pulmonary	Non-pelvic	<200 ml		57	77% (66-88)	58% (45-71)	C
7	Pulmonary	Non-pelvic	≥200 ml		62	60% (48-72)	48% (36-60)	D
8	Pulmonary	Pelvic	<200 ml		17	82% (67-95)	76% (56-96)	D
9	Pulmonary	Pelvic	≥200 ml		46	54% (39-69)	45% (30-60)	D
10	Extrapulmonary	Non-pelvic	<200 ml		63	36% (24-48)	29% (17-41)	E
11	Extrapulmonary	Non-pelvic	≥200 ml		74	33% (22-44)	31% (20-42)	E
12	Extrapulmonary	Pelvic	<200 ml		22	46% (25-67)	46% (25-67)	E
13	Extrapulmonary	Pelvic	≥200 ml		57	21% (10-32)	17% (7-27)	E

Table 3 – Overall survival at 3 and 5 years for each prognostic group

Creation of 13 prognostic groups based on disease extent, tumor localization, volume and age showing overall survival (OS) with corresponding 95% confidence interval (CI) at 3 and 5 years. Last column shows the risk category based on clinical expertise (n=1314).

Category	n	2-year OS (95%CI)		3-year OS (95%CI)		5-year OS (95%CI)	
		Clinical	Cross-validated	Clinical	Cross-validated	Clinical	Cross-validated
A	296	93% (91-96)	93% (91-96)	90% (86-93)	90% (86-83)	88% (84-92)	88% (84-92)
B	450	85% (82-88)	84% (81-87)	77% (73-81)	76% (73-80)	68% (64-72)	66% (62-70)
C	227	74% (68-80)	76% (68-84)	68% (62-75)	70% (62-79)	52% (46-60)	56% (47-67)
D	125	57% (49-66)	57% (50-66)	50% (42-59)	50% (42-58)	41% (33-51)	40% (33-49)
E	216	39% (32-48)	36% (29-45)	30% (24-39)	28% (22-37)	28% (21-36)	25% (19-33)

Table 4 – Overall survival at 2, 3 and 5 years for clinical and cross-validated categories.

Detailed comparison of overall survival (OS) with corresponding 95% confidence interval (CI) in each of the clinical and cross-validated risk categories at 2, 3 and 5 years (n=1314).

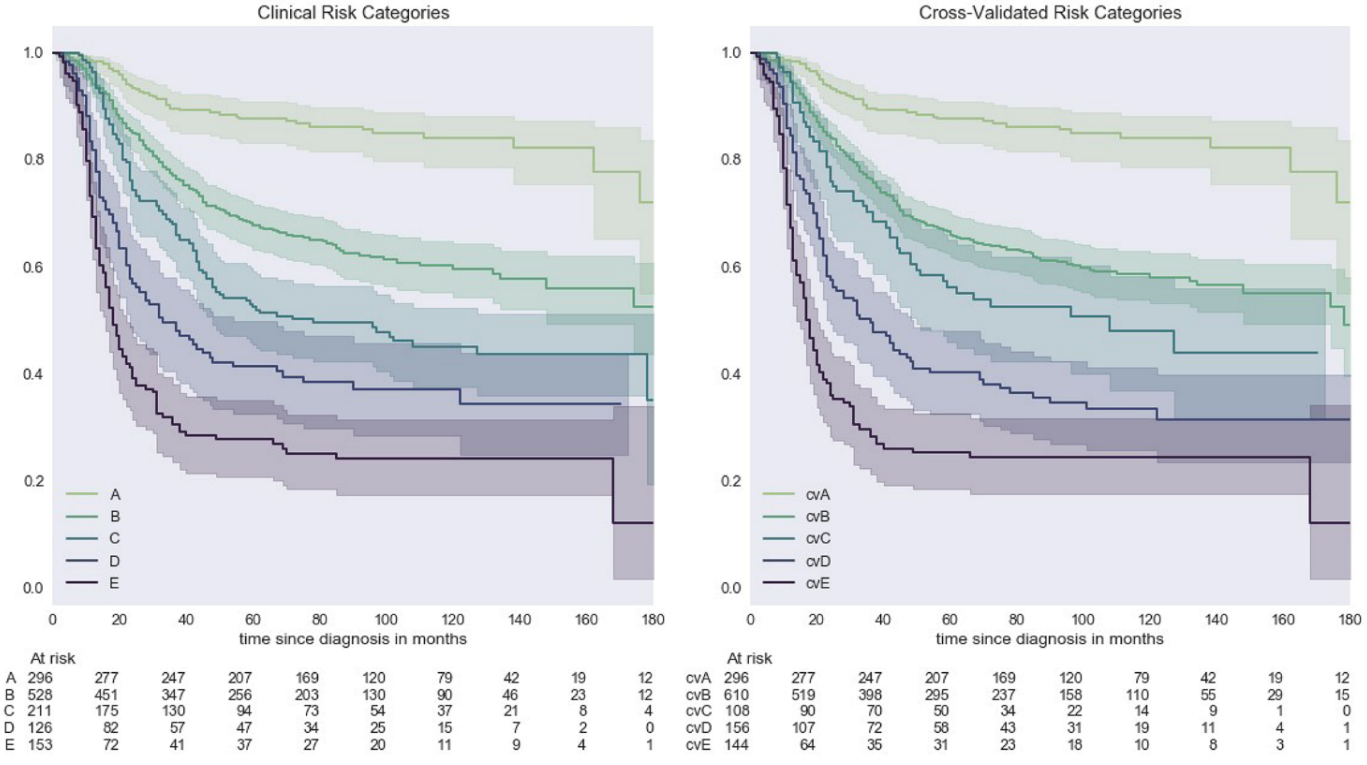

Figure 3 – Kaplan-Meier curves for overall survival of clinical risk categories and cross-validated risk categories

Survival is measured in months from diagnosis. On the left the Kaplan-Meier survival curves of the clinical risk categories (A-E) based on the 13 prognostic groups. On the right the Kaplan-Meier survival curves of the cross-validated risk categories (cvA-cvE).

Prognostic factors known at time of surgery

Table 5 shows the effect of prognostic factors known at surgery in univariate and multivariate analysis. Univariate analysis showed that age, volume at diagnosis, primary tumor localization, disease extent, number of metastatic lesions, surgical margin and histological response are significantly associated with OS. In multivariate analysis age ≥16 years (HR 1,38; 95%CI 1,08-1.77; p=0,01), pulmonary metastasis (HR 1,99; 95%CI 21.47-2,70; p<0.001), extrapulmonary metastasis with or without pulmonary metastasis (HR 3.18; 95%CI 2.23 – 4.53; p<0,001), ≥2 metastatic lesions (HR 2.53; 95%CI 1.93 – 3.32; p<0,001) and histological response of 90-99% (HR 1.58; 95%CI 1.16 – 2.16; p=0,04) and of < 90% (HR 2.90; 95%CI 2,15 – 3,93; p<0,001) remained significant prognostic factors for OS.

Effect of histological response on overall survival

A multivariate Cox model with prognostic factors histological response, risk categories and an interaction term was estimated. The interaction between histological response and risk category was not significant, meaning that the effect of histological response does not vary significantly across the risk categories. The association between histological response and OS was therefore assessed by fitting a Cox model with risk category and histological response, details are presented in Table 6.

Figure 4 presents a flowchart to stratify patients at surgery based on the Cox model. For patients in category A with 100% necrosis, 5y OS increased to 92% (95%CI 87-97), but decreased to 76% (95%CI 67-85) when necrosis was <100%. For patients in category B, 5y OS increased to 79% (95%CI 71-87) when necrosis was 100% and decreased to 62% (95%CI 55-69) when necrosis was <100%. In category C, survival increased to 65% (95%CI 55-75) when necrosis was ≥90% and decreased to 38% (95%CI 22-54) when necrosis was <90%. In category D, 5y OS increased to 65% (95%CI 52-78) when necrosis was ≥90% but decreased to 11% (95%CI 0-26) when necrosis was <90%. The same pattern accounts for category E where 5y OS increases to 52% (95%CI 38-66) when necrosis was ≥90% necrosis but drastically decreases to 7% (95%CI 0-19) when necrosis was <90%.

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	**p**	**HR (95%CI)**	**p**
Gender				
Female	1			
Male	1.08 (0.84-1.37)	0.564		
Age				
<16 years	1		1	
≥16 years	1.53 (1.20-1.94)	**<0.001**	1.38 (1.08-1.77)	**0.010**
Origin				
Osseous	1			
Extra-osseous	1.23 (0.87-1.74)	0.245		
Volume				
<200 ml	1		1	
≥200 ml	1.65 (1.30-2.09)	**<0.001**	1.29 (0.99-1.66)	0.053
Location				
Extremity	1		1	
Axial (excl pelvic)	1.09 (0.82-1.43)	0.564	1.05 (0.79-1.41)	0.735
Pelvic	1.59 (1.18-2.15)	**0.002**	1.30 (0.94-1.79)	0.110
Disease extent				
Localized	1		1	
Pulmonary metastasis	2.09 (1.55-2.81)	**<0.001**	1.99 (1.47-2.70)	**<0.001**
Extrapulmonary metastasis	2.88 (2.03-4.08)	**<0.001**	3.18 (2.23-4.53)	**<0.001**
Number of metastatic lesions				
None	1		1	
One	1.52 (0.85-2.73)	0.159	1.62 (0.90-2.92)	0.108
≥2	2.54 (1.96-3.29)	**<0.001**	2.53 (1.93-3.32)	**<0.001**

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	**p**	**HR (95%CI)**	**p**
Margin status				
Wide	1		1	
Marginal	**1.48 (1.08-2.03)**	**<0.001**	1.06 (0.76-1.47)	0.736
Intralesional	2.43 (1.55-3.81)	**<0.001**	1.47 (0.91-2.93)	0.120
Histological response				
100%	1		1	
90-99%	1.66 (1.22-2.25)	**<0.001**	1.58 (1.16-2.16)	**0.004**
<90%	2.86 (2.15-3.81)	**<0.001**	2.90 (2.15-3.93)	**<0.001**
Radiotherapy				
No	1			
Pre-operative radiotherapy	1.19 (0.71-1.99)	0.503		
Post-operative radiotherapy	1.10 (0.85-1.41)	0.478		

Table 5 - *Hazard ratio (HR) with corresponding 95% confidence interval (CI) from univariate and multivariate analysis at time of surgery (n=792).*

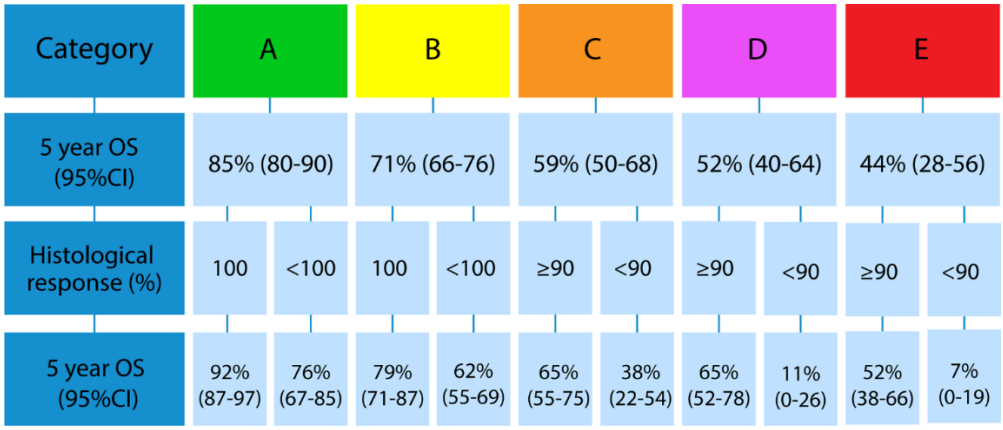

Figure 4 – Flowchart for stratification of Ewing sarcoma patients at surgery assessed by Kaplan Meier method.

		Cox model	
	N	**HR (95%CI)**	**p**
Histological response			
100%	360	1	
90-99%	224	1.57 (1.15-2.12)	**0.004**
<90%	208	3.15 (2.37-4.19)	**<0.001**
Risk category			
A	199	1	
B	316	2.07 (1.42-3.03)	**<0,001**
C	135	3.68 (2.46-5.52)	**<0,001**
D	73	4.38 (2.64-7.28)	**<0,001**
E	69	6.23 (3.72-10.44)	**<0,001**

Table 6 – Cox model for overall survival from surgery.
Hazard ratio (HR) along with 95% confidence interval (CI) (n=792).

Discussion

To further improve survival in Ewing sarcoma development of risk- and response adaptive treatment strategies are necessary to allow decision making at different disease stages. Accurate survival estimations are challenging. We developed and validated an easy-to-use survival estimation tool for EwS, based on age, volume, primary tumor localization and disease extent. Furthermore, we show that during the course of treatment survival changes as more information becomes available.

The model presented is based on a cohort of 1314 EwS patients with uniformity in diagnostics and treatment and availability of all relevant prognostic factors. The provided flowcharts are easy-to-use and based on assessable variables. The 13 prognostic groups provide detailed insight in expected survival and could assist in fine-tuning individual treatment. The prognostic groups were narrowed down to 5 risk categories (A-E) based on clinical expertise. The risk categories defined on clinical criteria are consistent with cross-validated risk categories defined on predicted 5-year survival probability. The information gained after surgery offers a second time-point for multidisciplinary decision-making, at this point histological response is an strong additional prognostic factor for OS.

The prognostic significance of the variables in both models has previously been reported. Disease extent is the foundation of the model and strongest prognostic factor in this study. This is consistent with previous studies demonstrating that the

presence of metastasis is a strong prognostic factor for survival (22-24); patients with extrapulmonary metastasis do significantly worse than patients with pulmonary metastasis alone. (2, 25, 26) Disease extent is also used to define risk groups in previous and current European EwS trials. We also found that primary tumors in the pelvic strongly affect survival, consistent with previously published studies. (27) Other studies suggested an adverse effect on survival for axial localizations (including pelvic) compared to tumours in the extremities. (11, 28-30) Volume has also been used to design EwS trails (31); research shows that larger volumes are associated with poorer survival. Cut-off points at 100 ml (26) and 150 ml (32) have been evaluated, but 200 ml seems the most appropriate (33, 34) and was therefore used in this study. Age is an independent prognostic factor for survival in the current study, but only shows strong impact on outcome in two prognostic groups. Cut-of points at 18 (22, 29, 30) and 14 years (35) have been evaluated. Strong evidence for a specific cut-off point is lacking. All studies consistently show that older age is associated with poorer survival. We chose 16 years as cut-off, as it is at the interface of pediatric and adult treatment. Histological response, used to tailor treatment in European EwS, is considered of high prognostic value as confirmed in this study. According to literature patients with 100% necrosis have the best survival (28, 32), other studies showed similar results using cut-of points at 95% (36) and 90% necrosis (33).

To our knowledge, only three studies described combining prognostic factors into risk groups. Rodriquez-Galindo et al. (9) used Cox proportional hazards models to identify four risk groups in 220 EwS patients based on age (</≥ 14 years), primary tumor site (pelvic/non-pelvic) and disease extent (localized/isolated lung metastasis/extrapulmonary metastasis). Although based on a small cohort and not validated, our risk groups are similar, with the exception that we added volume to the model. Although they found that tumor size was an independent prognostic factor for survival, they did not include it in the final model. Biswas et al. (11) developed a prognostic model for localized EwS based on 244 patients. Cox models were estimated showing that patients with axial tumors and elevated white blood cell count (WBC) (>11×109/L) had poor OS (HR 4.44 (95%CI 2.1-9.4; p<0.001) and patients with symptoms >4 months, tumor size ≥8 cm and elevated WBC had poor event-free survival (HR 3.89 (95%CI 1.63-9.26; p=0.002). These models were not validated and are based on a small unmixed cohort limiting its usefulness for clinical decision-making. Additionally, in the systematic review we performed before the start of the current study a consistent association between several biomarkers, such as neutrophil to lymphocyte ratio, hemoglobin and WBC count could not be found, in contrast to the model of Biswas et al. (11) and another study (37). Lastly Karski et al. (12) derived prognostic groups from 2124 EwS patients in the Surveillance, Epidemiology, and End Results (SEER) database. Using Cox models for OS they constructed five prognostic groups: 1) Localized, <18 years, non-pelvic; 2) Localized, <18 years, pelvic or localized, ≥18 years, White/non-Hispanic; 3) Localized, ≥18 years, other ethnicities; 4) Metastatic, <18 years; 5) Metastatic, ≥18 years. Validation

was performed on a cohort of 1680 EwS patients from the Children's Oncology Group trials, which showed significantly different OS based upon this classification. Although validated, the primary model did not include all relevant variables as the SEER database lacks information on metastatic site. In addition tumor size was missing in 40% of the patients and therefore not included, limiting the strengths of the models.

Limitation of this study include the fact that the local treatment choice was left to discretion of the threatening multidisciplinary teams and might have influenced the results discussed in this article. Secondly, a good prediction model should provide accurate prediction of events by using a comprehensive dataset. In addition, the model should be relatively simple and clinically easy to use. Inaccurate estimates of future events will mislead physicians to provide insufficient treatment. On the other hand, a model with high predictability but which is complex or has too many factors will not be useful. Achieving the optimal balance between predictability and simplicity is the key to a good prediction model. (13-17) Cohorts often contain more variables than can reasonably be used for prediction and for sufficient power one needs at least 10 events per variable. We therefor choose to select the most predictive and sensible predictors to be included in the univariate analysis based on our systematic review. (18) Using a more extensive variable profile could have given useful insights, but we feel that by doing so we would lose simplicity while not improving predictability. Third, surgical margins and histological response were assessed by local pathologists and not by a reference pathologist. Differences between centers in analyzing specimens are possible. Last, the retrospective study design using data form a prospectively performed trial led to some missing data (11%), despite this, a large cohort of EwS patients was available for analysis.

Conclusion

This study presents an easy-to-use clinical tool to predict OS from diagnosis in EwS, based on age, tumor volume, tumor localization and disease extent. After surgery, the second multidisciplinary decision point, histological response is a strong additional prognostic factor for OS.

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CHAPTER 4

Individual risk evaluation for local recurrence and distant metastasis in Ewing sarcoma: a multistate model

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Abstract

Background
Here we investigate the effect of surgical margins, histological response and radiotherapy, on local recurrence (LR), distant metastasis (DM) and survival in Ewing sarcoma.

Procedure
Disease evolution was retrospectively studied in 982 patients with Ewing sarcoma undergoing surgery after chemotherapy using a multistate model with initial state surgery, intermediate states LR, pulmonary metastasis (DMpulm), other DM±LR (DMother) and final state death. Effect of risk factors was estimated using Cox proportional hazard models.

Results
Median follow-up was 7.6 years (95%CI 7.2–8.0). Risk factors for LR are pelvic location, HR 2.04(1.10-3.80); marginal/intralesional resection, HR 2.28(1.25-4.16) and radiotherapy, HR 0.52(0.28-0.95); for DMpulm are <90% necrosis, HR 2.13(1.13-4.00), and previous pulmonary metastasis, HR 4.90(2.28-8.52); for DMother are 90-99% necrosis, HR 1.56(1.09-2.23), <90% necrosis, HR 2.66 (1.87-3.79), previous bone/other metastasis, HR 3.08(2.03-4.70) and risk factors for death without LR/DM are pulmonary metastasis, HR 8.08(4.01-16.29), bone/other metastasis, HR 10.23(4.90-21.36) and <90% necrosis, HR 6.35(3.18-12.69). Early LR (0-24 months) negatively influences survival, HR 3.79(1.34-10.76). Once DMpulm/DMother arise only previous bone/other metastasis remain prognostic for death, HR 1.74(1.10-2.75).

Conclusion
Disease extent and histological response are risk factors for progression to distant metastasis or death. Tumor site and surgical margins are risk factors for LR. If disease progression occurs, previous risk factors lose their relevance. In case of isolated LR, time to recurrence is important for decision-making. Radiotherapy seems protective for LR especially in pelvic/axial. Low percentages of LR in extremity tumors and associated toxicity questions the need for radiotherapy in extremity Ewing sarcoma.

Introduction

Ewing sarcoma is an aggressive primary bone tumor, predominantly affecting children and young adults.(1) At the time of diagnosis 20 to 25% of the patients present with pulmonary (70-80%) and/or osseous (40-50%) metastases. A multimodal approach to treatment drastically improved survival of patients with localized Ewing sarcoma, with a ten-year overall survival of 55-65% nowadays. However, local recurrence, distant metastasis, and poor survival in patients with metastatic disease with a five-year overall survival of 20-35%, still remain of great concern. (2, 3) One of the strongest risk factors is the presence of metastasis at diagnosis (4, 5) and site of metastatic lesions, patients with extrapulmonary metastasis do significantly worse than patients with pulmonary metastasis alone. (2, 6) Other well-known risk factors are the primary tumor site (7-9) and tumor volume and/or size. (6, 10-12) Principles of treatment consist of neo-adjuvant chemotherapy followed by local treatment of the primary tumor, either by surgery, radiotherapy or both, and adjuvant chemotherapy. The histological response, assessed after surgery, is a strong additional prognostic factor for OS. (7, 10, 11). The effect of surgical margins on survival is controversial. The risk of local relapse is significantly lower after wide resection compared to marginal or intralesional resections. (13, 14). How the occurrence of a local recurrence may affect overall survival is not yet clearly established. (9, 15) If surgery with or without radiotherapy is superior compared to radiotherapy only in order to maximize local control alone is also under debate. Existing evidence is based on retrospective, non-randomized trials. (16, 17) Several studies show advantage of post-operative radiotherapy (PORT) for patients with marginal or intralesional resections in terms of improved local control and event-free survival. (10, 12, 13, 16, 18) Possible association between PORT and overall survival, and between local recurrence and overall survival are not yet clearly established. The main problem in current studies on prognostic factors for Ewing sarcoma is that they are hampered by the choice of outcome variable. In general, overall survival, local recurrence free survival, or disease-free survival are reported. Multiple analyses for these different endpoints are usually utilized, however the relationship between those different endpoints cannot be investigated by using separate models. Multistate models can overcome these problems since the evolution of the disease and the occurrence of intermediate events such as local recurrence and distant metastasis which occur after surgery are incorporated in the model, which provides useful insights into their relation with the considered endpoint, usually death. (19-21)

This study aims to investigate the effect of surgical margins, histological response, and radiotherapy, on local recurrence (LR), distant metastasis (DM), and overall survival in a large cohort of patients with Ewing sarcoma treated according to the EURO-E.W.I.N.G 99 protocol (EUROpean Ewing tumor Working Initiative of National Groups-Ewing Tumor Studies).

Methods

This retrospective study was reviewed and approved by the institutional review board of Leiden University Medical Center (Leiden, The Netherlands) and a waiver for informed consent was granted. A retrospective analysis of patients from the GPOH registry (Gesellschaft für Pädiatrische Onkologie und Hämatologie) treated in or according to the EURO-E.W.I.N.G 99 (EE99) protocol (22) was performed. All patients were treated between 1999 and 2009, and followed up until the end of 2017. All patients were treated according to the protocol with the aim to administer six cycles of VIDE (vincristine, ifosfamide, doxorubicin, etoposide) induction chemotherapy followed by local treatment of the primary tumor. The choice of local treatment, surgery with or without radiotherapy or definitive radiotherapy was directed by specific guidelines in the protocol however the choice of the local multidisciplinary team prevailed. According to the EE99 protocol surgery was favoured whenever feasible, only in case of an inoperable lesion that cannot be completely resected or a tumors in a critical site where complete surgery would cause unacceptable morbidity, definitive radiotherapy is indicated. Pre-operative radiotherapy was indicated in case of clinical progression under chemotherapy or anticipated marginal or intralesional respectability. PORT was indicated in intralesional or marginal surgery and advised in cases with a poor histological response (<90% necrosis) regardless of surgical margins. Advised radiotherapy doses were 44.8 Gy to 54.4 Gy with a boost to a maximum of 64 Gy using a shrinking field technique. After local treatment patients received maintenance chemotherapy. Only patients that underwent surgery (with or without radiotherapy) of the primary tumor after induction chemotherapy were eligible for inclusion in this study. A total of 982 patients, 470 study patients and 512 registry patients (that were treated according to the protocol but not randomized), was found to be eligible for inclusion in this study.

Measures

The following data was extracted from the GPOH registry: age (0-10 years; 11-18 years; >18 years), gender, disease extent (localized, pulmonary metastasis only, other metastasis), tumor volume (<200ml / ≥200 ml), tumor location (extremity / axial non-pelvic/ pelvic), PORT (yes / no), surgical margin (wide / marginal / intralesional), histological response (<90% / 90-99% / 100% necrosis), and follow-up data on local recurrence (LR), distant metastasis pulmonary (DMpulm), distant metastasis extrapulmonary with or without pulmonary metastasis (DMother). Histological response and resection margins were assessed on the surgical specimen by experienced local pathologists. Local recurrence was defined as local regional recurrence after initial complete response. Distant metastasis was defined as new metastatic disease or recurrence of metastatic disease after initial complete response.

Statistical analysis

Overall survival (OS) was measured from date of surgery, until last day of follow-up or date of death and evaluated using Kaplan-Meier's estimates. To model disease progression, the multistate model illustrated in Figure 1 was estimated. The following five states are considered: alive after surgery without adverse events (state 1 - surgery); alive with LR (state 2 - LR); alive with pulmonary DM (state 3 - DMpulm); alive with other DM (state 4 - DMother); death (state 5). The effect of risk factors on each specific transition was estimated by using a Cox proportional hazard regression model; hazard ratios (HR) along with their 95% confidence intervals (95% CI) were estimated.

Missing data

For 776 (79%) of the 982 patients, information on all the covariates of interest was complete. Missing data was observed for the variable histological response (19%) and surgical margins (5%). In order to make full use of the available data, missing values were imputed using multiple imputation. Five complete datasets were generated. The multistate model was estimated on each of the imputed datasets and the results were then combined using Rubin's rule. (23) Multiple imputation is a well-known technique used to reconstruct data when there is a small percentage of missing data. Another common approach is to drop cases with missing values and only analyze complete cases, however this reduces the amount of patients and therefore the power of the statistical tests and may even lead to biased results in some scenarios (24) All analysis were performed using R version 3.5.1 (25). The R-package mstate (26) was used to estimate the multi-state model and to compute the occupation probabilities. The R-package Amelia II was used to impute the missing data. (27)

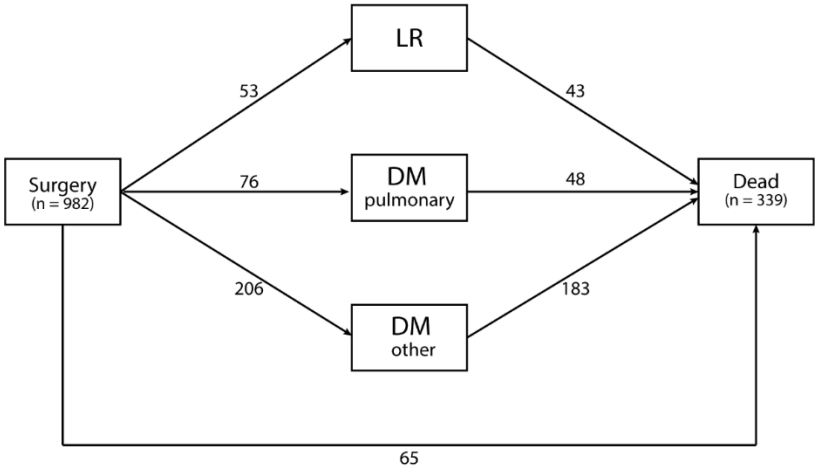

Figure 1 – Multistate model for Ewing sarcoma

Results

Table 1 summarizes patient- and tumor characteristics and treatment for the 982 included patients, 470 study and 512 registry patients, at the time of surgery. The median follow-up, estimated with reversed Kaplan-Meyer analysis, was 7.6 years (95% CI 7.2 – 8.0 years). The 5-year OS was 74% (71-77%) for localized disease, 56% (47-55%) for pulmonary metastasis and 43% (33-53%) for extrapulmonary metastasis. For patients that only had surgery as local treatment 5-year OS was 75% (71-79%) for localized disease, 52% (39-65%) for pulmonary metastasis and 41% (28-54%)) for extrapulmonary metastasis. For patients that had surgery with radiotherapy the 5-year OS was 74% (69-79%)) for localized disease, 59% (47-71%) for pulmonary metastasis only and 48% (31-65%) for extrapulmonary metastasis. In the group of patients treated with surgery and radiotherapy there were more pelvic tumors (21% versus 15% in the surgery group), more marginal and intralesional surgical margins (39% versus 21% in the surgery group) and less patients with 100% tumor necrosis (33% versus 52% in the surgery group). The other patient- and tumor characteristics were similar between both groups, see also supplementary file 1.

In total 10% (99 out of 982) of the patients developed LR. With respect to the location of the primary tumor, 13% of pelvic tumors (21 out of 169); 14% (55 out of 388) of non-pelvic axial tumors, and 5% (23 out of 425) of extremity tumors developed LR. 53 patients developed isolated LR, 8% (14/169) of pelvic tumors, 8% (30/388) of non-pelvic axial tumors and 2% (9 out of 425) of extremity tumors. The percentage of LR was similar for patients treated with surgery and surgery with radiotherapy, 6% versus 5% respectively. 28 (out of 128) patients with isolated pulmonary metastasis at diagnosis developed new pulmonary metastasis during follow-up. The percentage of patients that developed new pulmonary metastasis was similar for patients treated with surgery and surgery with radiotherapy, 7% versus 8% respectively. 39% (33 out of 84) patients with previous bone/other metastasis and 21% (27 out of 128) of patients with pulmonary metastasis only developed new extrapulmonary metastasis during follow-up. The percentage of patients that developed new extrapulmonary metastasis was similar for patients treated with surgery and surgery with radiotherapy, 20% versus 22% respectively. Table 2 provides more details of the patient- and tumor characteristics of patients that developed local recurrence, pulmonary metastasis and other/bone metastasis with or without local recurrence with respect to the local treatment modality used.

Characteristic	N (%)	Study	Registry
Total	982	470	512
Gender			
Male	590 (60)	280 (60)	310 (60)
Female	392 (40)	190 (40)	202 (40)
Age			
0-10 years	252 (26)	117 (25)	135 (26)
11-18 years	452 (46)	225 (48)	227 (44)
>18 years	278 (28)	128 (27)	150 (30)
Primary tumor localization			
Pelvic	169 (17)	75 (16)	94 (18)
Non-pelvic	813 (83)	395 (84)	418 (82)
Extremity	425 (43)	224 (48)	201 (40)
Axial	388 (40)	171 (36)	217 (42)
Volume at diagnosis			
<200 ml	577 (59)	311 (66)	266 (52)
≥200 ml	405 (41)	159 (34)	246 (48)
Disease extent at diagnosis			
Localized	770 (78)	417 (89)	353 (69)
Pulmonary metastasis	128 (13)	53 (11)	75 (15)
Extrapulmonary metastasis	84 (9)	0 (0)	84 (16)
Surgical margin			
Wide	717 (73)	352 (75)	365 (71)
Marginal	161 (16)	74 (16)	87 (17)
Intralesional	104 (11)	44 (9)	60 (12)
Histological response			
100%	426 (43)	225 (48)	202 (39)
90-99%	284 (29)	151 (32)	133 (26)
<90%	271 (28)	94 (20)	177 (35)
Post-operative radiotherapy			
No	550 (56)	284 (60)	266 (52)
Yes	432 (44)	186 (40)	246 (48)

Table 1 – Patient demographics and treatment characteristics after surgery for the 982 included patients.

In the multistate model (Figure 1) estimated to study the evolution of the disease after surgery the following five states are considered: alive after surgery without adverse events (state 1); alive with LR (state 2); alive with pulmonary DM (state 3); alive with DMother (state 4); death (state 5). State 5 is called absorbing: once a patient has entered the state, she/he stays there. This leaves us with a model with seven transitions which describe the possible paths a patient may follow after surgery. For each transition in the model the number of patients that moved from one state to the other are reported. 53 (5%) of the patients moved from surgery to LR, 76 (7%) of the patients moved from surgery to DMpulm, 206 (21%) of the patients moved from surgery to DMother, and 65 (7%) of the patient died without the occurrence of LR or DM. 60% (39/65) of these patients had metastatic disease at diagnosis and died of progressive disease. 9% (6/65) died of therapy related complications, and 15% (10/65) due to a secondary malignancy. For the remaining 10 patients the cause of dead was unknown. In total, 339 patients (35%) died. Hazard ratios (HRs) for each risk factor along with their 95% confidence interval (95%CI) for each transition were estimated using a multivariate Cox proportional hazard regression model (Table 3).

As table 3 shows, the effect of risk factors is different for each transition in the model. The main prognostic factors for transition 1 (surgery to LR) are primary tumors located in the pelvis (HR 2·04; 95%CI 1·10-3·80) and marginal or intralesional resection margins (HR 2·28; 95%CI 1·25-4·16). The administration of radiotherapy seems protective for LR for all tumor sites combined (HR 0·52; 95%CI 0·28-0·95). Radiotherapy was not randomized in this study, but was recommended, in the EE99 protocol, in case of intralesional or marginal resection and in case of poor histological response (defined as less than 90% necrosis) regardless of surgical margins. However, guidelines were not always followed. 143 patients (26%) treated with surgery alone had, based on the protocol guidelines, an indication for post-operative radiotherapy and 190 patients (44%) who received post-operative radiotherapy had no indication for it based on the protocol guidelines. The main prognostic factor for patients moving from surgery to new pulmonary metastasis or recurrence of pulmonary metastasis after initial complete response (transition 2) is a histological response of less than 90% necrosis (HR 2·13; 95%CI 1·13-4·00) and previous pulmonary metastasis (HR 4·90; 95%CI 2·28-8·52). Risk factors for the transition surgery to new bone/other DM with or without LR (transition 3) are histological response (HR 1·56; 95%CI 1·09-2·23 for 90-99% necrosis and HR 2·66;95%CI 1·87-3·79 for <90% necrosis) and previous bone/other metastasis with or without pulmonary metastasis (HR 3·08; 95%CI 2·03-4·70).

Characteristic	Total n (%)	Surgery (n=550)	Surgery + radiotherapy (n=432)
Local recurrence	**53/982 (5)**	**33 (6)**	**20 (5)**
Location primary tumor			
Extremity	9/425 (2)	7	2
Non-pelvic axial	30/388 (8)	16	14
Pelvic	14/169 (8)	10	2
Surgical margin			
Wide	30/717 (4)	23	7
Marginal	12/161 (7)	4	8
Intralesional	11/104 (11)	6	5
Histological response			
100%	16/426 (4)	13	3
90-99%	19/284 (7)	11	8
<90%	18/271 (7)	9	9
Distant metastasis – pulmonary	**76/982 (8)**	**41 (7)**	**35 (8)**
Disease extent			
Localized	46/770 (6)	24	22
pulmonary metastasis	28/128 (22)	15	13
Bone/other metastasis	2/84 (2)	2	0
Histological response			
100%	25/426 (6)	18	7
90-99%	26/284 (9)	15	11
<90%	25/271 (9)	8	17
Distant metastasis – bone/other with or without LR	**206 (21)**	**110 (20)**	**96 (22)**
Disease extent			
Localized	146/770 (19)	76	70
Metastatic pulmonary	27/128 (21)	11	16
Metastatic bone/other	33/84 (39)	23	10
Histological response			
100%	65/426 (15)	37	28
90-99%	60/284 (21)	35	25
<90%	81/271 (30)	38	43

Table 2 – Patient-, tumor and treatment characteristics of patients that developed local recurrence, pulmonary metastasis and other/bone metastasis with or without local recurrence

Disease extent (HR 8·08; 95%CI 4·01-16·29 for pulmonary metastasis and HR 10·23; 95%CI 4·90-21·36) for bone/other metastasis) and histological response (HR 6·35; 95%CI 3·18-12·69 for <90% necrosis) are risk factors for transition 4 (surgery to death). The administration of radiotherapy, which is not given randomly, seems to be protective for transition surgery to death (HR 0·45; 95%CI 0·26-0·76). The effect of time to recurrence is prognostic for survival with a HR of 3·79 (95%CI 1·34-10·76) for recurrence in the first 0-24 months. Histological response and disease extent are prognostic value for the transition surgery to new pulmonary metastasis (DMpulm) but in the presence of new pulmonary disease no statistically significant effect of histological response and disease extent on survival was observed (transition DMpulm death). Histological response was also a risk factor for transition 3 (surgery to DMother), but in the presence of new metastatic disease at diagnosis histological response is not a prognostic factor for survival anymore. Only previous bone/other metastasis with or without pulmonary metastasis remain of prognostic value in the presence of new metastatic disease (HR 1·74; 95%CI 1·10-2·75 for transition 7).
The estimated multistate model was used to estimate outcome probabilities for specific patients Estimations of these probabilities are based on the results obtained from the Cox model on the transition hazards between different states. Different patient- and tumor characteristics are considered. Figure 2 and 3 visualize the effect of local treatment modality on the patient specific state occupation probabilities at different time points after surgery. The distance between two curves represents the probability of being in a specific state at a specific time point. Figure 2 illustrates two treatment scenarios (surgery with radiotherapy (left panel) versus surgery alone (right panel)) for a patient with a localized non-pelvic Ewing sarcoma less than 200 ml and marginal/intralesional margins for <90% necrosis, 90-99% necrosis, and 100% necrosis. Figure 3 shows two treatment scenarios (surgery with radiotherapy (left panel) versus surgery alone (right panel)) for a patient with a localized pelvic Ewing sarcoma and wide margins for <90% necrosis, 90-99% necrosis, and 100% necrosis. After surgery, the probability of occupying the state “local treatment” decreases. The probabilities of occupying the states “local recurrence”, “DMpulm”, and “DMother” are similar for patients treated with surgery and surgery with radiotherapy regardless of the tumor site, surgical margins and histological response. However, radiotherapy was not randomized so these results should be interpreted with caution.

Discussion

In Ewing sarcoma local recurrence, distant metastasis, and poor survival in patients with metastatic disease remain of great concern. Associations between local treatment modality, local recurrence, distant metastasis, and death are not yet clearly established. In this study we investigated the effect of surgical margins, histological response, and radiotherapy, on the intermediate events local recurrence,

distant metastasis, and on survival in a large cohort of patients with Ewing sarcoma using a multistate model.

Marginal or intralesional surgical margins are an important risk factor for transition from surgery to LR and when a patient reaches the LR state it was observed that the probability of death is higher in case of early LR (0-24 months), so time to recurrence could be considered as most relevant in these situations. Histological response is a strong prognostic factor for transition from surgery to distant metastasis and death. When a patient experiences new distant metastasis (either pulmonary, bone, other or combined), histological response loses relevance as a risk factor as the occurrence of distant metastasis more dramatically affects survival. Administration of radiotherapy seems to be protective for LR. Other prognostic factors identified in this study were the primary tumor site and disease extent. A pelvic tumor site is an important risk factor for transition from surgery to LR. Previous pulmonary metastasis is a risk factor for transition to new pulmonary disease, but when a patient experiences new pulmonary disease, previous pulmonary metastasis is no longer prognostic factor for survival. Previous pulmonary or bone/other metastasis is a risk factor for transition to new bone/other metastasis with or without simultaneous LR. When reaching the DMother state only previous bone/other metastasis remain of prognostic value for survival.

The prognostic value of disease extent (2, 4-6), histological response (7, 10), primary tumor site (7-9) and surgical margins (10, 13-15) observed in this study is consistent with previous studies. Several large studies show advantage of PORT for patients with marginal or intralesional resections. (10, 12, 13, 16, 18) In addition to previous studies, this study has extended the knowledge about the effect of prognostic factors for intermediate events and final event death in Ewing sarcoma. We showed that prognostic factors have different effects on different transitions and that the impact on the next state in the evolution of the disease depends on the state a patient occupies. Apart from the patient's history, the time-element is also of paramount importance for decision-making. LR within 2 years or the occurrence of distant metastasis with or without subsequent LR significantly affect survival chances, and despite our efforts as physicians almost all patient that experience such an event died of progressive disease. Therefore, the balance between the toxicity of intensive salvage treatments and quality of life in the remaining life span of these patients should be carefully considered. In case of late local recurrence (at least 2 years after treatment) there is no standard approach. The patients' age and preferences, previous treatment and tumor characteristics such as location, should all be considered and discussed in a multidisciplinary setting.

	Transition													
	1: Surgery → LR		2: Surgery → DMpulm		3: Surgery → DM other		4: Surgery → death		5: LR → death		6: DMpulm → death		7: DMother → death	
Predictor	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Age														
0-10 years					1								1	
11 – 18 years					**1.55**	**1.05 – 2.27**							0.85	0.56 – 1.31
>18 years					**1.85**	**1.24 – 2.76**							0.97	0.60 – 1.55
Tumor site														
Non-pelvic	1		1		1								1	
Pelvic	**2.04**	**1.10 – 3.80**	1.64	0.96 – 2.83	1.32	0.93 – 1.86							0.98	0.66 – 1.44
Disease extent														
Localized + <200ml			1		1		1				1		1	
Localized + ≥200 ml			1.47	0.82 – 2.62	1.34	0.96 – 1.86	1.87	0.91 – 3.85			1.34	0.62 – 2.88	1.06	0.73 – 1.53
Metastatic pulmonary			**4.90**	**2.82 – 8.52**	1.52	0.98 – 2.36	**8.08**	**4.01 – 16.29**			0.77	0.38 – 1.57	0.96	0.59 – 1.56
Metastatic other			0.62	0.15 – 2.62	**3.08**	**2.03 – 4.70**	**10.23**	**4.90 – 21.36**			1.38	0.16 – 11.67	**1.74**	**1.10 – 2.75**
Surgical margin														
Wide	1				1								1	
Marginal / intralesional	**2.28**	**1.25 – 4.16**			0.79	0.57 – 1.10							1.31	0.92 – 1.87

Histological response														
100%	1		1		1		1		1		1		1	
90-99%	1.43	0.73 – 2.79	1.49	0.84 – 2.63	**1.56**	**1.09 – 2.23**	1.17	0.50 – 2.74	1.02	0.37 – 2.81	1.79	0.82 – 3.92	0.91	0.60 – 1.38
<90%	1.13	0.48 – 2.66	**2.13**	**1.13 – 4.00**	**2.66**	**1.87 – 3.79**	**6.35**	**3.18 – 12.69**	0.86	0.18 – 4.14	1.32	0.60 – 2.92	1.14	0.77 – 1.69
Radiotherapy														
No	1		1		1		1		1				1	
Yes	**0.52**	**0.28 – 0.95**	0.82	0.51 – 1.31	0.99	0.73 – 1.32	**0.45**	**0.26 – 0.76**	1.53	0.65 – 3.64			1.08	0.78 – 1.49
Time to recurrence														
>24 months									1				1	
0-24 months									**3.79**	**1.34 – 10.76**			1.06	0.65 – 1.73

Table 3 – Hazard ratios and 95% confidence intervals for all prognostic factors and the different transitions in the multistate model.

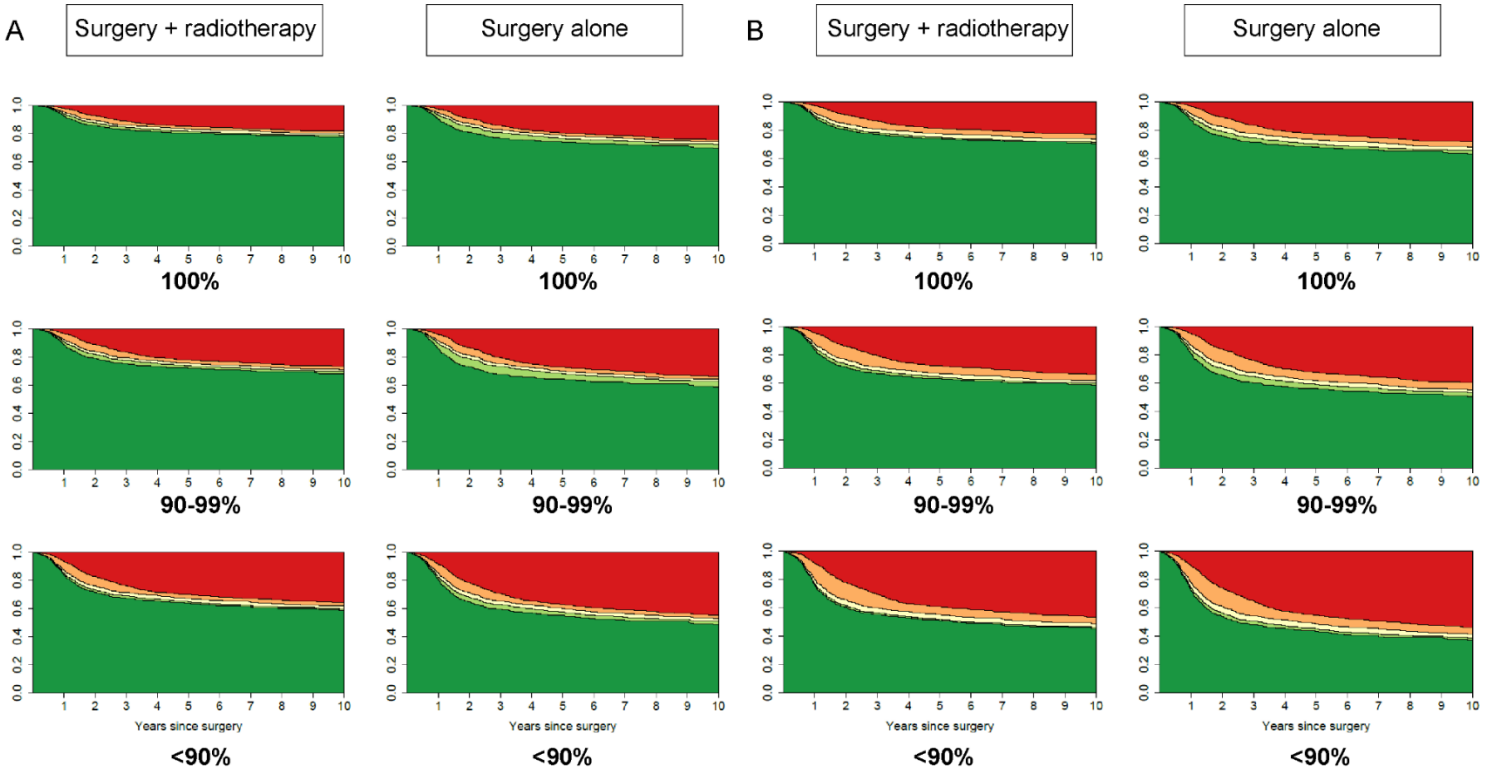

Figure 2 - State occupation probabilities for patients with different local treatment strategies

Estimations are based on the model presented in Figure 1 and Table 2 and shows state occupation probabilities for A) patient 1: aged 10-18 with a localized non-pelvic Ewing sarcoma and marginal or intralesional resection margins who is treated with surgery + radiotherapy (left panel) or surgery alone (right panel) for 100% necrosis, 90-99% necrosis and <90% necrosis. B) patient 2: aged 10-18 with a localized pelvic Ewing sarcoma and wide resection margins who is treated with surgery + radiotherapy (left panel) or surgery alone (right panel) for 100% necrosis, 90-99% necrosis and <90% necrosis.

Radiotherapy seems protective for LR in all tumor sites combined, even in case of good histological response. However, radiotherapy is not given randomly and is strongly correlated to patient- and tumor characteristics therefore a note of caution in the interpretation of the results is required here. Patient treated with post-operative radiotherapy generally have more tumor located in the pelvic, more inadequate surgical margins and poorer histological response which could have biased the results (see also supplementary file 1). The incidence of local recurrence, especially in extremity Ewing sarcoma, is low. Only 2% (9 out of 425) of the patients with extremity tumors developed isolated LR versus 8% (14 out of 169) of the pelvic tumors and 8% (30 out of 388) of the non-pelvic axial tumors. The number needed to treat (NNT) with surgery and radiotherapy to prevent the occurrence of a single LR is 72 for all tumor sites combined. In contrast, the NNT for extremity tumors is 80 and the NNT for pelvic tumors is 10. Which questions the value of radiotherapy in patients with an extremity Ewing sarcoma, were an individual patient with an extremity Ewing sarcoma might benefit only few really are in need for this potentially toxic treatment, especially in the growing child. Radiotherapy is associated with a significant risk for secondary radiotherapy induced malignancies, growth disturbance and postoperative complications of surgical reconstructions. (28) In case of Ewing sarcoma in a high-risk location, such as the pelvic or axial skeleton, this study showed that the administration of radiotherapy seems protective for LR, proton beam therapy could in theory be the solution in these cases, however long-term data on radiation induced late effects of proton beam radiation is not available yet. Prevention of distant metastasis and local recurrence appears to be the key to improve outcome in Ewing sarcoma, but distant metastases are still the main cause of treatment failure and the results suggest that the use of radiotherapy is not protective for the occurrence of distant metastasis.

We compared the results presented in this article, which were computed using multiple imputation for missing data, to 776 complete cases and found that HRs were of similar magnitude. More details can be found in the Supplementary file 2 (only available in online publication).We used a large cohort of patient with Ewing sarcoma which strengthens this study. However, several limitations exist. Some subgroups are small, therefor we cannot ensure that our findings of no effect of certain risk factors are not a result of the low number of events in these subgroups. Secondly, histological response and surgical margins were assessed by the local pathologist. The design of the study, in which a retrospective analysis was performed using a prospectively collected cohort, made revision of surgical margins and histological response not possible. Clear definitions were stated in the protocol, but differences in interpretation and evaluation could still exist. Third, cohorts often contain more variables than can reasonably be used for prediction and for sufficient power one needs at least 10 events per variable. We therefor choose to select the most predictive and sensible predictors to be included in the

analysis. Using a more extensive variable profile would have led to reduced predictability. Lastly, the recommendations for the use of radiotherapy were not consistently followed, and the results from this study are subjected to confounding by indication. Therefore, caution is needed when interpreting these results. Since the cohort used in this study is large and treated according to one protocol we feel that the cohort adequately represents the population of interest and that the results are generalizable.

Conclusion

Disease extent at diagnosis and histological response are the main risk factors for progression to distant metastasis or death after surgery. Tumor site and surgical margins are important risk factors for local recurrence. In case disease progression occurs, previous risk factors lose significance. Only time to recurrence is important for decision-making, since early LR (0-24 months) negatively influences survival. Both local recurrence and distant metastasis significantly affect survival, and despite our efforts as physicians almost all patient that experience an event died of progressive disease. Therefore, the balance between the toxicity of intensive salvage treatments and quality of life in the remaining life span of these patients should be carefully considered in these cases. Radiotherapy seems protective for LR when all tumor sites are combined. However, a very low percentage of local recurrence in extremity tumors and the associated long-term toxicity with the use of radiotherapy questions the indication of radiotherapy in all extremity cases. Indications for radiotherapy should be explored further, preferable in a prospective randomized setting.

Supplementary file 1

Characteristic	Surgery n (%)	Surgery + radiotherapy n (%)
Total	550	432
Gender		
Male	335 (61)	255 (59)
Female	215 (39)	177 (41)
Age		
0-10 years	149 (27)	103 (24)
11-18 years	235 (43)	217 (50)
>18 years	166 (30)	112 (26)
Primary tumor localization		
Pelvic	80 (15)	89 (21)
Non-pelvic	470 (85)	343 (79)
Extremity	272 (50)	153 (35)
Axial	198 (35)	190 (44)
Volume at diagnosis		
<200 ml	336 (61)	241 (56)
≥200 ml	214 (39)	191 (44)
Disease extent at diagnosis		
Localized	431 (78)	339 (79)
Pulmonary metastasis	62 (11)	66 (15)
Extrapulmonary metastasis	57 (10)	27 (6)
Surgical margin		
Wide	453 (82)	264 (61)
Marginal	58 (11)	105 (24)
Intralesional	39 (7)	65 (15)
Histological response		
100%	284 (52)	142 (33)
90-99%	165 (30)	119 (28)
<90%	100 (18)	171 (39)

Characteristic	Surgery n (%)	Surgery + radiotherapy n (%)
Transition to state		
Local recurrence	33 (6)	20 (5%)
DMpulm	41 (8%)	36 (8)
Extrapulmonary metastasis	113 (21)	99 (23)
Alive without disease	359 (65)	284 (66)

Table 1 – Patient demographics of the patients treated with surgery and surgery with radiotherapy.

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PART II

Pre-operative and intra-operative imaging techniques

CHAPTER 5

^{18}F-FDG PET-CT versus MRI for detection of skeletal metastasis in Ewing sarcoma.

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Abstract

Objective
To determine the level of discrepancy between magnetic resonance imaging (MRI) and ^{18}F-FDG PET-CT in detecting osseous metastases in patients with Ewing sarcoma.

Methods
20 patients with histopathological confirmed Ewing sarcoma between 2000 and 2017 who had ^{18}F-FDG PET-CT and MRI performed within a 4-week range were included. Each imaging modality was evaluated by a separate observer. Reference diagnosis of each lesion was based on histopathology or consensus of an expert panel using all available data, including at least 6 months follow-up. Sensitivity, specificity, and predictive values were determined. Osseous lesions were analyzed on patient- and lesion-basis. Factors possibly related to false-negative findings were evaluated using Pearson's chi-square of Fisher's exact test.

Results
112 osseous lesions were diagnosed in 13 patients, 107 malignant and five benign. Seven patients showed no metastases on either ^{18}F-FDG PET-CT or MRI. Forty-one skeletal metastasis (39%) detected with MRI did not show increased ^{18}F-FDG uptake on ^{18}F-FDG PET-CT (false-negative). Lesion-based sensitivities and specificities were 62% (95%CI 52-71%) and 100% (48-100%) for ^{18}F-FDG PET-CT; and 99% (97-100%) and 100% (48-100%) for MRI, respectively. Bone lesions were more likely to be false-negative on ^{18}F-FDG PET-CT if hematopoietic bone marrow extension was widespread and active ($p=0.001$), during or after (neo)-adjuvant treatment ($p=0.001$) or when the lesion was smaller than 10 mm ($p<0.001$).

Conclusion
Although no definite conclusions can be drawn from this small retrospective study, it shows that caution is needed when using ^{18}F-FDG PET-CT for diagnosing skeletal metastases in Ewing sarcoma. Poor contrast between metastases and active hematopoietic bone marrow, chemotherapeutic treatment and/or small size significantly decrease the diagnostic yield of ^{18}F-FDG PET-CT, but not of MRI.

Introduction

Ewing sarcoma is an aggressive primary bone sarcoma, predominantly affecting children and young adults.(1, 2) At the time of diagnosis, 20 to 25% of the patients present with pulmonary (70-80%) and/or osseous (40-50%) metastases. A multimodal approach to treatment drastically improved survival. In non-metastatic Ewing sarcoma 10-year overall survival is currently 55 to 65%, but survival in metastatic Ewing sarcoma is still dismal, with a 5-year overall survival of only 20 to 35%. (3-5) Principles of treatment consist of neo-adjuvant chemotherapy followed by local control of the primary tumor, either by surgery, radiotherapy or both, and adjuvant chemotherapy. (2, 4) Detection of all metastatic lesions in patients with oligometastatic disease has become relevant, as a curative rather than a palliative treatment objective aimed at achieving local control at these sites has been reported to improve clinical outcome. (6) Pre-treatment imaging of newly diagnosed patients with Ewing sarcoma includes local staging with magnetic resonance imaging (MRI) and chest computerized tomography (CT) to detect pulmonary metastases (15). Bone marrow biopsies and bone scintigraphy have been used to detect or exclude skeletal metastases. More recently 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography with CT (^{18}F-FDG PET-CT) and whole-body MRI have been proposed to replace bone scintigraphy, because of higher sensitivity, and thus negative predictive value, to exclude skeletal metastasis. (7-12) With ^{18}F-FDG PET-CT reflecting glucose metabolism of the lesions and MRI imaging revealing morphologic characteristics of metastatic deposits, these two screening techniques display different properties of the cancerous lesions: either functional or anatomical. No published literature directly comparing ^{18}F-FDG PET-CT with whole-body MRI for detection of skeletal metastases in Ewing sarcoma is currently available. Literature comparing both modalities for skeletal metastases in other cancers shows conflicting results, with some suggesting superiority for ^{18}F-FDG PET-CT (10, 13, 14) and others superiority for MRI. (7, 15)

In our clinical practice we normally use both techniques. We frequently observed a mismatch between ^{18}F-FDG PET-CT and MRI; in some patients, metastatic skeletal lesions detected by MRI were not detected with ^{18}F-FDG PET-CT. Therefore, our purpose of this study was to retrospectively compare the diagnostic yield of ^{18}F-FDG PET-CT to whole-body MRI for detection of skeletal metastasis in Ewing sarcoma with final diagnosis of an osseous lesion made by an expert panel using all follow-up data or histopathology (where available).

Methods

Study design and patients

The local ethical board approved this retrospective study and waived the requirement for informed consent. We searched the database of our tertiary referral bone sarcoma center for all patients diagnosed with Ewing sarcoma between the

first of January 2010 and the first of January 2018. Patients were eligible for inclusion when fulfilling all of the following criteria: 1) histopathological confirmed Ewing sarcoma; 2) treatment and diagnostic work-up according to the EURO-E.W.I.N.G. (EUROpean Ewing tumor Working Initiative of National Groups-Ewing Tumour Studies) 2008 or 2012 protocol; 3) whole-body ^{18}F-FDG PET-CT scan and whole body or large field of view regional MRI scan performed within a 4-week range. All sets of scans performed at baseline were executed before the start of treatment. All sets of scans performed during follow-up were executed at the same treatment stage or moment in follow-up. In case of multiple paired ^{18}F-FDG PET-CT and MRI scans of a single patient, the first available set was used. We performed an additional analysis for therapy naïve patients and patients who were already treated separately to check if this had an impact on detection.

We identified 52 patients with histopathological confirmed Ewing sarcoma and included 20 patients who had undergone both ^{18}F-FDG PET-CT scan and MRI scan, either at diagnosis or during follow-up, within a 4-week range.

Figure 1 shows a flowchart of the inclusion process.

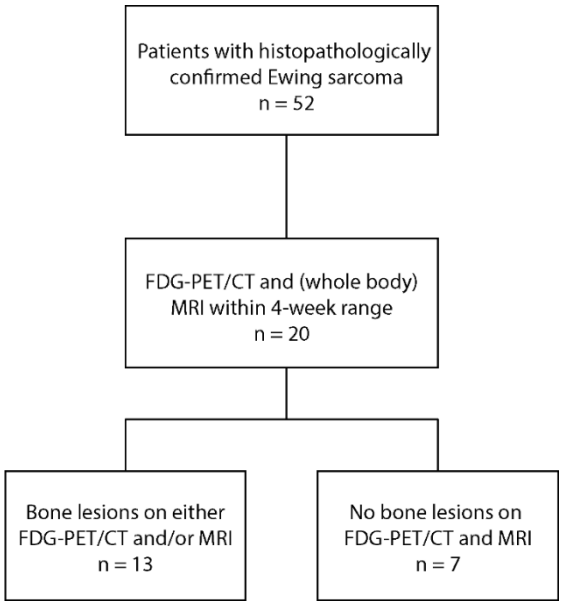


Figure 1 - Flowchart of the inclusion process

^{18}F-FDG PET-CT acquisition and evaluation

After at least six hours of fasting (sugar-free liquids were allowed) and validation of normoglycemia (<11.1 mmol/L), patients were intravenously injected with ^{18}F-FDG (dose dependent on bodyweight, scanner sensitivity and acquisition duration). After a ~60-minute resting period, low-dose CT and PET-images were acquired from vertex to toes on multiple PET-CT scanners (Siemens Biograph Horizon, Siemens Biograph mCT and Philips Gemini TF) in our own and six referring centers according to the European Association of Nuclear Medicine (EANM) procedure guidelines for tumor imaging in FDG PET-CT (version 2.0). (16) Analysis of ^{18}F-FDG PET-CT-images was repeated for all scans and primarily done by visual assessment. The decision of the conspicuity of a skeletal lesion was determined by an experienced PET-CT-reader (D.V., nuclear medicine physician, 10 years of experience), blinded for clinical and histopathological information and other imaging examinations. Visible lesions on ^{18}F-FDG PET-CT were scored positive (i.e. suspect for malignancy), negative (i.e. suspected benign) or inconclusive. Focal bone uptake visual in thee orthogonal plans, higher than the surrounding bone marrow without clear benign cause (e.g. growth plate) was scored as suspect for malignancy (positive). In case additional imaging was suggested for confirmation, it was scored as 'inconclusive'. All other lesions were scored benign (negative). Semi quantitative assessment of PET-positive lesions by measurement of their maximum standardized uptake value (SUV_{max}) was performed and related to the SUV_{max} of the mediastinal blood pool and healthy right liver activity, resulting in the 6-point scale presented in Table 1. Last, metabolically active hematopoietic bone marrow extension and activity was quantified using a visual 4-point scale defined *a priori* based on literature. (17-19) The visual 4-point scale was defined as followed: 0) metabolically active hematopoietic bone marrow only present in spine/pelvis without increased activity (SUV_{max} lower than or equally to liver); 1) metabolically active hematopoietic bone marrow only present in spine/pelvis with increased activity (SUV_{max} higher than the liver); 2) metabolically active hematopoietic bone marrow extension up to pertrochanteric femoral and/or subcapital humeral regions with increased activity; 3) metabolically active hematopoietic bone marrow extension beyond pertrochanteric femoral and/or subcapital humeral regions with increased activity. For analysis we dichotomized the data, normal hematopoietic bone marrow was defined by a score of 0 or 1 and widespread hematopoietic bone marrow extension and activity was defined as a score of 2 or 3.

Score	Description
0	No uptake
1	Notable uptake < mediastinal blood pool
2	Notable uptake > mediastinal blood pool, but < liver
3	Notable uptake ≈ liver uptake (±10%)
4	Intense uptake > liver, but ≤ 2,5x liver
5	Intense uptake > 2.5x liver uptake

Table 1 – Semi-quantitative assessment of lesion ^{18}F-FDG uptake according to a 6-point scale.

MRI acquisition and evaluation

Whole body MRI was performed in 14 patients using a 1.5T system (Philips Healthcare, Best, the Netherlands). Standard protocol included T1-weighted turbo spin echo (TSE) with slice thickness: 5 mm, repetition time (TR) of 727 ms, echo time (TE) of 15 ms, and short-Tau inversion recovery (STIR) sequences using four stations in the coronal plane with slice thickness: 5 mm , TR 7192 ms, TE 50 ms, inversion time 210 m, and sagittal T1 and STIR sequences of the entire spine using the above mentioned MRI parameters for T1 and STIR. In six patients a large field of view regional MRI scan using the same parameters was obtained. Additional sequences that were made in these regional scans were not reviewed for current analysis. In these six patients, only regions imaged by both modalities were evaluated and compared. In one of these six patients, ^{18}F-FDG PET-CT showed three osseous lesions outside the MRI field of view, which were not included for current analysis.

MRI images were evaluated by one radiologist specialized in MRI imaging (J.L.B., >10 years of experience), blinded for clinical and histopathological information and other imaging examinations. Malignancy on MRI was based on the assessment of morphological and signal characteristics. A nodule presenting with a lower signal than the surrounding bone marrow on T1 and a higher signal on STIR sequences was scored positive (i.e. suspect for malignancy). All other lesions were considered benign (negative). Next, quantitative assessment of MRI positive lesions was performed by measuring the size (defined as maximum diameter) of the lesion. Lesions were dichotomized at the 10mm diameter level.

Reference method

Histopathological correlation for every depicted osseous lesion was not available in the majority of the lesions for ethical reasons: in only two patients, confirmation of skeletal metastasis by biopsy was available. In the other 11 patients with osseous lesions on imaging, the final decision of the true status of the osseous lesion was made by consensus using an expert panel consisting of a board-certified radiologist and nuclear physician. All available clinical information, including therapy schedules, response to treatment and follow-up imaging examinations (^{18}F-FDG PET-CT, MRI, diagnostic CT) were used to reach the overall decision. Patients were routinely evaluated every 3 months by ^{18}F-FDG PET-CT and/or MRI. The mean imaging follow-up was 15,7 months (range 1,8 to 31,3 months). Two patients deceased due to progressive disease shortly (1,8 and 3,8 months) after imaging was performed and no obduction was performed. In all other 18 patients (7 without osseous lesions and 11 with osseous lesions) at least 6 months of follow-up imaging examinations was available to determine the true status of a bone lesion. Change in imaging characteristics, increase in size of the entire lesion or the extra-osseous component or increased ^{18}F-FDG uptake of the lesions indicated malignancy. Response to treatment was used as a sign of malignancy and was defined as decrease in ^{18}F-FDG-uptake, decrease in size of the lesions or complete disappearance of the lesion. A lesion was considered benign if a specific diagnosis could be made, if it showed no change over time, especially when other lesions changed in response to treatment, or if there was progressive disease diagnosed in other sites of the skeleton.

Data analysis

Each visible lesion was scored separately as being malignant, benign or inconclusive on either imaging modality. Number of lesions and location were determined for both ^{18}F-FDG PET-CT and MRI. Location was defined using eleven predefined skeletal body regions: 1) skull; 2) ribs; 3) pelvis; 4) cervical spine; 5) thoracic spine; 6) lumbar spine; 7) proximal upper extremity; 8) distal upper extremity; 9) proximal lower extremity; 10) distal lower extremity; 11) other regions (scapula, sternum, clavicles). If a patient presented with multiple lesions in one region a maximum of 4 lesions was included for analysis to avoid bias of few patients with very large number of lesions. In case of discordance between ^{18}F-FDG PET-CT and MRI, we searched for potential causes in a separate consensus meeting by the expert panel, after all patients had been scored by the individual observers. Additionally, if osseous lesions showed no ^{18}F-FDG uptake we evaluated whether these lesions were visible on the low-dose CT of the ^{18}F-FDG PET-CT, using MRI as guidance.

Statistical analysis

Both patient-based analysis and lesion-based analysis were performed and the results are described as true-positive, true-negative, false-positive and false-negative. Lesions that were scored as inconclusive on imaging were considered

positive for this analysis. Osseous lesions were also evaluated and reported as true-positive, false-positive, true-negative, and false-negative in patient-based and lesion-based analysis. In case of a discordant finding within a single patient, a true-positive lesion will supersede all other lesions, including false-negative, true-negative and false-positive lesions. Thus, if a subject presented with at least one true-positive lesion, that patient will be considered true-positive for this imaging modality. In the absence of a true-positive lesions, a false-negative lesion will supersede a true-negative or false-positive lesion. Therefore, if imaging is false-negative in at least one site, that patient will be considered false-negative overall for this modality. Using this approach, we address the question if recurrent/metastatic disease is present or not. We computed accuracy, sensitivity, specificity, positive and negative predictive values using the classical equations. The 95%-confidence intervals of these test characteristics were computed using the absolute Clopper-Pearson interval (using the beta-distribution). We explored the following factors to be related to false-negative findings: lesion size, location, hematopoietic bone marrow extension and treatment stage (before treatment, on treatment, recurrence after treatment) using Pearson's chi-square or Fisher's exact test, where appropriate.

Results

Patient population

In seven of the 20 patients ^{18}F-FDG PET-CT and MRI were both negative for the presence of osseous lesions. All these patients were routinely evaluated every 3 months by ^{18}F-FDG PET-CT and MRI and none of these patients was diagnosed with skeletal metastasis within the next six months. All these cases were considered true-negative on both imaging modalities. Later three of these patients developed pulmonary and/or skeletal metastasis during long term follow-up. At the termination of our study, the four other patients were alive with no evidence of disease and the three patients who later developed metastases, died due to recurrent or progressive disease.

In the remaining 13 patients, Table 2, osseous lesions on any or both imaging modalities were reported to be present. A total of 112 bone lesions were identified using our standard of reference; 89 in the axial skeleton (30 vertebral, 15 rib, 33 pelvic, 4 glenoid, 1 acromion, 3 clavicles, 2 sternum, 1 skull), and 23 in the peripheral skeleton (16 lower extremity, 7 upper extremity). Four patients had already been treated at the time of imaging, while all imaging was performed prior to start of treatment in the other nine patients. At the termination of our study seven patients had died due to progressive disease, six patients were alive of which four were undergoing palliative treatment and two were alive with no evidence of disease.

						^{18}F-FDG PET-CT		MRI	
No.	**Age/Sex**	**Primary tumor**	**Purpose of the study**	**Standard of reference**	**Number of lesions#**	**PB**	**LB**	**PB**	**LB**
1	23/M	Tibia	Follow-up*	CF	16	TP	6	TP	16
2	22/M	Femur	Follow-up	CF	2	TP	2	TP	2
3	23/M	Femur	Staging	CF	3	TP	3	TP	3
4	26/M	Pelvic	Staging	CF	25	TP	19	TP	24
5	17/M	Tibia	Follow-up	CF	16	TP	6	TP	16
6	17/F	Costa	Follow-up	CF	2	FP	2	TN	0
7	5/F	Tibia	Staging	HP	1	TP	1	TP	1
8	23/M	Humerus	Staging	CF	24	TP	19	TP	23
9	8/F	Tibia	Staging	HP	2	TP	1	TP	2
10	23/M	Costa	Staging	CF	11	TP	4	TP	11
11	22/M	Pelvic	Staging	CF	5	TP	3	TP	5
12	16/M	Fibula	Staging	CF	1	TP	1	TP	1
13	29/F	Femur	Staging	CF	2	TP	2	TP	2
Total					**112**		**69**		**106**

Table 2 - Patient-based and lesion-based diagnosis of bone lesions in patients with at least one abnormality

Abbreviations: CF = clinical follow-up; F = female; FN = false negative; FP = false positive; HP = histopathology; LB = lesion based positive lesions; M = male; PB = patient basis; TP = true positive; TN=true negative

**active chemotherapeutic treatment*

#on any of both imaging modalities

Patient-based analysis for PET-CT vs MRI

Twelve out of 13 patients (92.3%) with suspicion of skeletal metastasis on any of the two imaging modalities were correctly identified by ^{18}F-FDG PET-CT and MRI concordantly, and thus were considered true positive.

In one patient (7.7%) ^{18}F-FDG PET-CT showed two almost symmetric lesions with subtle sclerosis and ^{18}F-FDG-uptake in both distal femoral diaphysis of which the true nature could not be clearly defined. Based on the information available these lesions were classified as inconclusive. On MRI and CT a diagnosis of bilateral bone infarctions was made as confirmed by the expert panel (Figure 2). During follow-up the patient presented with progressive disease, and eventually died 16.3 months later. No metastatic lesions developed at the distal femora during the disease progression and the bone infarctions didn't change, the ^{18}F-FDG PET-CT was therefore considered false-positive. There were no false-positive MRI-scans and there were no false-negative scans. The positive predictive values (PPV) with corresponding 95%-confidence interval (95%CI) of ^{18}F-FDG PET-CT and MRI therefore were respectively 92% (62-100%) and 100% (72-100%), respectively. The sensitivities were 100% (72-100%) for ^{18}F-FDG PET-CT and 100% (72-100%) for MRI.

Lesion-based analysis

A total of 112 lesions in 13 patients were identified and characterized as malignant or benign by the expert panel using the predefined standard of reference. Of these 112, 107 lesions (95.5%), present in 12 patients, were considered malignant . Five lesions in four patients were considered to be benign.The data from the lesion-based analysis are presented in Table 3.

^{18}F-FDG PET-CT and MRI were concordantly positive in 65 (58%) osseous lesions, whereas 41 (37%) osseous lesions in seven patients were observed on MRI only, compared to 4 (4%) osseous lesions in three patients observed on ^{18}F-FDG PET-CT only. Two osseous lesions (1%) in one patient were defined as being benign on both imaging modalities.

		PET		
		+	-	Total
MRI	+	65	41	106
	-	4	2	6
	Total	69	43	112

Table 3 – Lesion-based analysis of all osseous lesions.

The 41 lesions visible on MRI only were all considered to be malignant according to the standard of reference and therefor true-positive. These 41 lesions were thus false-negative on ^{18}F-FDG PET-CT. The majority of these 41 lesions (36 lesions; 88%) was located in the axial skeleton; spine (20 lesions; 49%), rib (2 lesions; 5%), pelvis (7 lesions; 17%), other axial regions (glenoid and clavicles; 7 lesions, 17%). Only 5 lesions (12%) were found in the extremities. Lesions were not more likely to be false-negative on ^{18}F-FDG PET-CT when located in the axial skeleton compared to an extremity location (40% versus 30%; p=0.522). In addition to location in the axial skeleton we evaluated potential cofounders potentially explaining the false-negative lesions on ^{18}F-FDG PET-CT. In the nine therapy-naïve patients, lesions were less likely to be false-negative on ^{18}F-FDG PET-CT compared to the four patients that already started treatment (26% versus 58%; p=0.001). In three patients with false-negative lesions on ^{18}F-FDG PET-CT widespread hematopoietic bone marrow extension and activity was present. Lesions were more likely to be false-negative on ^{18}F-FDG PET-CT when widespread active red bone marrow was present (55% versus 22%, p=0.001). In one patient recent chemotherapy led to bone marrow rebound on ^{18}F-FDG PET-CT obscuring ten lesions all located in the axial skeleton (Figure 3). Ten lesions in five patients were smaller than 10 mm and all but one of these lesions were located in the axial skeleton. Lesion size below 10 mm lead to more false-negative lesions on ^{18}F-FDG PET-CT (100% versus 30%, p<0.001). Figure 4 and 5 provide examples of the false negative lesions on ^{18}F-FDG PET-CT.

Of these 41 false negative lesions on ^{18}F-FDG PET-CT, 39 could not be identified on the low-dose CT of the ^{18}F-FDG PET-CT by the expert panel. The expert panel identified two skeletal metastasis present in two patients that were visible on the low-dose CT as small osteolytic lesions, positive on MRI but interpreted as negative on ^{18}F-FDG PET-CT. One of these two false-negative lesions, was in close proximity to the physiologically ^{18}F-FDG positive growth plate and (thus) falsely interpreted as negative. The other false-negative lesion was located at the in the posterior iliac crest and interpreted as reactive uptake due to bone-marrow biopsy for its location. However, no bone marrow biopsy had been performed, and the small lytic lesion on low-dose CT had been interpreted as iatrogenic. All other 39 false-negative lesions showed no ^{18}F-FDG uptake on PET-CT. On MRI and during imaging follow-up this lesion was classified as malignant and thus interpreted false-negative by ^{18}F-FDG PET-CT.

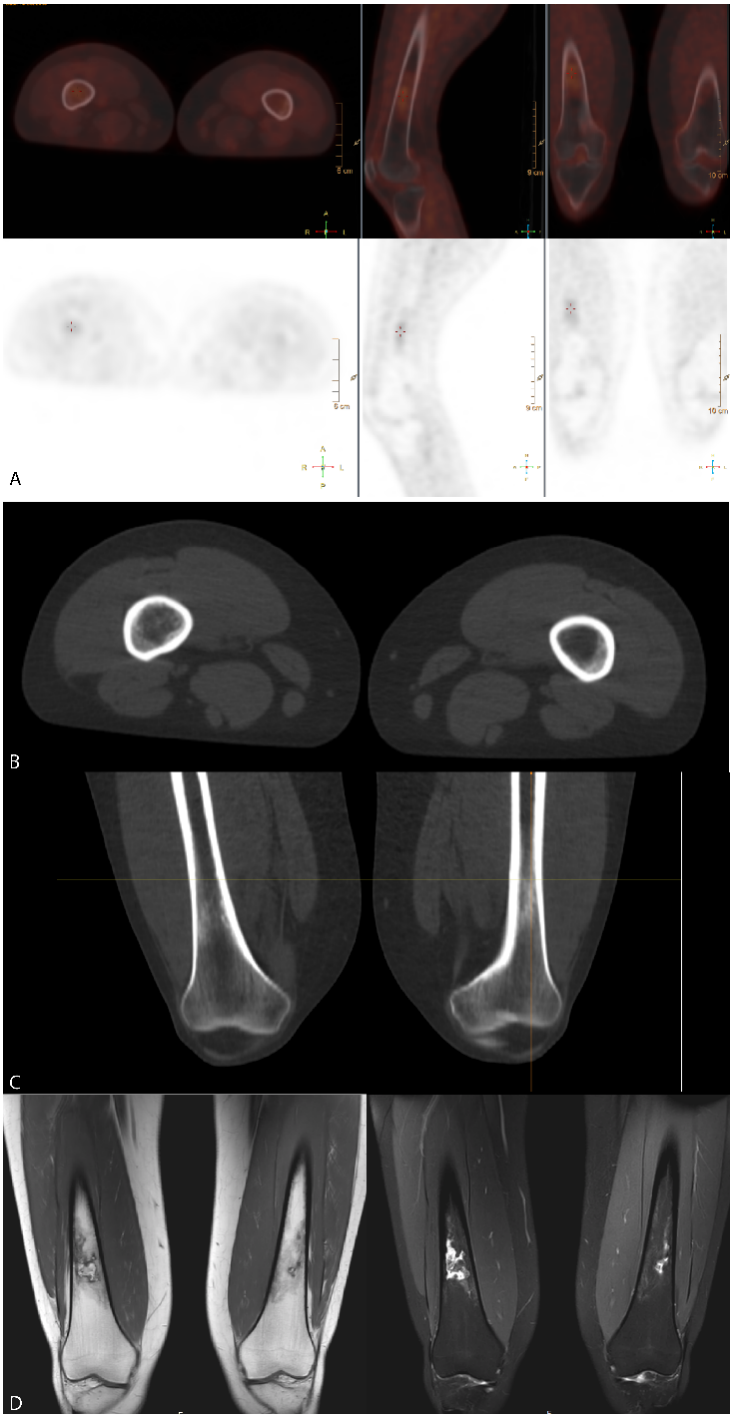

A
B
C
D

Figure 2 - False-positive lesions on 18F-FDG PET-CT.
A 19-year-old woman diagnosed with localized Ewing sarcoma of the seventh rib. Six months after initial treatment consisting of six courses of vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) chemotherapy, 8 courses of vincristine, actinomycin-D, and ifosfamide (VAI) chemotherapy, radiation therapy and surgery, imaging was performed because of chest pain, with local recurrence suspected. A) 18F-FDG PET-CT showed two lesions with 18FDG-uptake in both femora of which the true nature could not be clearly defined; based on the information available they were classified as inconclusive (positive). B) and C) Low-dose CT images in the transverse and coronal planes of the suspected bone lesions showing sclerosis. d MRI T1- and T2-weighted images show bilateral bone infarctions and no sign of malignancy

Three out of four bone lesions visible on ^{18}F-FDG PET-CT only, were considered false-positive. These included two lesions diagnosed as bone infarctions in a single patient (Fig 1) and a bone lesion in the 8th thoracic vertebral body. During imaging follow-up of over a year no change of the lesion in the 8th thoracic vertebral body was seen. A diagnosis could not be made, however since no progression or change of the lesion was seen in over a year, while the patient had progressive disease under treatment, the lesion was regarded as being benign according to our reference standard and therefor as false-positive on ^{18}F-FDG PET-CT.
The one PET-positive lesion that was false-negative on MRI according to the standard of reference was missed due to partial volume effects. This small lesion (<1 cm) fell between two slices due to the slice gap of 10% with a slice thickness of 5 mm.
Table 4 provides an overview of the lesion-based analysis relative to the standard of reference for each imaging modality separately. The lesion-based PPV for ^{18}F-FDG PET-CT and MRI were respectively 96% (95%CI 91-100%) and 100% (97-100%). The lesion-based NPV for ^{18}F-FDG PET-CT and MRI were respectively 5% (0-11%) and 83% (54-100%). Sensitivities and specificities for these modalities were 62% (95%CI 52-71%) and 100% (95%CI 48-100%) for ^{18}F-FDG PET-CT and 99% (97-100%) and 100% (48-100%) for MRI, respectively. Accuracy was 63% (95%CI 54-72%) for ^{18}F-FDG PET-CT and 99% (95%CI 95-100%) for MRI.

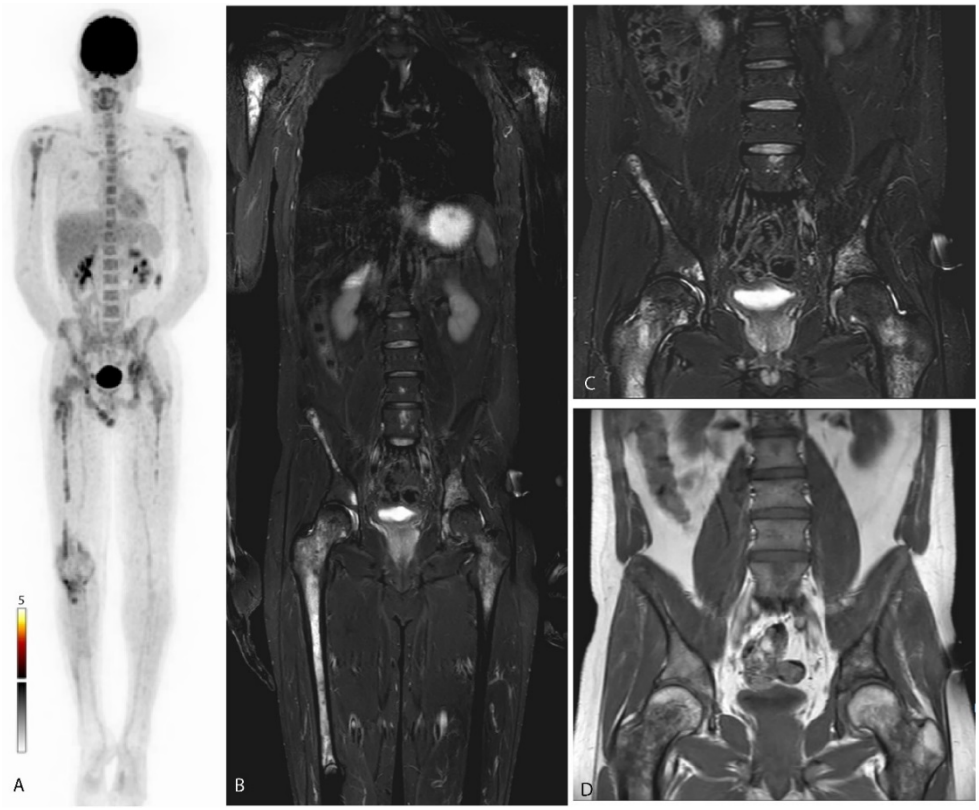


Figure 3 - False-negative lesions on 18F-FDG PET-CT with widespread hematopoietic bone marrow activity.

A 23-year-old man diagnosed with localized Ewing sarcoma of the right proximal tibia. Images obtained 6 months after initial treatment (6 × VIDE, surgery, 8 × VAI), at this time undergoing second-line chemotherapy because of recent distant metastasis. A) 18F-FDG PET-CT with symmetrical 18F-FDG uptake in the axial skeleton and proximal extremities. This was classified benign (negative) owing to anemia or recent chemotherapy. B) T1-weighted short tau inversion recovery (STIR) MRI images with multifocal metastatic lesions throughout the whole axial skeleton. C) STIR images with several skeletal metastases in the left and right ilium and fifth lumbar vertebral body. D) T1-weighted turbo spin echo (TSE) images with several skeletal metastases in the left and right ilium and fifth lumbar vertebral body

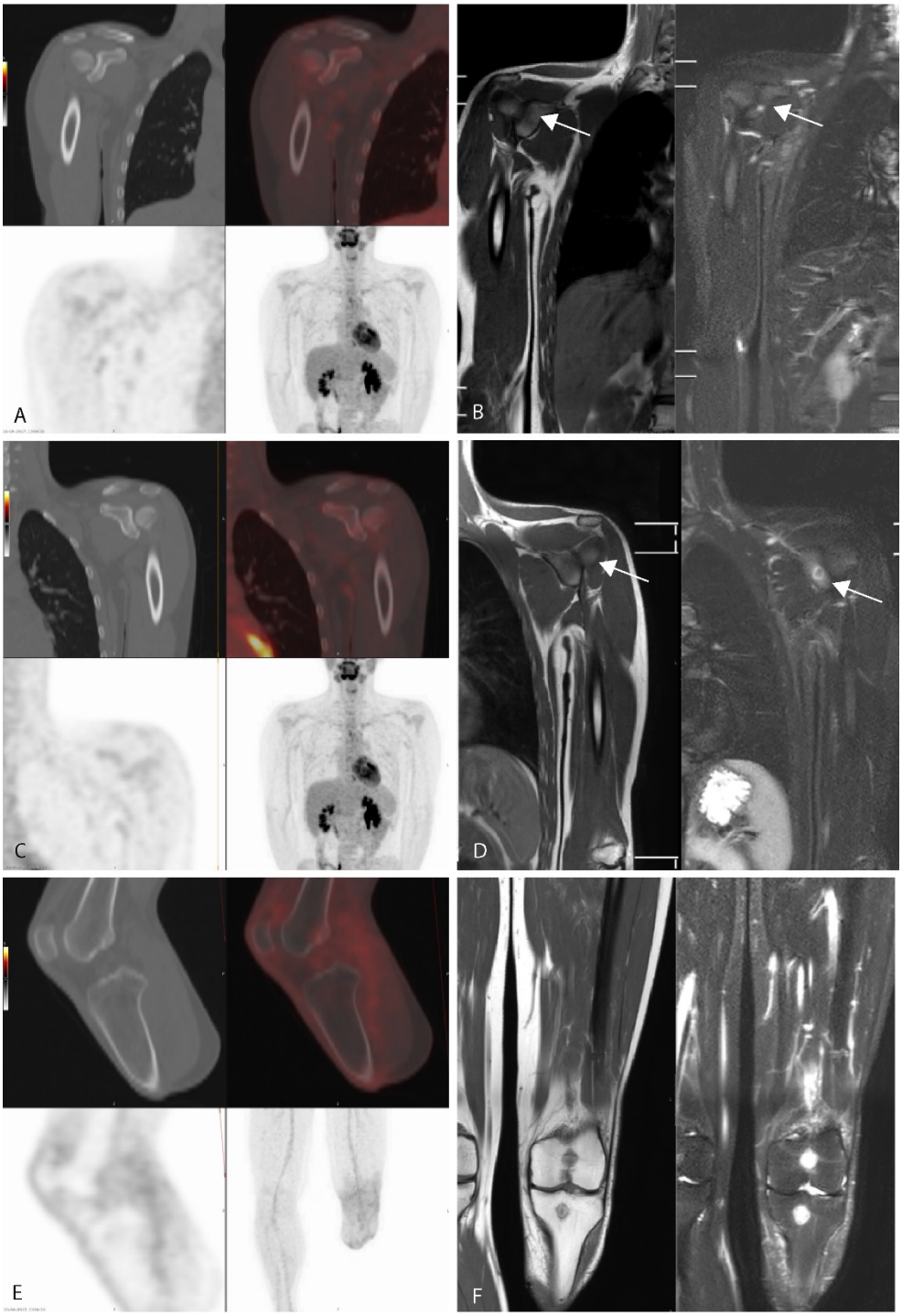
		Standard of reference	
		Malignant	**Benign**
PET	**+**	66	0
	-	41	2
	Indeterminate	0	3
MRI	**+**	106	0
	-	1	5

Table 4 – Lesion based analysis according to the standard of reference.

Semi quantitative assessment of ^{18}F-FDG PET-CT
Most of the true positive PET lesions (67/107, 63%) had a score of 3 (notable uptake with a SUV_{max} of ±10% compared to the liver uptake) or higher. The remaining 40 lesions showed no visible uptake on ^{18}F-FDG PET-CT or only showed low uptake and were considered as benign (SUV_{max} lower than bloodpool). See table 5.

			Score					
		total	**0**	**1**	**2**	**3**	**4**	**5**
Standard of reference	Malignant	107	36	2	2	3	52	12
	Benign	5	0	0	2	0	3	0
PET-CT interpretation	Positive/Indeterminate	69	0	0	4	2	53	10
	Negative	43	36	2	0	1	2	2

Table 5 – Scores of ^{18}F-FDG PET-CT lesions
Score based on maximum SUV_{max} divided into standard of reference and visual ^{18}F-FDG PET-CT interpretation.


A
B
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F

Figure 4 - False-negative lesions on 18F-FDG PET-CT.
A 17-year-old boy diagnosed with localized Ewing sarcoma of the distal tibia. Images obtained 1 year after finishing treatment (6 × VIDE, amputation, 8 × VAI). A) 18F-FDG PET-CT showing no increased 18F-FDG-uptake at the glenoid of the right shoulder. B) T1-weighted (left) and STIR (right) images showing a small nodule (arrow) with a high degree of suspicion for metastasis at the glenoid of the right shoulder. C) 18F-FDG PET-CT showing no increased 18F-FDG-uptake or lytic changes on low-dose CT at the glenoid of the left shoulder. D) T1-weighted (left) and STIR (right) images showing a nodule (arrow) with a high degree of suspicion for metastasis at the glenoid of the left shoulder. E) 18F-FDG PET-CT showing no increased 18F-FDG-uptake or lytic changes on low-dose CT at the left proximal tibia and distal femur. F) T1-weighted (left) and STIR (right) images showing two nodules with a high degree of suspicion for metastasis at the left proximal tibia and distal femur

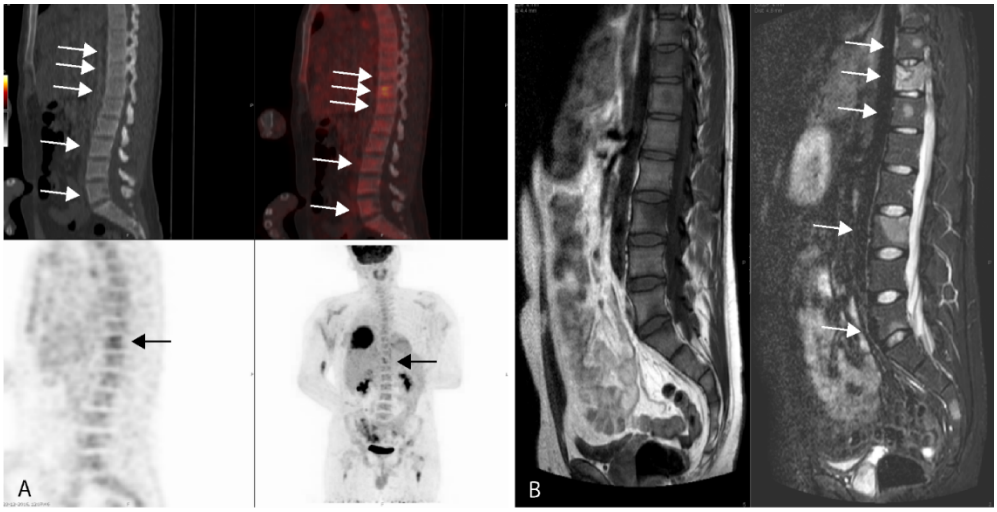

Figure 5 – False-negative lesions on 18F-FDG PET-CT (arrows).
A 23-year-old man presenting with metastatic Ewing sarcoma of the right seventh rib. Images obtained at diagnosis, before the start of treatment. A) 18F-FDG PET-CT showing increased 18F-FDG-uptake at the eleventh thoracic vertebrae only. B) T1-weighted (left) and STIR (right) images showing nodules with a high degree of suspicion for metastasis at the tenth, eleventh, and twelfth thoracic vertebrae and the third and fifth lumbar vertebrae

Discussion

Accurate detection and localization of all metastases in oligometastatic Ewing sarcoma is clinically relevant since metastasectomy or radiation of these sites potentially provides a curative approach. (6)

Of all detected lesions, 95.5% were considered malignant by our reference standard. The PPV of both ^{18}F-FDG PET-CT and MRI are high and the number of false positive lesions low. Thus in this young patient population, any lesion should be considered malignant until proven otherwise. In 39% of confirmed metastases detected with MRI no increased ^{18}F-FDG uptake was present and these were thus missed on ^{18}F-FDG PET-CT. Only two (5%) of these ^{18}F-FDG negative metastases could retrospectively be found by the expert panel on the low dose CT images. On patient basis ^{18}F-FDG PET-CT and MRI both performed well. In only one patient without skeletal metastasis, PET-CT showed inconclusive and thus, according to predefined criteria, false-positive findings, while MRI was true negative. All other patients with suspicion of skeletal metastasis were correctly identified by both imaging modalities. Our results cannot be compared to existing literature, since published reports on performance of MRI relative to ^{18}F-FDG PET-CT are normally based on inclusion of heterogeneous populations with different types of malignancy. In general ^{18}F-FDG PET-CT and MRI are performing well, but there is no consensus in literature about differences in performance in specific tumor types such as Ewing sarcoma.

The question is what can explain the difference between ^{18}F-FDG PET-CT which is based on glucose metabolism within the tumor, and MRI which is based on morphology of metastases in bone marrow. It seems that there are at least three factors that, in combination, are causing these false negatives; activity of normal bone marrow on ^{18}F-FDG PET-CT, small lesion size, and variation in glucose consumption. First, the presence of hematopoietic bone marrow has significant impact on performance of ^{18}F-FDG PET-CT as it decreases contrast between normal and abnormal ^{18}F-FDG uptake. Patients with Ewing sarcomas are young and have active hematopoietic bone marrow in the axial skeleton. Also anemia, previous treatment with chemotherapy or medication may lead to increased activity of hematopoietic marrow in Ewing sarcoma patients. ^{18}F-FDG has an increased uptake in hematopoietic marrow relative to yellow bone marrow, thereby increasing the background activity on ^{18}F-FDG PET-CT. Since hematopoietic bone marrow is typically located in the axial skeleton and proximal extremities, it is no surprise that most (88%) false negative lesions were located in the axial skeleton.

Second, lesion size also contributes to the large number of false-negative lesions on ^{18}F-FDG PET-CT. Ten out of 41 false-negative lesions (24%) were smaller than 10 mm and these smaller lesions were more likely to be false-negative on ^{18}F-FDG PET-CT.

Lastly, changes in the tumor micro-environment of Ewing sarcoma that affect the glucose metabolism may also contribute to the large amount of false-negative lesions of ^{18}F-FDG PET-CT. (20, 21)

This study has a few limitations. First, Ewing sarcoma is a rare disease, so numbers are low. In addition, we performed a retrospective study, so selection bias may play a role: there could have been a reason for the second imaging modality to be performed after the first one (i.e. no independency). Secondly, histopathological confirmation was not available in the majority of the lesions. Follow-up imaging was used as a reference method in the majority of the lesions. Although this is an accepted tool for lesion characterization it could affect the accuracy of our results. Third, imaging analysis was done by one experienced nuclear medicine physician and one experienced radiologist. In general ^{18}F-FDG PET-CT is evaluated by a nuclear medicine physician and MRI by a musculoskeletal (MSK) radiologist. The large number of radiologist allows for more specialization. If two MSK radiologist would have evaluated all imaging data two false-positive lesions (the two bone infarctions in one patients, Figure 1) and two false-negative lesions (that could in retrospect be found on low-dose CT) might have been prevented and could thus be considered as interpretation error. All other lesions did not show ^{18}F-FDG-uptake and were not visible on low-dose CT. Last, in 6 out of 20 cases no whole-body MRI was available for comparison and specificity of both techniques could therefore not be determined. However, there were only three osseous lesions visible on ^{18}F-FDG PET-CT not imaged by MRI.

In conclusion, although no definite conclusions can be drawn from this small retrospective study, we conclude that caution is needed when using ^{18}F-FDG PET-CT for diagnosing skeletal metastases in Ewing sarcoma, since 39% of metastases in this cohort seen on MRI are not detected with ^{18}F-FDG PET-CT. Suggestions of main causes are poor contrast between metastases and active hematopoietic bone marrow small size, and potentially changes in glucose metabolism in metastases of Ewing sarcoma. Further research is needed to evaluate the discrepancy in ^{18}F-FDG PET-CT and MRI findings and confirm our results.

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CHAPTER 6

Can Navigation Improve the Ability to Achieve Tumor-free Margins in Pelvic and Sacral Primary Bone Sarcoma Resections? A Historically Controlled Study

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Abstract

Background
Anatomic and surgical complexity make pelvic and sacral bone sarcoma resections challenging. Positive surgical margins are more likely to occur in patients with pelvic and sacral bone sarcomas than in those with extremity sarcomas and are associated with an increased likelihood of local recurrence. Intraoperative navigation techniques have been proposed to improve surgical accuracy in achieving negative margins, but available evidence is limited to experimental (laboratory) studies and small patient series. Only one small historically controlled study is available. Because we have experience with both approaches, we wanted to assess whether navigation improves our ability to achieve negative resection margins.

Questions/purposes
Are navigated resections for pelvic and sacral primary bone sarcomas better able to achieve adequate surgical margins than nonnavigated resections?

Methods
Thirty-six patients with pelvic or sacral sarcomas treated with intraoperative navigation were retrospectively compared with 34 patients undergoing resections without navigation. All patients underwent resections between 2000 and 2017 with the intention to achieve a wide margin. Patients in the navigation group underwent surgery between 2008 and 2017; during this period, all resections of pelvic and sacral primary bone sarcomas with the intention to achieve a wide margin were navigation-assisted by either CT fluoroscopy or intraoperative CT. Patients in the control group underwent surgery before 2008 (when navigation was unavailable at our institution), to avoid selection bias. We did not attempt to match patients to controls Non-navigated resections were performed by two senior orthopedic surgeons (10 years and >25 years of experience). Navigated resections were performed by one senior orthopedic surgeon with great experience in surgical navigation. The primary outcome was the bone and soft-tissue surgical margin achieved, classified by a modified Enneking system. Wide margins (≥ 2 mm) and wide-contaminated margins, in which the tumor or its pseudocapsule was exposed intraoperatively but further tissue was removed to achieve wide margins, were considered adequate; marginal (0-2 mm) and intralesional margins were considered inadequate.

Results
Adequate bone margins were achieved in more patients in the navigated group than in the nonnavigation group (29 of 36 patients [81%] versus 17 of 34 [50%]; odds ratio, 4.14 [95% CI, 1.43-12.01]; p = 0.007). With the numbers available, we found no difference in our ability to achieve adequate soft-tissue margins between the

navigation and nonnavigation group (18 of 36 patients [50%] versus 18 of 34 [54%]; odds ratio, 0.89 [95% CI, 0.35-2.27]; p = 0.995).

Conclusions

Intraoperative guidance techniques improved our ability to achieve negative bony margins when performing surgical resections in patients with pelvic and sacral primary bone sarcomas. Achieving adequate soft tissue margins remains a challenge, and these margins do not appear to be influenced by navigation. Larger studies are needed to confirm our results, and longer followup of these patients is needed to determine if the use of navigation will improve survival or the risk of local recurrence.

Introduction

The aim of surgery to treat bone sarcomas is to completely excise the tumor with negative margins while preserving as much normal tissue as possible. Preserving muscle, bone, and neurovascular structures may improve the surgeon's ability to achieve good reconstruction and reduce the likelihood of complications, thereby improving short- and long-term functional outcomes [1, 30]. Achieving wide surgical margins in the pelvis is challenging, and pelvic tumors are more likely to result in positive surgical margins than extremity tumors are [4, 12, 13, 24, 25]. Local recurrence occurs in 20%-40% of patients overall, and in those with positive margins, this number increases up to 70%, resulting in an increased risk of local recurrence and perhaps metastasis [4, 12, 13, 24, 25]. Neoadjuvant chemotherapy or radiotherapy may be used to increase the likelihood of adequate resection or treat positive surgical margins in some types of sarcomas, but it is considered less effective for treating chondrosarcomas and chordomas, which are the predominant tumor types in adults with pelvic and sacral sarcomas.

Intraoperative guidance techniques such as computer-assisted surgery may be useful in achieving wide margins during tumor resections, and thus may assist in improving oncologic outcomes. Computer-assisted surgery allows for three-dimensional preoperative planning of resection and reconstruction procedures. Intraoperatively, there is real-time feedback for the actual location and orientation, which allows for more precision [27, 30].

Available evidence about the putative benefits of computer-assisted surgery in resections for pelvic and sacral bone sarcomas are somewhat limited, consisting principally of experimental (laboratory) studies and small patient series [2, 5, 7, 16, 20, 22, 26, 30]. Only one small historically controlled study [19] comparing nine navigated resections for sacral and pelvic tumors with 12 nonnavigated resections is available. Although these studies have shown generally consistent results, with more accurate osteotomies, fewer intralesional resections, and increased benefits to the patient in terms of reduced operating time, less blood loss, and fewer

complications [2, 5, 7, 16, 19, 20, 22, 26, 30], they either do not have a control group and/or have small cohorts. To our knowledge, there has been no large and well performed, controlled study of navigated oncologic resections in the pelvis.
We therefore asked: are navigated pelvic and sacral primary bone sarcoma resections better able to achieve adequate surgical margins than nonnavigated resections?

Patients and Methods

We retrospectively studied patients undergoing resection of a primary pelvic or sacral bone sarcoma between 2000 and 2017. Our institutional review board reviewed and approved this study. All data were collected as part of routine patient followup examinations; therefore, the ethical review board waived the need to obtain individual informed consent.
Patients were identified from an intuitional database. Since 2008, computed-assisted surgery using CT fluoroscopy has been used for bone sarcoma resections at our institution; since 2015, intraoperative CT-based navigation has replaced CT fluoroscopy. Computer-assisted surgery was indicated for all patients presenting with a primary malignant pelvic or sacral bone sarcoma in a curative setting. Patients were eligible for inclusion if they had a pelvic or sacral primary malignant bone sarcoma and underwent resection with the intention to achieve a wide margin. Patients presenting with recurrent disease after a previous resection were excluded. Patients were included in the navigation group if computer-assisted surgery, either CT fluoroscopy or intraoperative CT-based surgery, was used with the intention to achieve a wide resection. Patients in the control group were selected from 2000 to 2008 because surgical navigation was not available at that time at our institution. One hundred four resections of primary pelvic and sacral bone sarcomas were performed. Twenty-four patients underwent debulking or intralesional curettage of low-grade chondrosarcomas and were excluded. In 10 patients—four who underwent intraoperative CT-based surgery and six who underwent CT fluoroscopy-based surgery—there were technical problems with the navigation software. These resections were performed without navigation and these patients were also excluded from the analysis. The remaining 70 patients were included in this study: 36 with surgical navigation and 34 without navigation (Figure 1).

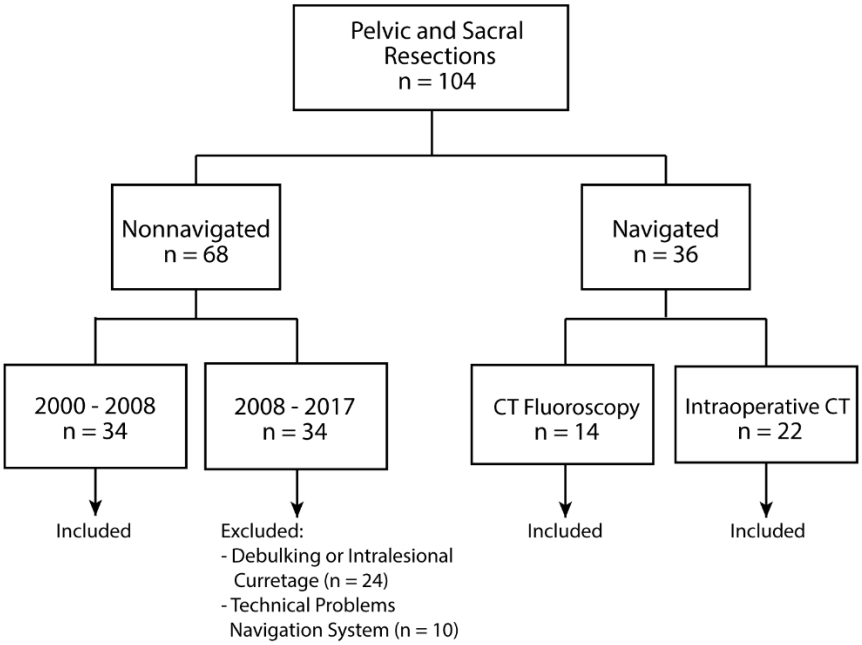

Figure 1 - This flowchart shows how patients were included in our study.

Two senior orthopaedic oncologic surgeons (PDSD, who has 10 years of experience in pelvic surgery, and AHMT, who has more than 25 years of experience in pelvic surgery) performed the nonnavigated resections. The navigated resections were all performed by a single surgeon (PDSD) experienced in this technique. The surgeon developed experience in surgical navigation first by practice on sawbones models and cadavers. He performed more than 10 navigated spondylodesis of the spine and resected at least 10 benign tumors of the pelvic and sacrum before performing navigated resection in primary malignant bone sarcoma.

Data on patient demographics, tumor characteristics, (neo)adjuvant treatment, surgery details, and complications were extracted from the patients' medical records. The mean age was 43 years ± 16.3 for patients in the navigation group versus 42 years ± 18.9 in the nonnavigation group ($p = 0.748$). No differences in the mean tumor size between the groups were observed (9.0 cm ± 4.4 for the navigation group versus 9.1 cm ± 3.4 for the nonnavigation group ($p = 0.923$). In the navigation group, the most prevalent type of sarcoma was chondrosarcoma (in 21 of 36 patients [58%], followed by osteosarcoma (seven of 36 patients [19%]), chordoma (five of 36 patients [14%]), and Ewing's sarcoma (two of 36 patients [6%]). In the nonnavigation group, the most prevalent types of sarcoma were chondrosarcoma (in 20 of 34 patients [59%], followed by Ewing's sarcoma (nine of 34 patients [27%]), chordoma (three of 34 patients [9%]) and osteosarcoma (two of 34 patients [6%]). No difference between the groups with regard to tumor type were observed ($p = 0.069$). The tumor grade also did not differ between the groups (22 of the 36 patients in the navigation

group [61%] had high-grade tumors, seven [19%] had intermediate-grade tumors, and seven [19%] had low-grade tumors; 18 of the 34 patients [53%] in the nonnavigation group had high-grade tumors, 14 [41%] had intermediate-grade tumors, and two [6%] had low-grade tumors ($p = 0.065$)] (Table 1). In both groups, all patients presenting with an osteosarcoma or Ewing's sarcoma received neoadjuvant chemotherapy. In the navigation group, four patients received radiation therapy, and in one, it was given preoperatively. In the nonnavigation group, seven patients received radiation therapy, and in one, it was given preoperatively. In all patients who had radiation, a dose of 54 or 55 Gy was administered. Resections were classified from P1 to P4 or a combination [9]. P1 resections involved the ilium; P2 resections involved the periacetabular regions, with or without involvement of the femur; P3 resections involved the pubis; and P4 resections involved the sacrum. No differences in the type of resection were observed between the groups ($p = 0.434$).

Variable	Total	Nonnavigated (2000-2008)	Navigated (CT fluoroscopy or intraoperative CT-based)	p value
n	70	34	36	
Male (%)	42 (59)	22 (65)	20 (56)	0.495
Age, years (mean ± SD)	43 (18)	42 (19)	43 (16)	0.748
Type of primary bone sarcoma (%)				
Osteosarcoma	9 (13)	2 (6)	7 (19)	0.069
Chondrosarcoma	41 (58)	20 (59)	21 (58)	
Ewing sarcoma	11 (16)	9 (27)	2 (6)	
Chordoma	8 (11)	3 (9)	5 (14)	
Other	1 (2)	0 (0)	1 (3)	
Tumor grade (%)				0.065
Low	9 (13)	2 (6)	7 (19)	
Intermediate	21 (30)	14 (41)	7 (19)	
High	40 (57)	18 (53)	22 (61)	
Location of primary tumor (%)				
Pelvis	53 (76)	28 (82)	25 (69)	0.233
Sacrum	17 (24)	6 (18)	11 (31)	
Tumor size, cm (mean ± SD)	9.0 (3.9)	9.1 (3.4)	9.0 (4.4)	0.923
Type of resection (%)				
P1	12 (17)	4 (12)	8 (22)	0.434
P1-2	10 (14)	7 (20)	3 (8)	
P1-2-3	8 (11)	4 (12)	4 (11)	
P1-4	3 (4)	1 (3)	2 (6)	
P1-2-4	1 (2)	0 (0)	1 (3)	
P2	8 (11)	6 (17)	2 (6)	
P2-3	5 (7)	2 (6)	3 (8)	
P3	7 (10)	4 (12)	3 (8)	
P4	16 (23)	6 (17)	10 (29)	

Table 1 – Demographics of the study groups

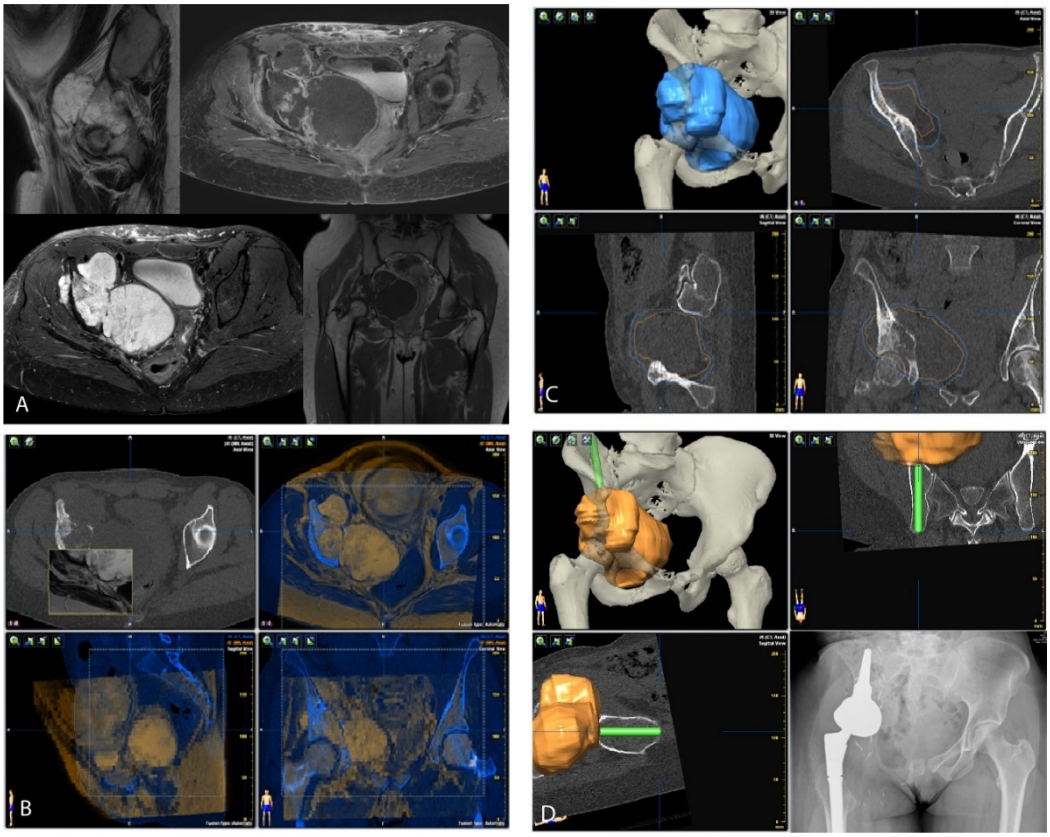

Figure 2 – Pre-operative planning

These images show preoperative surgical planning for tumor resection in a 34-year-old woman with a Grade II chondrosarcoma in the periacetabular region of the right side of the pelvis. A) Preoperative MR images demonstrate a high-grade tumor in the right periacetabular region. B) Computer screenshots of the navigation software show fusion of the MRI (orange) and CT scans (blue). C) This screen image from the navigation system shows the mapped tumor in orange and the planned safety margin in blue, in three planes (clockwise from top left: three-dimensional reconstruction in the transverse, sagittal, and coronal planes). D) This image shows the planning of a LUMiC® prosthesis (Implantcast, Buxtehude, Germany). The LUMiC® prosthesis is a modular device and consists of a separate cup and stem, both available in different sizes and with different coatings. An AP radiograph after navigation-assisted LUMiC® endoprosthetic pelvic reconstruction after resection is shown.

Preoperative Planning

All patients were treated with the intention to achieve a wide bone and soft-tissue resection margin. All patients underwent routine diagnostic workup, including biopsy (if indicated) and CT and MRI scanning with gadolinium. Treatment plans and decisions regarding (neo)adjuvant chemotherapy and/or radiation therapy were discussed at a multidisciplinary tumor board meeting for each patient. If navigation was used, preoperatively acquired CT and MR images were fused (Figure 2A-2D), allowing for an accurate three-dimensional model of the tumor that was correlated with the patient's anatomy during surgery. Images were used before surgery to plan margins and, if needed, reconstruction.

Intraoperative Navigation

Image-to-patient registration is the most-crucial step, in which the patient's anatomy is linked to preoperatively acquired imaging data. There are three registration methods in general. The most common registration method is manual, in which important predefined anatomical landmarks on the preoperative image dataset are located as accurately as possible on the patient during surgery, using a probe or pen (paired points matching). Accuracy is further improved by collecting more points from the patient's bone surface (surface matching) [26, 27].

Subsequent CT fluoroscopy matching allowed for semiautomatic registration. Fluoroscopic images (AP and lateral) taken intraoperatively were superimposed on the preoperative CT images. After manual image adjustment, they were displayed on the navigation monitor. Registration can also be performed automatically, in which matching is done using intraoperative CT-based navigation. The use of intraoperative CT improves the workflow and allows for intraoperative updates and, if needed, change of the plan [27, 30].

CT Fluoroscopy-based Navigation

For CT fluoroscopy navigation, we used a mobile C-arm (Philips BV, Eindhoven, the Netherlands) combined with a navigation platform (Curve™ Image Guided Surgery, Brainlab AG, Feldkirchen, Germany).

Intraoperative CT-based Navigation

For intraoperative CT-based navigation, we used a mobile AIRO® CT scanner (Mobius Imaging, Ayer, MA, USA) with spinal navigation software (Curve™ Brainlab AG, Feldkirchen, Germany). The AIRO scanner consists of a mobile CT gantry with a radiography tube. It is designed to function in any operating room and can be moved using an electrical drive system. With a 105-cm diameter, the bore is extralarge. Surgery is performed on a radiolucent, carbon-fiber CT examination table (TRUMPF TruSystem 7500, Trumpf Inc., Farmington, CT, USA) that can be turned in any direction greater than 360°. The entire setup has a 1.5-m^2 footprint (Figure 3A-3C).

Intraoperative Navigation and Surgical Resection

Standard surgical approaches were used, and soft-tissue dissection was performed. Next, stable attachment of a navigation tracking tree, with two 2.8-mm pins fixed to the iliac wing or a clamp to the spinous process, was performed. Image-to-patient registration was done using two-dimensional fluoroscopic images or intraoperative CT-based scanning (5-10 min). The operating room personnel stood behind a radiography protection screen, and an on-call radiology technician performed the scanning (Figure 3B). The acquired imaging data were automatically transferred to the DICOM and navigation software. A navigated pointer was used to validate the registration. A registration error of less than 1 mm was considered acceptable. All osteotomies were performed using a navigated oscillating saw or chisel (Figure 4).

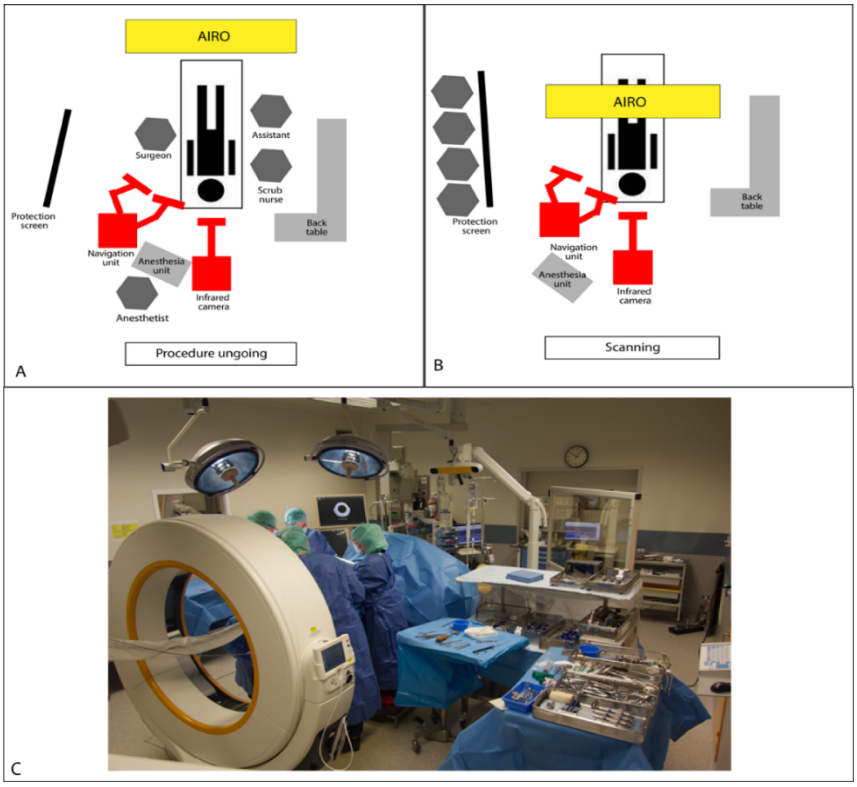

Figure 3 – General set-up of AIRO

This figure shows the general set up of the AIRO® scanner in our operating room during surgery and intraoperative CT-based scanning. A) This demonstrates the schematic setup of intraoperative CT-based navigation during the procedure. B) This demonstrates the schematic setup of the intraoperative CT position during scanning. C) This picture was made during a procedure in which intraoperative CT-based navigation was used.

Resection planes of the nonnavigated resections were based on preoperative plain radiographs, CT images, and MR images. During the operation, we determined the site of the osteotomies using measurements from landmarks that were based on the preoperative imaging data.

After resection, the specimens were sent for a histopathological analysis, including assessment of the surgical margin. In the navigation group. 25 of the 36 patients [69%] underwent reconstruction after resection, of whom 12 had an endoprosthesis, eight had spongiosaplasty with the TSRH 3D® (Medtronic Sofamor Danek, United States of America), and five had reconstructions using an autograft or allograft. In the nonnavigation group, 28 of the 34 patients (82%) underwent reconstruction after resection, of whom 13 had an endoprosthesis, two underwent surgery using a TSRH 3D® with spongiosaplasty, and 13 underwent reconstruction using an autograft or allograft. The mean total blood loss, accurately measured by an anesthesiologist, was similar in both groups: 2270 ml ± 2160 in the navigation group versus 2740 ml ±1660 in the nonnavigation group ($p = 0.308$). Operating time, measured from the start of incision to closure of the wound, was also similar in both groups: 352 min ± 195 in the navigation group versus 333 min ± 126 in the nonnavigation group ($p = 0.73$).

Major complications were defined by the need for reintervention or a prolonged hospital stay. Fourteen of the 36 patients (39%) in the navigation group had major complications, mainly wound infections with a need for reintervention (10 patients; 71%). Other complications were neurapraxia in one patient (7%), acute renal failure in one (7%), iatrogenic fracture in one (7%), and two surgical sponges were left behind in the wound that resulted in reoperation in one (7%). None of these complications were thought to be caused by surgical navigation nor were they thought to be complications that could have been avoided using surgical navigation. They were all directly related to the tumor resection itself or reconstruction of the bone and soft-tissue defect. In the nonnavigation control group, 13 of the 34 patients (38%) had a major complication; the most common were wound infections that indicated surgery (seven patients [54%]). Other complications consisted of neurapraxia in four patients (31%), excessive bleeding that was examined a second time the next day in one (8%), and screws in the sacral canal that impinged on the nerve roots in one patient (8%). The proportion of major complications was similar between the navigation and nonnavigation groups: 53% versus 47% (odds ratio [OR], 1.03 [95% CI, 0.39-2.69]; $p = 0.955$). Followup included imaging of the local site and chest every 3 months postoperatively for 2 years followed by every 6 months for 3 years.

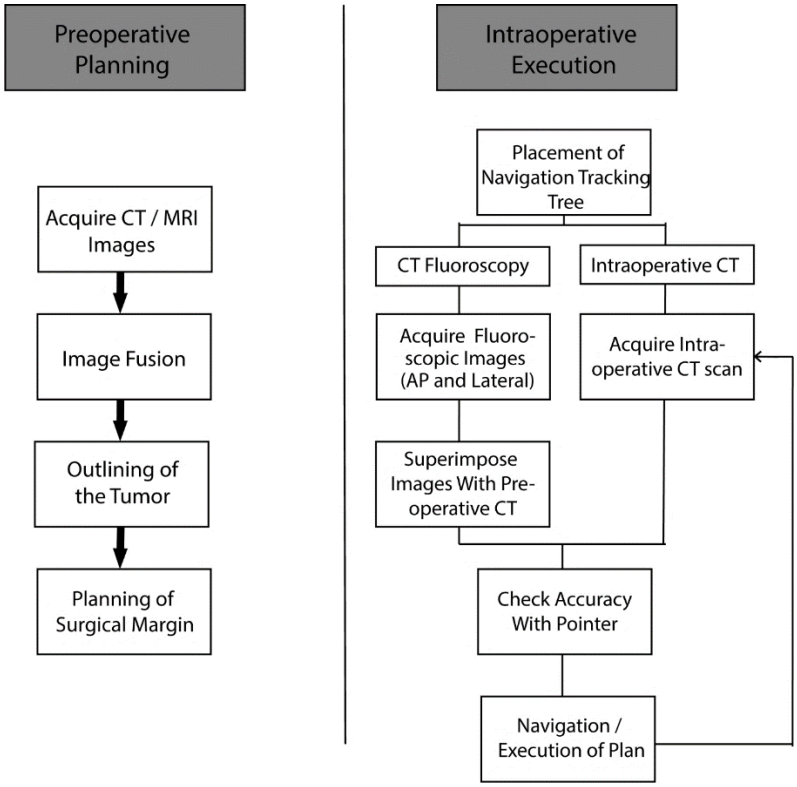

Figure 4 - This shows the schematic workflow for computer-assisted orthopaedic surgery.

Outcome Measures

Surgical margins were evaluated from two areas, the bone and soft tissue, for each patient. Margins were determined by a pathologist (AHGC using surgical specimens. Bone margins were assessed at the osteotomy and soft-tissue margins at the circumferential soft tissue. All bone and soft-tissue surgical margins were histologically defined based on the worst margin according to a modified Enneking's classification [10] into wide margins in patients with en bloc resection with a cuff of normal tissue of at least 2 mm, marginal margins in patients with viable tumor cells within 2 mm of the resection plane or a resection plane through the reactive zone, intralesional margins when tumor cells were present in the resection plane, and wide-contaminated if the tumor or its pseudocapsule was exposed intraoperatively but further tissue was removed to achieve wide margins. If the pathology report was inconclusive about either the bone or soft-tissue surgical margin or provided no information regarding the minimal margin, a senior pathologist who specializes in bone tumors (AHGC) reviewed the report and tissue slices and assigned a margin status. For analysis, wide and wide-contaminated margins were considered

adequate, and marginal or intralesional margins were considered inadequate [3, 17, 21, 23]. Because we were primarily interested in how navigation affects the bony and soft-tissue surgical margins, we did not examine oncologic outcomes in this study.

Statistical Analysis

Patient characteristics were compared using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. ORs with corresponding 95% CIs are provided. A p value < 0.05 was considered significant. For all analyses, IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA) was used.

Results

Adequate bone margins were achieved in a higher number of patients in the navigated group than in the nonnavigation group (28 of 36 patients [81%] versus 17 of 34 patients [50%]; OR, 4.14 [95% CI, 1.43-12.01]; p = 0.03). In the navigation group, the bone margin was wide in 28 of 36 patients (78%), wide contaminated in one (3%), marginal in three (8%), and intralesional in four (11%). In the nonnavigation group, the bone margin was wide in 16 of 34 patients (47%), wide contaminated in one (3%), marginal in eight (24%), and intralesional in nine (26%) (Table 2). With the numbers available, we found no difference in the ability to achieve adequate soft tissue margins between the navigated and nonnavigation groups (18 of 36 patients [50%] versus 18 of 34 [54%]; OR, 0.89 [95% CI, 0.35-2.27]; p = 0.995). However, fewer intralesional soft tissue margins were observed in the navigation group than in the nonnavigation group (two of 36 patients [6%] versus eight of 34 [23%]; OR, 0.19 [95% CI, 0.04-0.98]; p = 0.032) (Table 2). In the navigation group, seven inadequate margins were observed. In three patients, proximity to the nerves, which were not visible on preoperative images, made wide resection impossible. We might have prevented this by using better-quality imaging data and thinner slides. Furthermore, all three tumors originated in the sacrum. There was one wide contaminated margin. A no-touch technique, in which a Gigli saw is used instead of a chisel or saw, might have prevented this.

Variable	Total n (%)	Nonnavigated (2000-2008) n (%)	Navigated (CT fluoroscopy or intraoperative CT-based) n (%)	p value
n	70	34	36	
Surgical margin—bone[a]				
Classified by Enneking				0.025
Wide	43 (61)	16 (47)	28 (78)	
Wide contaminated[b]	0 (0)	1 (3)	1 (3)	
Marginal	16 (23)	8 (24)	3 (8)	
Intralesional	11 (16)	9 (26)	4 (11)	
Dichotomized[c]				
Adequate	43 (61)	17 (50)	29 (81)	0.007
Inadequate	27 (38)	17 (50)	7 (19)	
Surgical margin—soft tissue[a]				
Classified by Enneking				0.102
Wide	34 (49)	17 (51)	17 (47)	
Wide contaminated[b]	2 (3)	1 (3)	1 (3)	
Marginal	24 (34)	8 (23)	16 (44)	
Intralesional	10 (14)	8 (23)	2 (6)	
Dichotomized[c]				0.995
Adequate	36 (51)	18 (54)	18 (50)	
Inadequate	34 (49)	16 (46)	18 (50)	

Table 2 - Outcome measures for each of the study groups

a: Surgical margins were evaluated from two areas, the bone and soft-tissue, for each patient. Bone margins were assessed at the osteotomy and soft-tissue margins at the circumferential soft-tissue.

b: Wide-contaminated: if the tumor or its pseudocapsule was exposed intraoperatively, but further tissue was removed to achieve wide margins.

c: Wide and wide-contaminated margins were considered adequate, and marginal or intrale-sional margins were considered inadequate.

Discussion

Pelvic and sacral bone sarcoma resections are challenging because of anatomic and surgical complexity. Inadequate surgical margins are more likely to result in inadequate surgical margins in the extremities, which is likely associated with a higher risk of local recurrence. This may have a major impact on the oncologic outcome, especially in patients with chondrosarcomas, for which surgical resection is frequently the preferred treatment option. Computer-aided surgery could assist in achieving higher surgical accuracy and thus improve the oncologic outcome. Available evidence, although promising, is limited to experimental (laboratory) studies and small patient series. Only one small historically controlled study [19] comparing nine navigated and 12 nonnavigated pelvic resections is available (Table 3). We showed that navigation improved our ability to achieve tumor-free bony resection margins in our patients compared with patients in whom navigation was not used, but no differences in the ability to achieve adequate soft-tissue margins were observed.

This study had a number of limitations. First, while we reported a relatively large cohort of patients who underwent navigated resection for pelvic and sacral primary bone sarcomas, the subgroups were small and we cannot ensure that our finding of no differences between the groups in baseline characteristics such as tumor type, tumor grade, and type of pelvic resection does not reflect a Type II error.

Second, because we only included patients undergoing resection with the intention to achieve a wide margin, and because pelvic sarcomas are relatively rare, we did not enroll a large number of patients during the study period. Also, different surgeons might diagree with whether a procedure will result in a wide margin or not. This might have resulted in a selection bias. However, a large cohort was presented, and we therefore feel that our study groups adequately represent the general population.

Third, the tumor location and type of reconstruction, if any, strongly influence the total blood loss, operating time, and proportion of complications. Because we only had data on the total operating time and blood loss, including those for reconstruction, we were not able to adequately assess differences in blood loss, complications, and operating time between the groups.

Fourth, we attempted to avoid selection bias by selecting control patients who underwent surgery when surgical navigation was not available at our institution. When surgical navigation was available, it was used for all patients presenting with a primary pelvic or sacral tumor in which the aim was to achieve a wide margin. In 10 patients, there were technical problems with the navigation software, which caused us to switch to nonnavigated resection. Twenty-four patients presenting with primary malignant tumors underwent debulking or intralesional curettage; these patients were excluded from this study. Because it is unlikely that a randomized study will be performed, we think that our group of patients treated with resection for similar tumor types before we began using navigation serves as a reasonable comparison.

Fifth, all nonnavigated resections were performed by two senior orthopaedic oncology surgeons who specialize in pelvic surgery. The potential differences in experience between the surgeons could have influenced the results. Most nonnavigated resections were performed by AHMT, who was at that time at the end of his learning curve and retired during the study period. All navigated resections were performed by one experienced senior orthopaedic surgeon (PDSD). Research by Farfalli et al. [11] shows that surgical time decreases as surgeons perform more navigated procedures. This learning curve might have influenced the operating time and therefore the results. However, this surgeon had much experience with surgical navigation for spondylodesis of the spine and benign tumor resections, and was familiar with the software before performing navigated resections for malignant bone sarcomas. Therefore, we feel that we minimized the risk of the learning curve because the surgeon was highly familiar with surgical navigation. Even though differences between the surgeons could have influenced the results, all resections were performed by experienced surgeons with more than 10 years of experience; therefore, we believe our study groups adequately represent the general population.
Sixth, in our study, wide and wide-contaminated margins were considered adequate. The definition of an adequate margin differs among tumor types and grades. For a low-grade chondrosarcoma, a marginal resection could be considered adequate, especially in the extremities, whereas for grade 2 or 3 chondrosarcomas, a 4-mm margin (or at least more than 2 mm) is advised to reduce the risk of local recurrence [23]. For a high-grade osteosarcoma, a wide margin (classified as > 2 mm) is prognostic for local recurrence-free survival [17]. Furthermore, in patients with Ewing's sarcoma, the local recurrence rate and event-free survival in patients with or without distant metastasis is better after wide resection than after marginal or intralesional resections [3, 21]. Research showed that only wide surgical margins are associated with improved oncologic outcomes and less local recurrence of most tumor types. Wide-contaminated margins, in which the tumor or its pseudocapsule is exposed intraoperatively but further tissue is removed to achieve wide margins, were also considered adequate. The aim of our study was to assess our ability to achieve a wide margin using a certain technique; therefore, wide-contaminated margins were considered adequate. If the aim was to study the oncologic outcome and survival, a wide-contaminated margin should not have been considered adequate.
Finally, the short followup period for patients who underwent intraoperative CT-assisted resections and the wide variety of patient diagnoses, treatments, and ages inevitably resulted in missing information on clinical outcomes. However, the aim of this study was to assess our ability to achieve a negative margin in navigated and nonnavigated resections to treat pelvic and sacral primary bone sarcomas, and research showed that wide margins are associated with improved oncologic outcomes in most tumor types [3, 17, 21, 23]. For a true reflection of the oncologic

outcome, long-term followup data are required for a range of tumor types and stages. Only then will we know the true effect of computer-assisted surgery.

The use of navigation during surgery resulted in a higher number of patients with adequate bony surgical margins. No differences in adequate soft tissue margins were observed between the groups; however, using navigation, we observed fewer intralesional soft-tissue margins.

Computer-aided surgery for pelvic resections was first reported by Krettek et al. [18] and Hufner et al. [14]. They concluded that computer-aided surgery may increase accuracy in tumor resections involving anatomic and surgical complexity. Computer-aided surgery further evolved because of Wong et al. [28, 29], who described CT and MRI fusion in navigated tumor surgery. Two experimental studies showed improved bone cutting accuracy during pelvic resection using surgical navigation. Sternheim et al. [22] found that osteotomies using navigation within 5 mm of the planned cut resulted in a negative margin in more than 95% of the cuts. Cartiaux et al. [5] showed a mean location accuracy of 2.8 mm from the target plane using navigation and no intralesional cuts. These results imply that with a decrease in error, removal of less normal bone may be possible while achieving negative bony margins, which may translate to better reconstruction possibilities. Laitinen et al. [19] presented the first historically controlled study comparing nine navigated and 12 nonnavigated pelvic resections. In the navigation group, no intralesional margins and 22% local recurrence were observed, versus three intralesional margins (25%) and 50% local recurrence in the nonnavigation group. The authors also noted less intraoperative blood loss and reduced surgical time in the navigation group. Several studies used local recurrence as an endpoint (Table 3). However, surgical navigation is a relatively new technique; therefore, long-term followup data to assess oncologic outcomes are not available from any center we know of to date, which questions the strength of these conclusion. The use of navigation does not guide or help to achieve more adequate soft tissue margins. However, better visualization during surgery could contribute to the lower proportion of intralesional soft-tissue margins observed in this study. Compromised soft-tissue margins are considered a poor independent prognostic factor, and most local recurrences occur in the soft tissue (margin) and not in osteotomy [7, 11, 15, 26].

We showed that computer-aided surgery improved our ability to achieve adequate margins of bony resections as defined in this study in patients with pelvic and sacral bone sarcomas. Achieving adequate soft tissue margins remains a challenge, and we could not document whether patients in whom sarcomas are resected using navigation will have reduced local recurrence rates or improved survival. Computer-aided surgery could also aid in pelvic reconstruction by facilitating allograft planning and three-dimensional printed endoprostheses, although we did not evaluate this in the current study [6, 8, 27]. Prospective multicenter studies with long-term followup are needed to further investigate the potential benefits of this promising technique.

Author (year)	Study design	Study period	Follow-up	Patient population	Navigation	Results
Abraham (2018) (10)	Case series	2009 – 2015	27 (12 – 52)	Sample size = 23 - 13 pelvic - 10 sacrum Type = 2x OS, 8x CS, 3x ES, 6x chordoma, 4 other. - Navigation used in all patients	Brainlab Kolibri Medtronic O-arm	Margins = 21/23 negative - 2 positive soft-tissue margins (sacrum) LR = 26% Complication = 56%
Laitinen (2017) (11)	Case control	1987 - 2015	Overall = 45 Case = 23 Control = 61	Sample size - Navigated : 9 - Non-navigated: 12 Type = iliosacral bone tumors (OS, CS, ES) - 12 high grade in non-navigated group vs 5 in navigated group	Orthopmap 3D	Margins = 100% negative margins in navigated group vs 75% negative in non-navigated group. LR = 22.% in navigation group vs 50% in non-navigation group. Complication = 33.3% in the navigation group versus 33.3% in the non-navigation group. Blood loss = 1170 ml for navigated vs 3800 ml for non-navigated resections (p=NS) Duration surgery = 190 min for navigated vs 355 min for non-navigated resections (p=NS)
Ould-Slimane (2016) (12)	Case series	2014 – 2014	NA	Sample size = 6 Type = pelvic resections (2x CS, 1x chordoma, 1x sarcoma, 1x non-union, 1x GCT)	Medtronic O-arm	Margins = 100% negative
Young (2015) (2)	Case series	2009 - 2015	27 (6 – 45)	Sample size = 9 Type = pelvic bone tumors (3x OS, 4x CS, 3x ES)	Orthomap 3D	Margins = 100% negative - All within 5 mm from planned planes.
Sternheim (2015) (16)	Experimental	NA	NA	Sample size - 90 navigated osteotomies (sawbone) - 54 non-navigated osteotomies (sawbone) - 16 navigated osteotomies (cadaver)	Mobile C-arm iCT with custom 3D visualization software	Accuracy = navigated entry and exit cuts in sawbones more accurate than non-navigated; 1.4±1 and 1.9±1.2 versus 2.8±4.9 and 3.5±4.6 respectively. (p<0,001). In cadavers the navigated entry and exit cuts were 1.5±0.9 and 2.1±1.5 respectively.

Jeys (2013) (13)	Case series	2010 – 2012	13 (3 – 34)	Sample size = 31 - 20 pelvic - 11 sacrum Type = 23 primary bone sarcoma, 4 metastatic lesions, 4 rectum tumors.	Orthomap 3D	Margins = 91.3% negative - 71% negative without navigation. LR = 13% Complication = 32% Duration surgery = 260 min
Wong (2013) (9)	Case series	2006 – 2009	39 (5 – 69)	Sample size = 11 - 6 pelvic - 5 sacrum Type = 2x OS, 5x chordoma, 2x metastatic, 2x other.	CT-based spinal navigation	Margin = 100% negative - 3x marginal (sacrum) LR = 27% (sacrum)
Cho (2012) (14)	Case series	2005 - 2008	48 (22 – 79)	Sample size = 10 - 7 pelvic - 3 sacrum Type = 2x OS, 5x CS, 3x ES	CT-based spinal navigation	Margin = 100% negative - 3x marginal (sacrum) LR = 22% (pelvic)
Cartiaux (2010) (15)	Experimental	NA	NA	Sample size = 156 osteotomies Type = pelvic sawbones	Praxim Surgetics navigation system	Accuracy = 2,8 mm error for navigated vs 5.2 mm error for non-navigated cuts compared to planned resection.

Table 3 - Overview of previous studies comparing navigated and nonnavigated pelvic resections

Abbreviations: NS = not significant, CS = chondrosarcoma, OS = osteosarcoma, ES = Ewing sarcoma, GCT = giant cell tumor, LR = local recurrence

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CHAPTER 7

Introducing fluorescence guided surgery into orthopedic oncology: A systematic review of candidate protein targets for Ewing sarcoma

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Abstract

Ewing sarcoma (ES), an aggressive bone and soft-tissue tumor, is treated with chemotherapy, radiotherapy and surgery. Intra-operative distinction between healthy and tumorous tissue is of paramount importance but challenging, especially after chemotherapy and at complex anatomical locations. Near infrared (NIR) fluorescence guided surgery (FGS) is able to facilitate determination of tumor boundaries intra-operatively, improving complete resection and therefore survival. This review evaluates potential ES-specific proteins from the literature as targets for NIR fluorescence guided surgery.

Introduction

Ewing sarcoma (ES) is a small, round cell sarcoma that shows pathognomonic molecular findings, and varying degrees of neural differentiation. [1] ES is the second most common primary osseous malignancy in children and young adults after osteosarcoma, with a peak incidence in the second decade of life. [2-4] Treatment generally consists of chemotherapy followed by surgery and/or radiotherapy. This multimodal approach drastically improved survival, from a 10-year overall survival (OS) of approximately 10% up to 55 to 65% in patients with localized disease and 20 to 35% for patients with metastatic disease. [5-10] During the last decades local treatment has changed from routinely amputation to limb-salvage, in which preservation of a functioning limb is at the essence of achieving clear margins. [7]

ES arises from the diaphysis of long bones with early involvement of the surrounding soft tissue. The soft tissue mass is usually large, circumferential around the involved bone and might even exceed the intra-osseous component in size. [1] Neoadjuvant treatment causes shrinkage of both the bony and soft tissue component, but tumor boundaries can still consist of vital tumor cells. The infiltrative rather than pushing type of tumor outgrowth of ES impedes border definition, but achieving wide surgical resection is of paramount importance for survival in ES. Incomplete resection occurs in 20-30% of the cases. [11-13] A large study of 244 patients registered in the Cooperative Ewing's Sarcoma Studies (CESS) showed that the local recurrence rate in patients with or without systemic metastasis was significantly lower after wide resection compared to marginal or intralesional resection (5% versus 12%). [13] Another large study of 512 ES patients showed that local control and 5-year disease-free survival are significantly better when adequate surgical margins are achieved (96.6% versus 71.7% and 69.6% versus 46.3% respectively). [12]

Developments in intra-operative imaging, like CT-based systems, make accurate defining and localization of the osseous margins possible. MRI enables adequate pre-operative visualization of soft tissue involvement and can show possible ingrowth in nearby neurovascular tissue, which is essential knowledge for surgical planning. However intra-operative definition of soft tissue margins remains challenging, especially after neoadjuvant treatment. [7,14,15] During surgery the surgeon relies mostly on his eyes and hands when distinguishing tumor issue margins. Furthermore, about 50% of the Ewing sarcomas arise in the axial skeleton with the pelvic and spine as common locations. Accurate surgery with clear margins is challenging in these complex anatomical locations. [5] This emphasizes the need for tools to define surgical margins of soft tissue involvement during surgery.

Targeted imaging uses membrane proteins that are over-expressed on tumor or tumor-associated cells to visualize tumors. One of the most eye catching technologies in targeted imaging is near infrared (NIR) fluorescence imaging. It provides optical contrast between tumor and surrounding healthy tissue in a broad range of (pre)clinical tumor types and might have the potential to delineate soft tissue involvement of Ewing sarcoma during surgery. NIR light is less absorbed than visible light and thus penetrates tissue much deeper. Furthermore, lower auto-fluorescence

is observed at NIR wavelengths which enables good contrast. Because NIR light is invisible for humans, the surgical field remains unstained, but a dedicated NIRF camera system and screen are needed for visualization. [16-19]
Over the past 10 years clinical research has focused mainly on non-specific fluorescent agents like indocyanine green (ICG), which are primarily used for vascular imaging and sentinel lymph node procedures. [20] Unfortunately these simple non-targeted dyes are not useful to target malignant cells. Therefore, many oncologic targets have been explored and indeed sub-millimeter sized tumor nodules could be detected in animal models. [21] With the first tumor-targeting clinical trial performed in 2011 by van Dam et al. [22], FGS is at the doorstep of clinical translation to oncologic surgery and many targets are being explored using numerous detection platforms like antibodies, peptides and RNA aptamers. [23]
So far, NIR-based FGS has not been used to define margins in ES. Finding a good target might be challenging because of it's uniqueness and the limited similarities with other tumor types. Sand et al. [24] studied membranous CXCR4 expression on Ewing sarcoma cell lines using a fluorescently labelled CXCR4 targeting peptide. They showed that the fluorescently tagged CXCR4 targeting peptide was able to detect CXCR4 on living ES cells. Nevertheless, data are not explored *in vivo* yet.
When defining a potential biomarker for targeting, the following characteristics are of utmost importance: extracellular biomarker localization, expression pattern, tumor-to-healthy tissue ratio, percentage of positive tumors, reported successful use of the biomarker in *in vivo* imaging studies and internalization. [23,25]
The aim of this study was to provide an overview of possible tumor-specific biomarkers in Ewing sarcoma. For this purpose, a systematic analysis of scientific literature was conducted, using the recently published ES surfaceome database, based on three ES cell lines (A673, TC-32, and TTC-466) as a reference. [26]

Materials and methods

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. [27] The review protocol for this study was prospectively registered at PROSPERO[2] (registration number CRD42017080720).

Search strategy

A search strategy was developed and searches were ran in the following databases in October 2017: PubMed, Embase, Cochrane Library, Academic Search Premier and Web of Science. Search strategies for all databases were adapted from the PubMed strategy. The search strategy consisted of the keywords "Ewing sarcoma",

[2] http://www.crd.york.ac.uk/prospero

"biomarker", "target" and abbreviations thereof. See supplementary file 1 (only available in online publication) for the complete search strategies for each database.

Eligibility criteria

Multiple study designs were considered for this review, including clinical trials (phase I, II, and III) and prospective or retrospective cohort studies. Animal studies, case reports, reviews, viewpoints, or conference reports were excluded. We searched for systematic reviews on this topic and only included original articles in our review. Studies were eligible for inclusion if they met the following criteria: (1) Report of cell surface protein expression in the primary ES tumor; (2) Cell surface protein expression was evaluated by flow cytometry, western blot or immunohistochemistry; (3) Positive expression in 50% or more of the Ewing sarcoma samples tested; (4) Study was published in the English language. The eligibility of the studies was assessed by two authors (SB and PD). Disagreements were resolved by discussion during a consensus meeting. Persistent disagreements were settled by consultation of a third reviewer (PH).

Data extraction

The following data were extracted from eligible studies: target characteristics, sample size, type of sample, percentage of positive Ewing sarcoma samples and pattern of expression.

Target selection: scoring system

Considerations to select the optimal target for tumor imaging are: (1) The location and accessibility of the target on the cell membrane; (2) Up-regulation on tumor cells compared to cells in adjacent normal tissue. Note that targets/biomarkers like FLI1 and NKX2.2, which are considered standards for ES in diagnostics and pathology, cannot be used for *in vivo imaging,* because of nuclear or cytoplasmic expression. [28-32]

In order to select the best biomarkers useful for NIR FGS in Ewing sarcoma, we developed a scoring system based on the Target Selection Criteria (TASC) of van Oosten et al. [25] to classify the targets based on their characteristics and the evidence from the literature search (Table 1).

The scoring system is based on five domains:

I) Number of samples tested. This provides a measurement of the evidence from the literature search.

II) Pattern of expression. This concerns the expression pattern evaluated in the single tissue samples tested. In the most ideal situation a target is expressed by all tumor cells but in reality intra-tumoral heterogeneity is more likely. When a target is equally distributed through the tumor tissue, resulting in a strong diffuse pattern of expression, the target is more applicable for imaging than when it shows a focal expression. Intensity of the expression pattern was defined as + for mild expression (5-20% of cells in one sample positive), ++ for moderate expression (25-50% of cells in one sample positive) and +++ for strong expression (>50% of cells in one sample positive).

III) Up-regulation in Ewing sarcoma. To assess this we used the recently published ES surfaceome database of Town et al. [33], based on three ES cell lines (A673, TC-32, and TTC-466). They used next-generation RNA sequencing and coupled this to a database of known genes encoding for cell surface proteins (the surfaceome) to define a cell surface proteome of Ewing sarcoma compared with mesenchymal stem cells (MSC). A large list of genes encoding for cell surface proteins was created ordered by differences in expression level between Ewing sarcoma and MSC. The first 1000 genes on this list show a very high upregulation in ES compared to MSC. From place 5000 onwards genes show a small increase in ES compared to MSC or even higher levels in MSC than in ES. Obviously, enhanced RNA expression does not consequently mean protein upregulation.

IV) Percentage of tested Ewing sarcoma samples that showed expression. The percentage is presented as a mean together with the range.

V) Previously imaged: if a target is previously used for *in vivo* targeted imaging (either pre-clinical or in PET/SPECT studies) it indicates that a target is suitable for imaging purposes.

The maximum score for a target is 10 points. We chose 7 points as the cut-off value for potentially suitable targets for targeted imaging in Ewing sarcoma.

Results

Study selection

The initial search strategy identified 4943 records (PubMed *n* = 2203; Embase *n* = 1585; Web of Science *n* = 1054; Cochrane Library *n* = 19; Academic Search Premier *n* = 82). After removal of 2128 duplicates, 2815 records were available for screening. After screening of the titles and abstracts, 197 full-text articles were obtained, of which 111 eventually did not meet the eligibility criteria: 83 studies did not report cell

surface expression, 12 studies reported expression levels <50%, 13 studies were not about cell surface expression in Ewing sarcoma, 2 studies were not about expression in primary Ewing sarcoma tumors and of 1 study no full text was available. In total 86 studies were included studying 47 biomarkers (Figure 1). The reviewers initially disagreed on 21 inclusions during the selection process, but eventually consensus was reached for all studies.

Study characteristics

The characteristics of the 47 included biomarkers are presented in supplementary file 2 (online available in online publication).

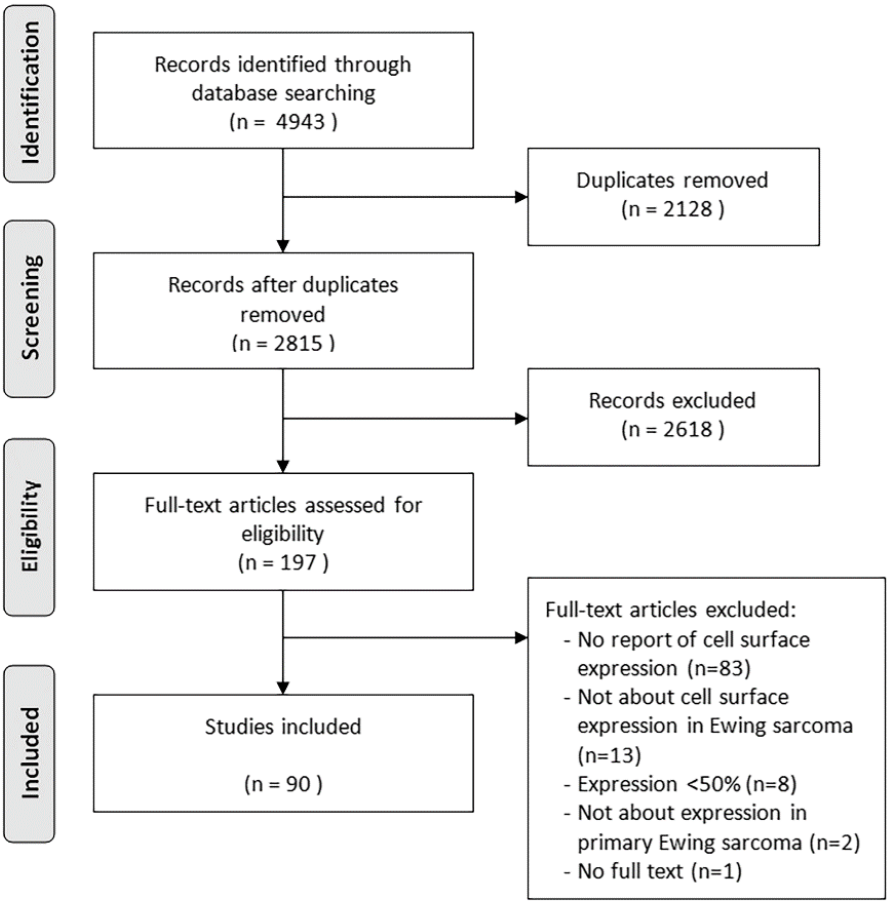

Figure 1 - Flowchart study selection process.

Candidate proteins for targeted imaging
The target scoring system (Table 1) was applied on the 47 targets listed in appendix II. Nine targets scored 7 points or higher. Table 2 provides an overview of the 9 targets that are candidates for NIR FGS in Ewing sarcoma surgery. Targets are subdivided in receptors and cell adhesion molecules (CAM) or anchoring proteins. The following 9 targets are considered potentially suitable (Table 2): CD99, LINGO-1, IGF-1R, C-kit, NOTCH receptor, CXCR4, NPY receptor Y1, Claudin-1, Occludin, TEM-1 (endosialin). These targets are discussed in more detail.

Target scoring system		**0**	**1**	**2**
I	Sample size	0 – 9	10 – 50	>50
II	Pattern of expression	Focal	Diffuse, mild or heterogenic	Diffuse, moderate or strong (++/+++)
III	Upregulation (based on the surfaceome of Town et al.)	≥5000	1000 – 5000	<1000
IV	Percentage expression	50 – 69 %	70 – 85%	>85%
V	Previously imaged	No		Yes

Table 1 – Targets scoring system.
Eligible biomarkers were granted points (0, 1 or 2) based on five domains: I) Sample size; II) Expression pattern, which comprises the intensity of the expression of the target; III) Upregulation compared to healthy tissue, using the recently published ES surfaceome database of Town et al. [33]; IV) Percentage positive cells; V) Previously imaged. The maximum score is 10. Targets that score 7 or higher are potentially suitable for targeted imaging.

Receptors

CD99
CD99 (also called MIC2, O13 or T-cell surface glycoprotein E2) is a transmembrane glycoprotein encoded by the CD99/MIC2X gene. CD99 is involved in differentiation of primitive neuroectodermal cells, migration of leukocytes, and apoptosis of T cells. It may promote growth and migration of tumor cells by down-regulation of the

potassium channel modulatory factor, KCMF1, which is thought to be a metastasis suppressor gene. [34] Overexpression of CD99 has been found in lymphoblastic lymphoma, rhabdomyosarcoma, synovial sarcoma, mesenchymal chondrosarcoma, and blastemal component of Wilms tumor. CD99 expression in ES is strong to moderate, diffuse and membranous and present in approximately 96% of ES tumors. (30, 34-60) The expression level is much higher in Ewing sarcoma compared with mesenchymal stem cells (MSC). [33] A preclinical study using ^{64}Cu labeled mouse monoclonal antibody against CD99 detected primary ES tumors and metastases with higher sensitivity than conventional FDG-PET *in vitro* as well as in xenograft mouse models. [35] Based on this study CD99-targeted FGS seems feasible, preferably using human or humanized antibodies to avoid human anti-mouse induced complications. Human CD99 antibody scFv-fragments have recently been developed using a synthetic phage antibody library. [36] Although intended for therapy, the specificity of these anti-CD99 scFv fragments for ES, in combination with the small size of these fragments in comparison with full size antibodies (27 versus 150 kDa), might offer an excellent agent for FGS of ES.

CXCR4

CXCR4 (CD184) is the receptor for chemokine SDF-1/CXCL12. CXCR4/SDF-1 signaling plays a role in chemotaxis of hematopoietic cells and in neuron generation during embryogenesis and adult life. It is absent in most healthy tissues but upregulated in the tumor microenvironment (TME) of many tumor types, where it is associated with metastasis, angiogenesis, and tumor growth. [24] Also in ES CXCR4 is associated with tumor progression and metastasis. [37-39] Overall 82% of ES tumors are positive for CXCR4 (range 64 – 100%), with cytoplasmic and membranous staining varying from weak to strong. [24,38] A surfaceome study of Ewing sarcoma by Town et al. [33] showed no expression of CXCR4 in mesenchymal stem cells (MSC), which makes CXCR4 an attractive target for targeted imaging. Sand et al. [24] studied membranous CXCR4 expression in ES cell lines using a fluorescently labelled CXCR4 targeting peptide (MSAP-Ac-TZ14011), indicating the feasibility of this agent for FGS. First in human experience with a radiolabeled version of a similar peptide (Pentixafor) in a small and heterogeneous patient cohort did not completely fulfill the expectations in comparison with standard (18)F-FDG PET. [40]

Target	Function	Score
Receptors		
CD99 (MIC2, O13 or T-cell surface glycoprotein E2)	Cell surface glycoprotein involved in differentiation of primitive neuroectodermal cells, apoptosis of T cells, T cell adhesion, migration of leukocytes; may promote growth and migration of tumor cells.	10
CXCR4	Receptor for chemokine SDF-1/CXCL12, which is involved in chemotaxis of hematopoietic cells and neuron generation. In cancer is plays a role in the tumor microenvironment, where it is associated with metastasis, angiogenesis and tumor growth.	9
NPY-R-Y1	Expressed in the central nerve system and periphery (heart, kidney, gastro-intestinal tract); activation is associated with modulation of the MAPK pathway, which leads to increased or uncontrolled cell proliferation and resistance to apoptosis.	8
LINGO-1	Functional component of Nogo receptor signaling complex. Important negative regulator of oligodendrocyte differentiation and axonal myelination.	8
IGF-1R	A tyrosine kinase receptor (TKR) that is activated by insulin-like growth factor 1 (IGF-1) and involved in hypertrophy of skeletal muscles and other tissues and cell survival; shows anti-apoptotic effects that allow cancer cells to resist the cytotoxic properties of chemotherapeutic drugs or radiotherapy.	8
C-kit (CD117)	Stem cell factor receptor that is important for development and survival of mast cells, hematopoietic stem cells, melanocytes, germ cells and interstitial cells of Cajal; plays role in cancer cell survival, proliferation and differentiation.	7
NOTCH-R	Involved in cell signaling; shows oncogenetic (suppress apoptosis; promote neo-angiogenesis, tumor cell growth and metastasis) and tumor-suppressive (inhibit angiogenesis and induced cell differentiation or apoptosis) functions.	7

Target	Function	Score
CAM / anchoring proteins		
Occludin	Integral plasma-membrane protein that is required for cytokine-induced regulation and formation of the tight junction paracellular permeability barrier; in cancer it attributes to increased invasion and reduced adhesion which promotes metastasis.	9
Claudin-1	Tight junction protein that contributes in cell-to-cell adhesion by forming continuous seals around cells; dysregulation of claudins plays a role in tumorigenesis, the exact underlying mechanism remains unclear.	8

Table 2 – Potentially suitable targets for NIR FGS in Ewing sarcoma

NPY receptor Y1

Neuropeptide Y (NPY) receptors are members of the G-protein coupled receptor superfamily. The NPY receptor Y1 is expressed in the central nerve system and periphery including heart, kidney, and gastro-intestinal tract. It mediates the function of a neurotransmitter neuropeptide Y (NPY), and a gastrointestinal hormone peptide YY (PYY). Activation is associated with modulation of the MAPK pathway, which leads to increased or uncontrolled cell proliferation and resistance to apoptosis. [41] NPY receptor Y1 is highly expressed in human cancers, for instance breast cancer. Körner et al. [42] studied NPY expression in several sarcomas, including Ewing sarcoma. ES samples showed a strikingly high NPY receptor Y1 expression. 84% of the tumors tested positive with high receptor density. Recently Li et al. [43] developed fluorescent nanobubbles (NBs) for specific targeting of Y1 receptors overexpressed in breast cancer. The fluorescent NBs were used as ultrasound contrast agents (UCAs) for targeted molecular imaging with contrast-enhanced ultrasound and fluorescent imaging using the Lumina XRMS system. The NBs showed high affinity and specificity for NPY receptor Y1, providing evidence that specific targeted imaging might also be applicable in ES.

LINGO1

Leucine-rich repeat and immunoglobulin domain containing protein 1 (LINGO1) is a functional component of the Nogo receptor. It plays a key role in the central nerve system where it is an important negative regulator of oligodendrocyte differentiation and axonal myelination. [44,45]. It is characterized by a large and well defined

extracellular domain. [46] Town et al. [33] explored the cell surface proteome of Ewing sarcoma and found that LINGO1 is highly expressed in 91% of ES and not in any other somatic tissue apart from the brain, suggesting it as an appropriate candidate for imaging. The human monoclonal antibody Opicinumab (Li81, BIIB033), has recently been developed to block LINGO1 as a treatment for multiple sclerosis. Li81 was isolated using Fab phage display technology and engineered into a human IgG1 monoclonal antibody with high affinity and specificity to LINGO1. [47,48] Initial clinical trials showed disappointing results, but regardless of its therapeutic efficiency this monoclonal antibody could in principle be applied for use of FGS in ES.

IGF-1R

Insulin-like growth factor 1 receptor (IGF-1R), a tyrosine kinase receptor, is found on the surface of many human cells. It is activated by IGF-1 or IGF-2, playing a role in transformation events like hypertrophy of skeletal muscles and other tissues. IGF-1R is upregulated in various types of tumors, including bladder, breast, prostate and lung cancer. IGF-1R signaling is thought to be play a role in survival and cell growth of cancer cells. It induces an anti-apoptotic effect, allowing cancer cells to resist the cytotoxic properties of chemotherapeutic drugs or radiotherapy. [49-53] IGF-1R is expressed in 78% of the Ewing sarcomas, but it shows to be very variable (range 33-100%). The pattern of expression is often diffuse, varying from weak to strong. [49-53] Several pre-clinical studies evaluated the use of IGF-1R antibody-conjugated fluorophores to identify tumor cells in animal models of various cancer types. Zhang et al. [54] used the humanized monoclonal antibody AVE-1642 conjugated to Alexa 680 to target xenograft tumor and was able to detect IGF1R down regulation, with little nonspecific targeting of other tissues or organs in mice. Park et al. [55] used IGF-1R antibodies conjugated with PEG-ylated 650 nm fluorophores to selectively highlight liver metastases in a liver metastasis model of colon cancer in nude mice. The IGF-1R targeted fluorophore-antibody conjugation enabled clear imaging of liver metastases compared to normal liver tissue, despite the relatively high expression level in normal liver tissue. Humanized anti-IGF-1R monoclonal antibodies, like AVE1642 and R1507, have been developed for therapy. These antibodies have been evaluated for radiolabel-based SPECT/PET as well, but only in pre-clinical settings. [56,57]

C-kit (CD117)

C-kit is a tyrosine kinase receptor important for development and survival of mast cells, hematopoietic stem cells, melanocytes, germ cells and interstitial cells of Cajal. It plays a role in tumor growth and progression. [58] C-kit is expressed by the KIT gene which is highly upregulated in ES cell-lines. [33] The intensity of the expression varies however and only 60% of ES show a strong membranous expression (range 31 – 100%). Staining intensity varies from weak to strong and is diffuse membranous and/or cytoplasmic. [59] Metildi et al. [60] used fluorescently labeled (AlexaFluor 488) rat-derived anti-KIT antibodies to label KIT-expressing Gastrointestinal Stromal

Tumors (GISTs) *in vivo* and laparoscopically. They provided proof of concept and confirmed that KIT could be accurately labeled in GISTs for detection of primary tumors, as well as for detection of small metastatic deposits that might be missed at the time of surgical resection. The recently developed humanized monoclonal antibody KTN0158 is being evaluated in dogs for therapeutic purposes and might eventually have potential for imaging. [61]

NOTCH receptor

NOTCH receptors 1-4 are membranous proteins that play a role in the development of numerous cells and tissue types. Their role in cancer is ambiguous: They can act oncogenic or tumor-suppressive, dysregulating apoptosis, angiogenesis, tumor cell growth and metastasis. [62,63] Bennani-Baiti et al. [64] investigated the expression of NOTCH receptors in ES and found that NOTCH receptors are highly expressed but do not appear to be active. 97% of the samples showed positive staining for at least one NOTCH receptor and 75% of the ESFT expressed two or more NOTCH receptors. The stainings showed to be diffuse and only moderate membranous. The genes encoding for the 4 different types of NOTCH receptors are expressed slightly higher in ES compared to healthy tissue. [33] Although potential therapeutic antibodies like bronctictuzumab against activated NOTCH1 are being evaluated for therapy in patient derived xenografts. Direct imaging of NOTCH has not been described yet.

Cell adhesion molecules (CAMs) or anchoring proteins

Cell adhesion molecules are membrane-bound proteins that affect cellular processes. Generally they are transmembrane receptors that consist of 3 parts: an intracellular domain interacting with the cytoskeleton, a transmembrane domain and an extracellular domain that interact with either other CAMs or with the extracellular matrix (ECM). CAMs form a large and diverse group of proteins and most of the members belong to either the immunoglobulin superfamily, or to the families of integrins, cadherins or selectins. [23,65]

Claudin-1

Claudin-1, encoded by the CLDN1 gene, is a tight junction protein and contributes to cell-to-cell adhesion by forming continuous seals around cells, serving as a physical barrier to prevent solutes and water from passing freely through the para-cellular space. [66,67] Abberant claudin expression has been reported in several cancer types, including lung, prostate and gastro-intestinal tumours. Reduced claudin expression results in loss of cell-to-cell adhesion and enhances cell motility, invasion and metastasis. Abnormally high levels are also associated with neoplastic growth. Schuetz et al. [66] studied the expression of claudin-1 in 30 ES tissue samples and found expression in 63% of the samples. Positive cases showed expression in more than 50% of the cells. In a similar study Machado et al. [67]

found a strong positive staining of claudin-1 in 285 of 415 tissue samples (76%). Rabinsky et al. [68] performed a study on real-time NIR fluorescence endoscopic imaging of mice bearing human colonic adenomas. They used a phage display derived peptide CLDN-1(53-80) labelled with near-infrared dye Cy5.5 at the C-terminus. After intra-rectal administration they found a significantly higher signal-to-background ratio for human colonic adenomas compared to the signal-to-background ratio of normal in vivo images. Alternatively, humanised monoclonal antibodies against claudin-1 are currently being evaluated for therapy, that could also be explored for imaging purposes. [69]

Occludin

Occludin is an integral plasma-membrane protein that is required for cytokine-induced regulation and formation of the tight junction para-cellular permeability barrier. Occludin is able to induce adhesion when cells lack tight junctions. [67] Although OCLN, the gene encoding occludin is often downregulated in cancers it is highly upregulated in Ewing sarcoma. [33] Machado et al. [67] studied the expression of several epithelial cell adhesion molecules in ES, including occludin. Occludin showed a moderate to strong, diffuse membranous staining in 287 out of 415 ES tissue samples (76%). Because occludin is actually down-regulated in many tumors, there are currently no occludin targeting agents developed for therapy or imaging.

Discussion

In this systematic review we provide an overview of tumor-specific biomarkers that could be used for NIR fluorescence guided surgery in Ewing sarcoma. Based on a scoring system, 9 potentially suitable biomarkers for targeted imaging were identified.

This study has several limitations. The studies evaluated in this systematic review are heterogenic in the evaluation of cell surface expression. Flow cytometry, western blot, immunohistochemistry or a combination of these techniques are used. Furthermore, some targets like C-kit and IGF-1R show a wide range in expression levels among studies whereas several other targets are only investigated in a single study. Also the expression of a target may depend on tumor stage. CXCR4 for example is associated with more advanced disease. [38] The results of the studies evaluated might therefore be less comparable. Finally, we used the recently published surfaceome database in our scoring system to evaluate the upregulation of a potential target in ES. This surfaceome is based on three ES cell lines (A673, TC-32, and TTC-466). [33] Serial passage of cell lines can cause genotypic and phenotypic variation over an extended period of time. Cell lines might therefor not adequately reflect the true ES surfaceome.

The scoring system used in this review is a guidance tool that helped in selecting potential targets. We chose a score of 7 as the cut-off point to identify potential targets. Other factors play a role in selecting the most optimal candidate, such as

toxicity of an antibody, the availability of a humanized antibody and results from previous studies.
Following this study, immunohistochemical analysis and cell line-based validation of the potential biomarkers should be performed. Next, imaging of a peptide or an antibody (derivative) conjugated to a fluorophore should be assessed *in vitro.* Finally, if a target still shows potential, *in vivo* testing with a specific binding ligand in a tumor mouse model is needed.

Conclusion

In Ewing sarcoma a large number of tumor-specific biomarkers is upregulated. With the use of a scoring system we identified CD99, LINGO-1, C-kit, NOTCH receptor, CxCR4, NPY receptor Y1, Claudin-1 and Occludin as the most interesting ES specific biomarkers for the use in NIR fluorescence guided surgery. Further immunohistochemical and cell line-based research of these potential targets should be performed to elucidate the most optimal candidate. With this study the first steps are made to explore this promising technique that is on the doorstep of optimizing orthopedic oncologic surgery

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Summary and general discussion

CHAPTER 8

Summary

One of the main questions in Ewing sarcoma treatment is to identify low-risk patients that can be treated with less intensive treatment so that toxicity and the occurrence of long-term adverse effects can be limited while still maintaining high cure rates or to identify those patients for whom treatment is expected to have limited benefit. Furthermore, to identify high-risk patients in which treatment needs to be intensified to improve outcome. Selection of risk groups and adjusted treatment allows for early decision making, will help to improve future outcomes and assists in clinical trial design. Additionally, treatment of Ewing sarcoma is multimodal and surgery, if feasible, is crucial for curative management. However, accurate detection and localization of tumor boundaries, especially in anatomical complex locations such as the pelvic is challenging. Inadequate surgical margins lead to a higher risk of local recurrence which has major impact on oncological outcome. Developments in intra-operative imaging, like CT-based navigation systems and near infrared (NIR) fluorescence guided surgery (FGS) make accurate defining and localization of surgical margins possible. They represent a whole new field of precision medicine and provide new treatment options for patients, thereby improving function outcome and healthcare quality. This thesis aimed to provide individual clinically advanced and response adaptive treatment strategies for Ewing sarcoma. The first chapters of this thesis describe the development of two prediction models for Ewing sarcoma. The following chapters focus on developments in pre- and intra-operative imaging.

During the last decades, many prognostic factors have been identified, and the most relevant ones are being used to tailor treatment and for clinical trial design. Cohorts, however, often contain more variables than can reasonably be used for prediction. Therefore, the most predictive and sensible predictors should be selected. The systematic review in **chapter 2** on the current known prognostic factors for overall survival (OS) and event-free survival (EFS) in Ewing sarcoma showed that the presence of metastasis at diagnosis, large tumors (volume ≥ 200 ml or largest diameter ≥ 8 cm), primary tumors located in the axial skeleton, especially pelvic, and a histological response of less than 100% are strongly associated with poorer survival in Ewing sarcoma. These factors should therefore be included as risk factors in the development of prediction models for survival in ES. Insight about the effect of surgical margins and local treatment modality requires further investigation. Surgical margins seemed to be associated only with EFS, their association with OS needs to be further established. Heterogeneity among centers in defining and evaluating surgical margins and the use of post-operative radiotherapy in case of inadequate margins might play part in these somewhat inconsistent results. Local treatment modality in relation with survival was evaluated by multiple studies which showed inconsistent results. The existing evidence available is based on retrospective, non-randomized trials. Many of these studies are affected by a selection bias, where radiotherapy is only indicated in specific groups of patients, for instance patients with less favorable prognostic factors. In order to assess the effect of local treatment on

survival, randomized trials aimed at comparing surgery, radiotherapy and a combination of both or prospective comparative studies are needed.

Achieving the optimal balance between predictability and simplicity is the key to a good prediction model. In **chapter 3** we developed and validated an easy-to-use clinical tool to predict OS in Ewing sarcoma at the time of diagnosis using a cohort of 1314 patients. Apart from predicting survival from diagnosis the model also shows how survival changes during the course of treatment as more information comes available. Independent prognostic factors at diagnosis are the patient's age, tumor volume, primary tumor localization and disease extent. Based on these factors 5 risk categories (A-E) are identified with a 5-year OS (95%CI) of 88% (86-94), 69% (64-74), 57% (50-64), 51% (42-60) and 28% (22-34) respectively. Figure 1 shows a flowchart to easily stratify patients into risk category A to E.

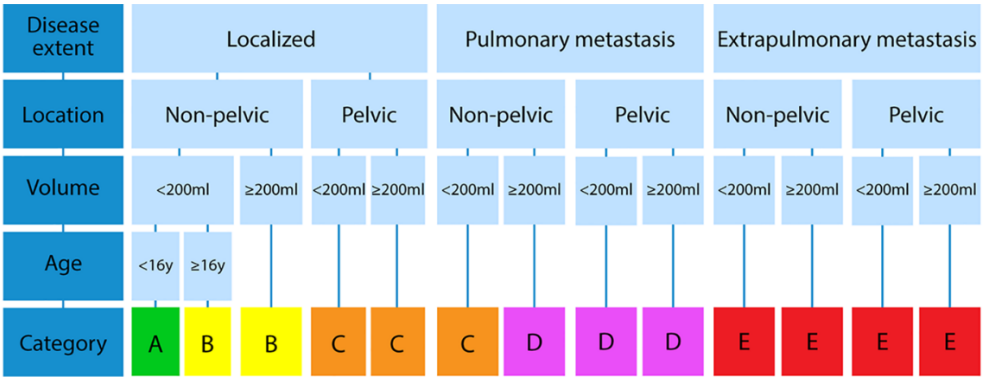

Figure 1 – Flowchart to easily stratify patients at diagnosis into risk category A to E.

The Harrell C-statistic of the model, a measure of goodness of the fit, was 0.70, indicating a good model. Next, we searched for associations between prognostic factors at surgery and OS and found that independent prognostic factors from surgery are the patient's age, tumor volume, disease extent and histological response. A proportional hazard Cox model from surgery including histological response and risk category was estimated. Figure 2 presents a flowchart to stratify patients at surgery based on the Cox model. In categories A-B, 5y OS increased to 92% (87-97) and 79% (71-87) respectively for 100% necrosis and decreased to 76% (67-85) and 62% (55-69) respectively for <100% necrosis. In categories C-E, 5y OS increased to 65% (55-75), 65% (52-78) and 52% (38-66) respectively for ≥90% necrosis and decreased to 38% (22-54), 11% (0-26) and 7% (0-19) respectively for <90% necrosis.

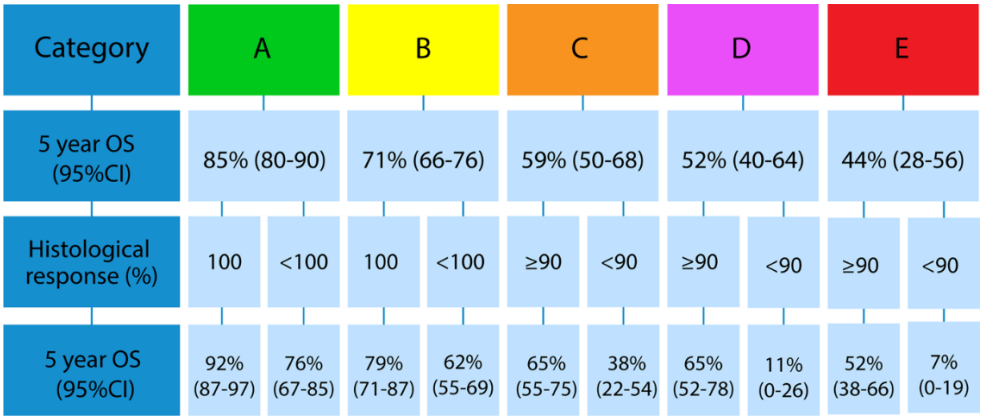

Figure 2 - Flowchart for stratification of Ewing sarcoma patients at surgery assessed by Kaplan Meier method.

The model we developed is based on a comprehensive dataset of 1314 ES patients with uniformity in diagnostics and treatment and availability of all relevant prognostic factors. The provided flowcharts are easy-to-use and based on assessable variables. Additionally, the 13 prognostic groups provide detailed insight in expected survival and could assist in fine-tuning individual treatment. This relatively simple and clinically easy to use character strengthens its usefulness. On top of that, the model provided new insights in how survival changes during the course of treatment. The information gained after surgery offers a second time-point for multidisciplinary decision-making, at this point histological response is a strong additional prognostic factor for OS in each risk category.

In Ewing sarcoma local recurrence, distant metastasis, and poor survival in patients with metastatic disease remain of great concern. Associations between local treatment modality, local recurrence, distant metastasis, and death are not yet clearly established. In **chapter 4** we provide a more in-depth analysis of disease evolution by development of a multistate model. Multistate models study the evolution of the disease and incorporate the occurrence of intermediate events such as local recurrence and distant metastasis which occur after surgery in the model. This provides useful insights into their relation with the considered endpoint, usually death. Disease evolution is retrospectively studied in 982 patients with Ewing sarcoma undergoing surgery after chemotherapy using a multistate model (Figure 3) with initial state surgery, intermediate states LR, pulmonary metastasis (DMpulm) and other DM±LR (DMother) and final state death.

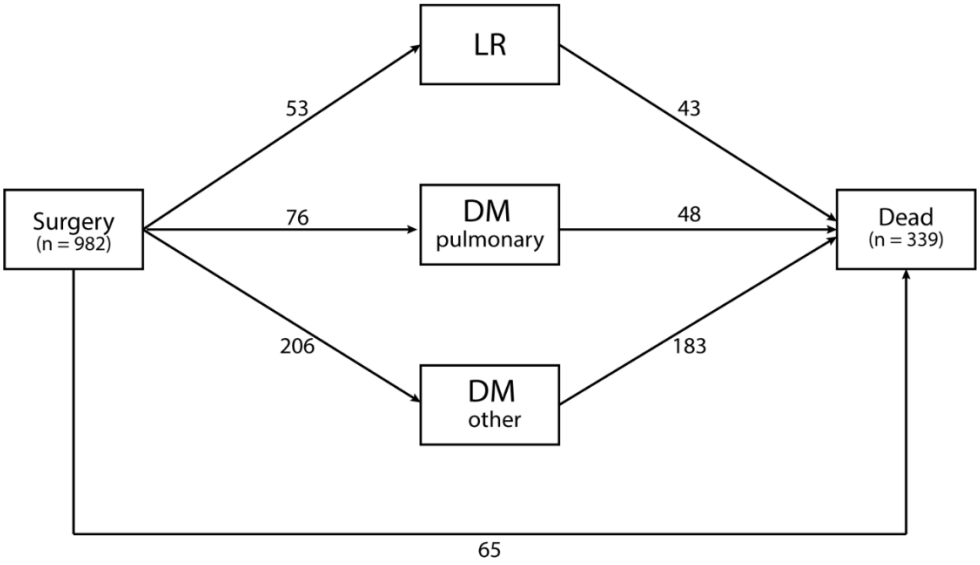

Figure 3 - Multistate model for Ewing sarcoma

Effect of risk factors is estimated using Cox proportional hazard models. Marginal or intralesional surgical margins are an important risk factor for transition from surgery to LR and when a patient reaches the LR state it is observed that the probability of death is higher in case of early LR (0-24 months), so time to recurrence could be considered as most relevant in these situations. Histological response is a strong prognostic factor for transition from surgery to distant metastasis and death. When a patient experiences new distant metastasis (either pulmonary, bone, other or combined), histological response loses relevance as a risk factor as the occurrence of distant metastasis more dramatically affects survival. A pelvic tumor site is an important risk factor for transition from surgery to LR. Previous pulmonary metastasis is a risk factor for transition to new pulmonary disease, but when a patient experiences new pulmonary disease, previous pulmonary metastasis is no longer prognostic factor for survival. Previous pulmonary or bone/other metastasis is a risk factor for transition to new bone/other metastasis with or without simultaneous LR. When reaching the DMother state only previous bone/other metastasis remain of prognostic value for survival. With this study we extended the knowledge about the effect of prognostic factors for intermediate events and final event death in Ewing sarcoma. We showed that prognostic factors have different effects on different transitions and that the impact on the next state in the evolution of the disease depends on the state a patient occupies. Apart from the patient's history, the time-element is also of paramount importance for decision-making. LR within 2 years or the occurrence of distant metastasis with or without subsequent LR significantly affect survival chances, and despite our efforts as physicians almost all patient that experience such an event died of progressive disease. Therefore, the balance

between the toxicity of intensive salvage treatments and quality of life in the remaining life span of these patients should be carefully considered. Radiotherapy seems protective for LR especially in pelvic/axial. However, the number needed to treat (NNT) with surgery and radiotherapy to prevent the occurrence of a single LR is 72 for all tumor sites combined. In contrast, the NNT for extremity tumors is 80 and the NNT for pelvic tumors is 10. This questions the value of radiotherapy in patients with an extremity Ewing sarcoma, where an individual patient with an extremity Ewing sarcoma might benefit only little. So is there really a need for this potentially toxic treatment, especially in the growing child, in this indication? Radiotherapy is associated with a significant risk for secondary radiotherapy induced malignancies, growth disturbance and postoperative complications of surgical reconstructions. Indications for radiotherapy should be explored further, preferable in a prospective randomized setting.

The second part of this thesis focusses on pre-operative and intra-operative imaging techniques. In **chapter 5** we retrospectively compare the diagnostic yield of 18F-FDG PET-CT to whole-body MRI for detection of skeletal metastasis in Ewing sarcoma. Since, accurate detection and localization of all metastases in Ewing sarcoma is very important because treatment of all these sites potentially provides a curative approach. In order to determine the level of discrepancy between magnetic resonance imaging (MRI) and ^{18}F-FDG PET-CT in detecting osseous metastases we included 20 patients with histopathological confirmed Ewing sarcoma between 2000 and 2017 who had ^{18}F-FDG PET-CT and MRI performed within a 4-week range. A total of 112 osseous lesions were diagnosed in 13 patients, 107 malignant and five benign. Seven patients showed no metastases on either ^{18}F-FDG PET-CT or MRI. Forty-one skeletal metastasis (39%) detected with MRI did not show increased ^{18}F-FDG uptake on ^{18}F-FDG PET-CT (false-negative). Bone lesions were more likely to be false-negative on ^{18}F-FDG PET-CT if hematopoietic bone marrow extension was widespread and active, during or after (neo)-adjuvant treatment or when the lesion was smaller than 10 mm. We showed that caution is needed when using ^{18}F-FDG PET-CT for diagnosing skeletal metastases in Ewing sarcoma. Poor contrast between metastases and active hematopoietic bone marrow, chemotherapeutic treatment and/or small size significantly decrease the diagnostic yield of ^{18}F-FDG PET-CT, but not of MRI.

Intra-operative distinction between healthy and tumorous tissue is of paramount importance but challenging, especially after chemotherapy and at complex anatomical locations. Intraoperative navigation techniques have been proposed to improve surgical accuracy in achieving negative margins, thereby reducing complications while still maintaining survival changes. About 25% of the Ewing sarcomas arise from the pelvic bones. Pelvic and sacral bone sarcoma resections are challenging due to anatomical and surgical complexity. Computer assisted surgery could assist in achieving higher surgical accuracy. In **chapter 6** we therefore

analyzed the accuracy in terms of surgical margin achieved by navigated pelvic and sacral primary bone sarcoma resections compared to non-navigated resections. Thirty-six patients with pelvic or sacral sarcomas treated with intraoperative navigation were retrospectively compared with 34 patients undergoing resections without navigation. Adequate bone margins are achieved in more patients in the navigated group than in the non-navigation group (29 of 36 patients (81%) versus 17 of 34 (50%); odds ratio, 4.14 (95% CI, 1.43-12.01); p = 0.007). With the numbers available, we found no difference in our ability to achieve adequate soft-tissue margins between the navigation and non-navigation group. We show that intraoperative guidance techniques improved our ability to achieve negative bony margins when performing surgical resections in patients with pelvic and sacral primary bone sarcomas. Achieving adequate soft tissue margins remains a challenge, and these margins do not appear to be influenced by navigation. Near infrared (NIR) fluorescence guided surgery (FGS) might be the solution to improve soft tissue margins. NIR FGS is able to facilitate determination of tumor boundaries intra-operatively, improving complete resection and therefore survival. It uses membrane proteins that are over-expressed on tumor or tumor-associated cells to visualize tumors. When defining a potential biomarker for targeting, the following characteristics are of utmost importance: extracellular biomarker localization, expression pattern, tumor-to-healthy tissue ratio, the percentage of positive tumors, reported successful use of the biomarker in *in vivo* imaging studies and internalization. **Chapter 7** provides an overview of possible tumor-specific biomarkers in Ewing sarcoma suitable for NIR FGS in Ewing sarcoma. In Ewing sarcoma a large number of tumor-specific biomarkers is upregulated. With the use of a scoring system we identified CD99, LINGO-1, C-kit, NOTCH receptor, CxCR4, NPY receptor Y1, Claudin-1 and Occludin as the most interesting ES specific biomarkers for the use in NIR fluorescence guided surgery. Further immunohistochemical and cell line-based research of these potential targets will be performed to elucidate the most optimal candidate. With this study the first steps are made to explore this promising technique that is on the doorstep of optimizing orthopedic oncologic surgery.

CHAPTER 9

General discussion and future perspectives

This thesis aimed at providing individual clinically advanced and response adaptive treatment strategies for Ewing sarcoma. Development of risk- and response adaptive treatment strategies is necessary to further improve clinical outcome and assists patients and multidisciplinary teams in shared decision making. In this chapter, previous chapters are summarized and discussed. Practical implications along with recommendations for future research are formulated.

Prognostic factors: classical and new

One of the main questions in Ewing sarcoma treatment is to identify patients that can be treated with less intensive treatment so that toxicity and the occurrence of long-term adverse effects can be limited while still maintaining high cure rates or to identify those patients for whom treatment is expected to have limited benefit, and also to identify high-risk patients in which treatment needs to be intensified to improve outcome. Selection of risk groups and adjusted treatment allows for early decision making, will help to improve future outcomes and assist in clinical trial design. As Hippocrates emphasized, prognosis is a core element of medicine. (1) In the treatment of Ewing sarcoma clinical prediction models that incorporate multiple variables are needed. A substantial amount of (ongoing) clinical research is devoted to the identification of prognostic factors. During the last decades, many prognostic factors have been identified, and the most relevant ones have been used to tailor treatment and for clinical trial design. Three studies combined prognostic factors into risk groups. Rodriquez-Galindo et al. (2) identified four risk groups in 220 Ewing sarcoma patients based on age (</≥ 14 years), primary tumor site (pelvic/non-pelvic) and disease extent (localized/isolated lung metastasis/extrapulmonary metastasis). Biswas et al. (3) developed a prognostic model for localized Ewing sarcoma in 244 patients based on tumor site (extremity/axial), white blood cell count (WBC) (</≥ 11×109/L), symptom duration (</≥ 4 months) and tumor size (</≥ 8 cm). Lastly, Karski et al. (4) constructed and validated five prognostic groups in a cohort of 2124 Ewing sarcoma patients based on disease extent (localized/metastatic), age (</≥ 18 years), primary tumor site (pelvic/non-pelvic) and ethnicity (white non-Hispanic / other). For accurate risk group stratification large representative and contemporary datasets that closely reflect the target population are needed to enhance the relevance, reproducibility and generalizability of the model. (5-9) The models of Rodriquez-Galindo et al. (2) and Biswas et al. (3) are based on small homogeneous cohorts that do not meet this requirement. Cohorts often contain more variables than can reasonably be used for prediction. Therefore, the most predictive and sensible predictors should be selected. In out systematic review on prognostic factors for survival in Ewing sarcoma (chapter 2) a consistent association between risk factors race/ethnicity and WBC count could not be found, in contrast to the models of Biswas et al. (3) and Karski et al. (4) described above. Additionally, all models described above only included pre-treatment factors. There are two major groups of prognostic factors in Ewing sarcoma: pre-treatment factors, known at diagnosis, and treatment factors, that come available during treatment. Survival is not static, but changes

constantly. Prediction models should thus not only be based on pre-treatment factors but also incorporate treatment depending factors. Finally, the predictive ability of the model should be evaluated using an independent dataset, preferably using an external dataset. Only Karski et al. (4) performed external validation. Due to missing data they did not include all relevant prognostic factors in the model, which limits the strength and usefulness of this model for clinical decision-making. One of the primary aims of this thesis is to develop and validate a prediction model stratifying treatment according to the individual patients' risk profile, before, but also during treatment. In order to provide all relevant risk factors for such a prognostic model a systematic review (chapter 2) on the current known prognostic factors for overall survival (OS) and event-free survival (EFS) was performed. The presence of metastasis was identified as most significant factor influencing survival. (2, 10-12) Other prognostic factors that consistently showed to be strongly associated with poorer survival are tumor size of ≥ 8 cm (2, 3, 13, 14), tumor volume of ≥ 200 ml (15-18), histological response of <100% (14, 16, 17, 19) and an axial, especially pelvic, location of the primary tumor. (3, 10, 13-15, 17, 18, 20-23) Age was evaluated in almost all studies, showing that older age is associated with a poorer survival. Strong evidence for a specific cut-off point was lacking and cutoff points varied from 14 to 28 years. (20, 21, 23, 24) Surgical margins seemed to be associated only with EFS, their association with OS needs to be further established. (11, 16) A good prediction model should provide accurate prediction of events by using a comprehensive dataset. In addition, the model should be relatively simple and clinically easy to use. Inaccurate estimates of future events will mislead physicians to provide insufficient treatment. On the other hand, a model with high predictability but which is complex or has too many factors will not be useful. Achieving the optimal balance between predictability and simplicity is the key to a good prediction model. (5-9) Therefore we developed and validated an easy-to-use clinical tool to predict OS in Ewing sarcoma from diagnosis using a cohort of 1314 patients (chapter 3). In addition to previous models, this model included all relevant prognostic factors and additionally, shows how survival changes during the course of treatment as more information, such as histological response and surgical margins, becomes available. The model is based on prognostic factors age (</≥ 16 years), tumor volume (</≥ 200 ml), tumor localization (pelvic / non-pelvic) and disease extent (localized / solitary pulmonary metastasis / bone and other metastasis). Based on these prognostic factors five risk categories are made. All variables are easy assessable and the provided flowcharts are easy to use. The Harrell's C-statistic of our model was 0.70. Discriminatory ability was further evaluated using cross validation. The overall agreement is very good (precision 90.26%; recall 89.57%). After surgery, new prognostic factors become available. We showed that histological response is a strong additional prognostic factor for OS. For patients in risk category A and B and 100% necrosis 5-year OS decreased significantly in case of less than 100% necrosis. For patients in categories C to E 5-year OS increased significantly in case of ≥90% necrosis and drastically decreased for patients with <90% necrosis, especially in categories D and

E (5-year OS 11% and 7% respectively). Histological response was divided into three categories: 100% necrosis, 90-99% necrosis and <90% necrosis. Cutoff points for good response differed among risk categories, in A and B 100% necrosis and in C to E 90% necrosis. But patients with 100% necrosis after induction chemotherapy had better survival compared to patient with 90-99% necrosis, HR 1.58 (95%CI 1.16-2.16;p=0.004), and patients with <90% necrosis, HR 2.90 (95%CI 2.15-3.93;p<0.001). Previous studies (16, 17, 19, 25) dichotomized histological response and considered patients with 90% necrosis or more as good responders. The cutoff at 90% is also used to tailor treatment in clinical trials (EURO-EWING 99, Ewing 2008 and Euro-Ewing 2012 study). By dichotomized the data, important information might be lost. The results in this study (chapter 3) and a recent study by Albergo et al. (26) show that it is desirable to only consider patients with 100% necrosis as good responders.

To gain more insight into disease evolution and the effect of surgical margins, histological response and radiotherapy, on local recurrence (LR), distant metastasis and OS we developed a multistate model (chapter 4). Instead of considering one endpoint, like other studies, the occurrence of intermediate events like local recurrence and distant metastasis that may occur after treatment and their relations to the considered endpoint, death, were estimated. By using a multi-state model the effect of possible prognostic factors on different disease states in Ewing sarcoma can be estimated. Results show that strong prognostic factors for transition from surgery to distant metastasis or death are the disease extent and histological response. A pelvic tumor site and marginal or intralesional surgical margins are important risk factors for transition from surgery to LR. In addition, we found that if LR or distant metastasis occurs the prognostic value of disease extent and histological response notably decrease and that intermediate events such as local recurrence or distant metastasis strongly negatively influence survival. The time-element is also of great importance. For local recurrence it was observed that the probability of death is lower if the event occurred after 2 years from surgery. The multistate model we developed can be used to predict some future clinical events given the history of a specific patient i.e. estimating a path a patient may follow after surgery. This information can assist in deciding on the optimum patient specific treatment strategies.

With disease extent being one of the strongest prognostic factors in Ewing sarcoma adequate staging is of paramount importance. Accurate detection, localization and treatment of all metastatic sites potentially provides a curative approach. (27) To evaluate the presence of metastasis a CT of the lungs to detect small lesions and whole-body imaging is required. Whole-body MRI and FDG-PET/CT are increasingly used to replace bone scintigraphy, because of higher sensitivity. (28-33) Current Ewing sarcoma guidelines of the European Society for Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) advise FDG-PET/CT for staging and follow-up. (34, 35) There is no published literature directly comparing FDG-PET/CT with whole-body MRI in Ewing sarcoma. In chapter 5 we compared

the diagnostic yield of FDG-PET/CT to whole-body MRI for detection of skeletal metastasis in Ewing sarcoma. 39% of metastases seen on MRI were not detected by FDG-PET/CT. Possible reasons include, poor contrast between metastases and active hematopoietic bone marrow, small lesion size, and potential changes in glucose metabolism in metastases of Ewing sarcoma.

Local treatment: away from a one size fits all approach

Chemotherapy alone cannot eradicate Ewing sarcoma tumor cells and local treatment, either surgery, radiotherapy or both, is a crucial element of Ewing sarcoma treatment. A main question in Ewing sarcoma treatment concerns the optimal local treatment strategy, more specifically the indications for the use of radiotherapy. The choice of local treatment is influenced by multiple factors such as age, tumor site, tumor size and volume, disease extent and clinic-radiological response to chemotherapy. No randomized trials have compared surgery with radiotherapy. Direct comparison between surgery, radiotherapy and surgery with radiotherapy in retrospective studies is difficult, drawing conclusions based on these studies is subjected to a patient selection bias. (21, 36-38) Patients who receive radiotherapy with or without surgery generally have more unfavorable prognostic factors, such as a tumor location or size not amenable to resection, incomplete resection, poor response. A systematic review on optimal local treatment strategies for Ewing sarcoma (39) concluded that both surgery and radiotherapy seem reasonable options for local control. Surgery is preferred over radiotherapy. If complete tumor resection is not feasible, radiotherapy alone might be the optimal choice. The combination of both was not recommended as initial treatment option due to the complications and toxicity of both treatment modalities. Postoperative radiotherapy (PORT) may be a treatment option in patients with residual tumor or positive margins. (40) Up to today, the optimal approach to local treatment still remains topic of debate. Tumour resection is performed whenever a marginal or wide resection seems possible, because surgical resection seems to show better results compare to definitive radiotherapy for local control. (41-48) Pre-operative radiotherapy seems a good alternative in cases where complete resection is not feasible after chemotherapy to further reduce the tumour and make surgery possible, for instance in a pelvic or spinal location. (47) A recent study comparing two patient cohorts, German and United Kingdom (UK), recruited within the international randomised EICESS-92 trial showed unexpected higher OS and EFS for patients in het German cohort. This difference in outcome was not obviously accounted for by differences in baseline characteristics, delivery of chemotherapy or follow up. Differences were found in management of the primary tumour: the administration of pre-operative RT was 45% in the German cohort versus 3% in the UK cohort. Less aggressive methods of local control have resulted in a higher rate of local recurrence and this was associated with a higher risk of metastatic disease and subsequent death. (49) Intralesional resection or debulking procedures followed by radiotherapy do not offer better local control or survival compared with definitive radiotherapy and

should be avoided. (46) When it comes to the indications for PORT research shows conflicting results. A lower risk of local relapse for patients with positive margins and PORT was observed. (40, 50-52) However, a combined analysis of CESS and EICESS trails showed similar percentage of local relapse for patients with or without PORT of 5.8 % vs 5,6% (46). Lin et al. (53) found a correlation between an axial tumor site and relevance of PORT. Foulon et al. (52) found a reduction in LR for patients who received PORT vs no PORT, even in case of good histological response. All studies are subjected to the patient selection bias mentioned above, where patients who are treated with PORT have less favourable characteristics. European trials (EURO-EWING 99, EWING 2008 and Euro-Ewing 2012) state that PORT is indicated in case of insufficient margins and/or poor histological response (≥ 10% viable tumour cells in the specimen). These indications might need to change in the futures as we (chapter 3) and Albergo et al. (26) showed that only patients with 100% necrosis should be considered good responders.

In chapter 4 we investigated the effect of surgical margins, histological response and radiotherapy, on local recurrence (LR), distant metastasis (DM) and overall survival. These results are based on a small number. On the other hand, only 2% (9 of 425) of the patients with extremity tumors developed isolated LR versus 8% (14 of 169) of the pelvic tumors and 8% (30 of 388) of the non-pelvic axial tumors. Which questions the clinical relevance of radiotherapy in extremity tumors. Distant metastases are still the main cause of treatment failure and the use of radiotherapy is not associated to occurrence of distant metastasis. The results should however be interpreted with caution since our study was subjected to the same limitation as previous studies, namely that radiotherapy is not given randomly and is strongly correlated to patient- and tumor characteristics which leads to confounding by indication.

Both surgery and radiotherapy are associated with short- and longterm toxicity. Surgical resection could result in functional deficits. Radiotherapy, on the other hand, is associated with bone growth disturbances in young children and increased risk of secondary malignancies. Secondary malignancy after Ewing sarcoma treatment is a great concern. Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) have been reported in 1-2% of Ewing sarcoma survivors (54, 55), they most commonly occur 2 to 5 years after treatment. There are also some dose intensive regimens that appear to be associated with a higher risk of hematological malignancies.(56, 57) Apart from hematological malignancies, Ewing sarcoma survivors develop more solid tumour through lifetime, most of these tumors occur in the radiation field. (58-60) A higher dose of radiation therapy, especially under the age of 18 years, is associated with an increased risk of sarcomas. (54, 55) The cumulative incidence of secondary malignancies at 25 years after diagnosis is 5 to 9%, most of these received (post-operative) radiotherapy, most of these occur 5 to 10 years after treatment. (61-64) Based on this, postoperative radiotherapy should be avoided and only indicated in specific cases. The introduction of proton beam therapy could be the solution to reduce this short- and longterm toxicity. It has unique

physical properties that allow for the reduction or elimination of unnecessary radiation to normal tissues. Follow-up is however relatively short and long-term benefits and toxicity needs yet to be evaluated. (112)

Pushing the limits of surgery: how high is the sky?

Achieving accurate surgical margins is of prognostic value for survival. Less local recurrence is observed after wide resection, compared to marginal or intralesional resection. (50, 53, 65) Survival after local recurrence is poor, as shown in chapter 4, and local recurrence should thus be prevented. The application of neoadjuvant chemotherapy and radiotherapy increases the possibility of complete surgical resection by shrinking the primary tumor, but makes accurate detection and localization of tumor boundaries challenging. Developments in intra-operative imaging, like CT-based navigation systems and near infrared (NIR) fluorescence guided surgery (FGS) make accurate defining and localization of surgical margins possible, could improve surgical resection and thereby outcome in Ewing sarcoma. About 25% of the Ewing sarcomas arise from the pelvic. Pelvic and sacral bone sarcoma resections are challenging due to anatomical and surgical complexity and are more likely to result in positive surgical margins, which significantly affects oncological outcome. In chapter 6 we showed that using navigation more wide osteotomies, 81% versus 50% for non-navigated resections ($p=0.03$) were achieved. The availability of surgical navigation allows you to execute the plan without having to see everything and to anticipate on unexpected situations by providing real-time feedback on the actual location and orientation. Additionally, it could provide treatment options for patients otherwise not found eligible for resection, thereby improving healthcare quality. It is recommended to train more orthopedic oncologic surgeons to use this promising technique. The main limit of surgical navigation is the fact that it is CT-based and only guides the osteotomy. The soft tissue margins did not significantly differ between both groups, but we did observe less intralesional surgical margins in the navigation group. Ewing sarcoma generally presents with a large soft tissue mass and most local recurrences occur in the soft-tissue (margin) and not in osteotomy. (66-69) It might be that better visualization during surgery contributed to the lower proportion of intralesional soft-tissue margins observed, but accurate determination of tumor boundaries intra-operatively is needed to further improve complete surgical resection. Near infrared (NIR) fluorescence guided surgery (FGS) might be the solution to this problem. Membrane proteins that are over-expressed on tumor or tumor-associated cells are labelled with a fluorophore, next, NIR light and an advanced camera system are used to visualize the tumor cells. Chapter 7 provides an overview of possible tumor-specific biomarkers in Ewing sarcoma suitable for NIR FGS in Ewing sarcoma. We identified CD99, LINGO-1, C-kit, NOTCH receptor, CxCR4, NPY receptor Y1, Claudin-1 and Occludin as the most interesting. With this study the first steps are made to explore this promising technique that is on the doorstep of optimizing orthopedic oncologic surgery.

Future perspectives

In this thesis we extended our knowledge on prognostic factors in Ewing sarcoma. "Classical" and currently used prognostic factors are still important, but how to use, define and determine them needs to change. We suggest that only patients with a 100% necrosis after induction chemotherapy should be considered good responders, since their survival is significantly better than that of patients with 90-99% necrosis. Also, the location of the primary tumor is a strong prognostic factor, as confirmed in this thesis and by a substantial amount of previous studies. (3, 14, 20, 21) Current trials (EWING 2008 and Euro-Ewing 2012) do not use tumor site to define risk groups and consider patients with >90% necrosis as good responders. New clinical trials should incorporate this into the stratification to further confirm and evaluate their prognostic significance. The model described in chapter 3 could be the base for the risk stratification. Adequate detection of risk factors like disease extent is also important, especially since disease extent is one of the strongest prognostic factors for survival in Ewing sarcoma. Current methods that preference FDG-PET/CT might not be sufficient for detection of skeletal metastasis and thus adequate staging. The optimal whole-body staging modality needs to be further investigated in a larger cohort and prospective setting. The implementation of PET-MRI scanners could be the ideal method to address this issue.

In this thesis we developed a prediction model for overall survival (chapter 3) using pre-treatment and treatment factors and gained insight in different risk factors for different time points in treatment by estimating a multistate model (chapter 4). We found that histological response is an additional prognostic factor associated to survival. If intermediate events, like local recurrence or distant metastasis, occur the prognostic value of disease extent and histological response notably decrease, implying that the effect of prognostic factors depends on the patient's history. Subsequently, if LR or distant metastasis occurs almost all patients die. The occurrence of LR or distant metastasis could thus be considered a prognostic factor that strongly negatively influences survival. In those patients focus should be on quality of life. Surgery, radiotherapy and chemotherapy are associated with short- and long-term toxicity and morbidity and should only be performed in a palliative setting. Finally, the time-element is also of importance, the probability of death is lower if LR occurred after 2 years from surgery. Not only the state a patient occupies after surgery, but also the time-element is relevant and should be considered when predicting survival in Ewing sarcoma. To transfer these results to the clinical practice the results from the easy-to-use model and the multistate model need to be incorporated into one predictive model for Ewing sarcoma. Once developed it needs to be validation on an external cohort. Using a web-based application and mobile app the model will be made available to clinician throughout the world. Since predictions are obtained from complex mathematical methodology a way to make results accessible to clinicians is through a web-based application where results from the model are implemented. A web-based application can enhance implementation. (7, 9) This work is currently in progress. The model will provide the scientific basis

that will improve treatment of Ewing sarcoma patients and assist in shared decision-making.

Future studies will have to focus on identification of new prognostic factors, specifically in the 'omics' fields. Radiomics use quantitative features from medical images to characterize data and can be used to monitor tumor status and treatment response. (70) The tumor necrosis induced by neoadjuvant chemotherapy, the histological response, is a strong prognostic factor for survival in Ewing sarcoma. Nowadays, the degree of histological response can only be assessed after resection of the tumor. Non-invasive imaging techniques are needed to predict the response to neoadjuvant chemotherapy prior to surgical resection. Early information regarding tumor response to initial chemotherapy will help tailoring treatment and contribute to patient specific treatment, especially in patients that do not undergo surgery. Chemotherapy schedules can be intensified earlier and timing of surgical resection can be adjusted, thereby possibly improving prognosis. (71-73) In an attempt to classify chemotherapy response changes in tumor size were considered. This led to the development of the one-dimensional (1D) Response Evaluation Criteria in Solid Tumors (RECIST)(74) and two-dimensional (2D) WHO measurements. (75-77) However, changes in tumor size do not always occur in al dimensions and with 1 and 2D images assessment of tumor volume is limited. (78) 3D tumor size measurements were suggested by the Childrens Oncology Group (COG) (79-81) and where found to be a better predictor of chemotherapy response than 1d RECIST and 2D WHO (82). However, changes in tumor size do not always correlate with histological response and lack of progression is sometime associated with good outcome. (83-90) Static imaging techniques like CT and MRI cannot accurate distinguish between active tumour cells and necrosis. (85, 87, 88, 90-92) FDG-PET/CT could be used for response monitoring and survival prediction. (73) SUVmax (maximum standard uptake value), a marker for metabolic activity, is the most popular metric and routinely used. (93, 94) Some studies (83, 84, 95-97) showed a significant correlation between SUVmax after chemotherapy and histological response. SUVmax measured by pre-treatment FDG-PET/CT seems to be an independent predictor of overall survival sarcoma (73, 95, 96, 98, 99) Dynamic MRI is based on the initial distribution of low-molecular weight gadolinium chelates after bolus injection and provides information about perfusion, capillary permeability and interstitial volume and can differentiated viable malignant tumour zones from slowly enhance avascular necrotic areas and zones of fibrosis. (100-103) To extract quantitative information a time/intensity curve is obtained and different parameters can be calculated from this curve, based on relative intensity of contrast enhancement and/or the slope of the curve. (100, 101, 104, 105) Compartment models precisely reflect tumor microvascularization by taking into account contrast uptake and elimination (wash out). These can in theory predict viability of residual tumor after treatment. (103, 105, 106) Which FDG-PET/CT or dynamic MRI parameter offer the best prediction of outcome and treatment response in Ewing

sarcoma needs to be further established. A study by Valieres et al. (70) suggested a joint radiomics model of information from FDG-PET/CT and dynamic MRI.

A better understanding of Ewing sarcoma biology is critical to understand oncogenesis and metastatic processes, relapse and resistance mechanisms. Prevention of metastasis and local recurrence appears to be the key to improve outcome. The outcomes of patients with metastatic or recurrent disease remain poor, since most are incurable with current available chemotherapy regimens. Selection of patients, matching patient-specific oncogenic pathways with the mode of action of specific drugs, could be (among others) one of the new trial designs. Increased collaboration among clinical cooperative groups is essential to further improve outcome in Ewing sarcoma. New therapeutic approaches such as targeted therapy are required to improve survival of patients with metastatic or recurrent disease and reduce toxicity profiles of patients with localized disease.

As for local treatment, the true effect of radiotherapy and indications for its use, especially in extremity Ewing sarcoma, need to be further established, preferably in a randomized setting.

In chapter 6 we showed that surgical navigation improves osteotomies of pelvic and sacral bone sarcoma resection. Surgical navigation could also aid in improving functional outcome and quality of life after surgical resection. In pelvic reconstruction surgical navigation can be used to facilitate allograft planning and (3D printed) endoprosthesis. (107) One case report showed excellent allograft fitting after navigated resection followed by navigated cutting and placing of the allograft in a pelvic sarcoma. (108) An experimental study using pelvic saw bones showed that accuracy of customized implant installation can be improved three to five times using navigation. (109) NIR FGS can be used to identify soft-tissue margins intra-operatively. Further immunohistochemical and cell line-based research of the potential targets described in chapter 7 should be performed to elucidate the most optimal candidate, this work is currently in progress. NIR FGS might also be the answer for the question whether resection should be carried out at the level defined at initial pre-chemotherapy MRI or as defined on the post-chemotherapy MRI. Neoadjuvant treatment causes shrinkage of both the bony and soft tissue component, but tumor boundaries can still consist of vital tumor cells. The infiltrative rather than pushing type of tumor outgrowth in Ewing sarcoma impedes border definition. Two studies (110, 111) investigated if post-chemotherapy MRI could be used to plan the osteotomy. Both concluded that the post-chemotherapy MRI is more accurate for planning and assessing osseous tumor limits than the pre-chemotherapy MRI. When it comes to the extension of soft-tissue margins there is no consensus which MRI should be used as base for planning of the resection. With NIR FGS we can solve this issue, since soft tissue margins can be visualized intra-operatively. Subsequently, surgical navigation with fusion of CT and MRI can be used to define osseous margins. Ideally a combination of both techniques could optimize surgical resection even more.

In conclusion, in this thesis we developed and validated an easy to use prognostic model for overall survival in Ewing sarcoma incorporating pre-treatment and treatment factors. Additionally, we gained insight in different risk factors for different time points in treatment by estimating a multistate model. Instead of considering one endpoint, like other studies, the occurrence of intermediate events like local recurrence and distant metastasis that may occur after treatment and their relations to the considered endpoint, death, were estimated. The next step now is to transfer these results into one predictive model for Ewing sarcoma. As for local treatment, the true effect of radiotherapy and indications for its use, especially in extremity Ewing sarcoma, need to be further established, preferably in a randomized setting. Imaging is important in Ewing sarcoma treatment, not only for accurate staging, but surgical resection can be perfectionated using intra-operative imaging, like CT-based navigation systems and NIR FGS. We became a little closer to personalize Ewing sarcoma treatment.

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CHAPTER 10

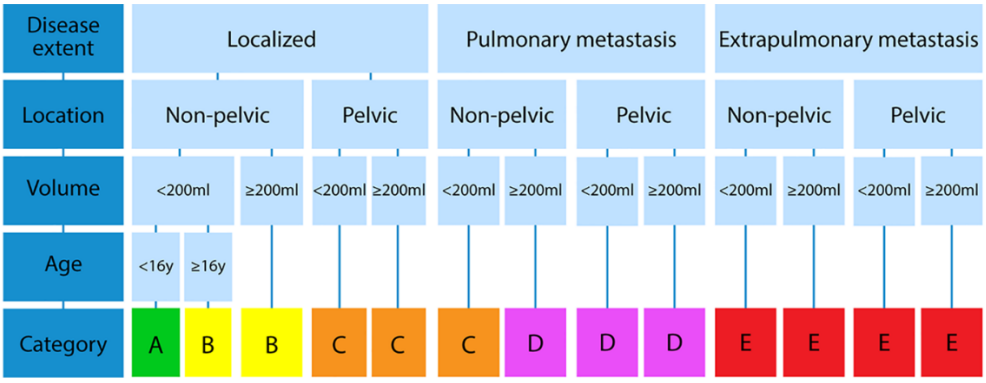
Nederlandse samenvatting

Een van de belangrijkste vragen bij de behandeling van Ewing sarcoom is het identificeren van laag risico patiënten die minder intensief behandeld kunnen worden, zodat toxiciteit en het optreden van bijwerkingen op de lange termijn beperkt kunnen worden met behoud van een hoog genezingspercentage. Daarnaast is het belangrijk om patiënten de identificeren waarbij behandeling maar weinig effect zal hebben en om hoog risico patiënten te identificeren waarbij een intensievere behandeling nodig is om de genezingskans te vergroten. Selectie van risicogroepen en daarop aangepaste behandeling maakt vroegtijdige besluitvorming mogelijk, helpt toekomstige resultaten te verbeteren en helpt bij het ontwikkelen van klinische studies. De behandeling van Ewing sarcoom is multimodaal en chirurgische verwijdering, indien mogelijk, is cruciaal voor genezing. Nauwkeurige detectie en lokalisatie van tumorgrenzen is echter een uitdaging, vooral op anatomische complexe locaties zoals het bekken. Inadequate chirurgische marges leiden tot een hoger kans op een lokaal recidief, wat op zijn beurt grote gevolgen heeft voor de oncologische uitkomst. De ontwikkelingen in intra-operatieve beeldvorming, zoals CT-geleide navigatiesystemen en nabij-infrarood (NIR) fluorescentie geleide chirurgie (FGS) maken een nauwkeurige bepaling en lokalisatie van chirurgische marges mogelijk. Ze vertegenwoordigen een geheel nieuw gebied van precisiegeneeskunde en bieden nieuwe behandelingsopties voor patiënten, waardoor de functionele uitkomst en de kwaliteit van leven kunnen worden vergroot. Dit proefschrift beschrijft individuele klinisch geavanceerde en responsadaptieve behandelingsstrategieën voor Ewing sarcoom. De eerste hoofdstukken van dit proefschrift beschrijven de ontwikkeling van twee predictiemodellen voor Ewing sarcoom. De hoofdstukken in deel twee zijn gericht op ontwikkelingen in pre- en intra-operatieve beeldvorming.

In de afgelopen decennia zijn er vele prognostische factoren voor Ewing sarcoom geïdentificeerd. De meest relevante zijn gebruikt om een behandeling op maat te bieden en voor het ontwerpen van klinische studies. Cohorten bevatten vaak meer variabelen dan redelijkerwijs gebruikt kunnen worden voor een voorspelling van de genezingskans. Het is daarom ook belangrijk de meest voorspellende en sensitieve prognostische factoren te selecteren. De systematische review in **hoofdstuk 2** over de huidige prognostische factoren voor algehele overleving en *event*-vrije overleving in Ewing sarcoom laat zien dat de aanwezigheid van metastasen bij de diagnose, de grootte van de tumor (volume ≥ 200 ml of grootste diameter ≥ 8 cm), primaire tumoren in het axiale skelet, vooral het bekken, en een histologische respons van minder dan 100% sterk worden geassocieerd met een slechtere overleving. Deze factoren moeten daarom als risicofactoren worden meegenomen bij de ontwikkeling van predictiemodellen voor overleving van Ewing sarcoom. Inzicht in het effect van chirurgische marges en lokale behandelingsmodaliteit vereist nader onderzoek. Chirurgische marges lijken alleen geassocieerd te zijn met event-vrije overleving, hun associatie met algehele overleving moet verder worden onderzocht.

Heterogeniteit tussen centra bij het definiëren en evalueren van chirurgische marges en het gebruik van postoperatieve radiotherapie in geval van inadequate marges kan een rol spelen bij deze enigszins inconsistente resultaten. De relatie tussen lokale behandelingsmodaliteit en overleving werd geëvalueerd door meerdere studies die inconsistente resultaten laten zien. De beschikbare resultaten zijn gebaseerd op retrospectieve, niet-gerandomiseerde studies. Veel van deze onderzoeken worden beïnvloed door een selectiebias, waarbij radiotherapie alleen wordt geïndiceerd bij specifieke groepen patiënten, bijvoorbeeld patiënten met minder gunstige prognostische factoren. Om het effect van lokale behandeling op de overleving te beoordelen, zijn gerandomiseerde studies, gericht op het vergelijken van chirurgie, radiotherapie en een combinatie van beide, of prospectieve studies noodzakelijk.

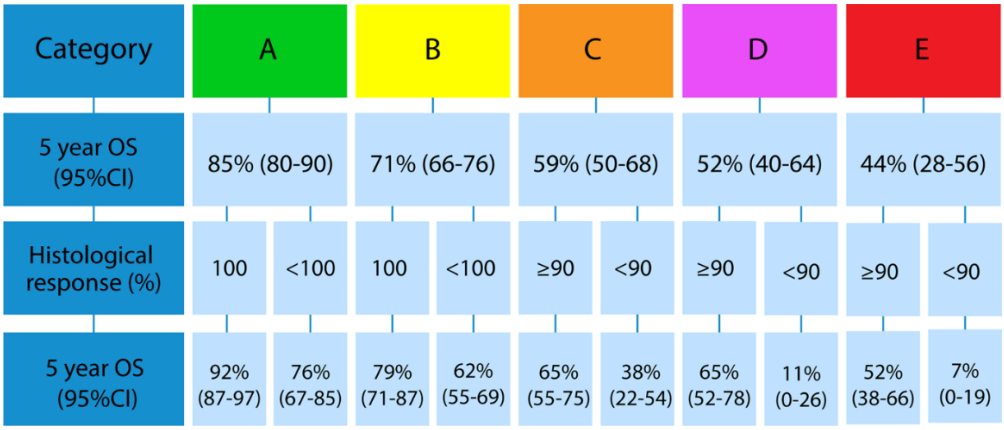
Een optimale balans tussen voorspellend vermogen en eenvoud is de sleutel tot een goed predictiemodel. In **hoofdstuk 3** hebben we aan de hand van een cohort met 1314 Ewing sarcoom patiënten een eenvoudig te gebruiken klinisch rekenhulpmiddel (*tool)* ontwikkeld en gevalideerd voor het voorspellen van de overleving vanaf het moment van diagnose. Daarnaast laat het model ook zien hoe de overleving verandert gedurende de behandeling naarmate meer informatie beschikbaar komt. Onafhankelijke prognostische factoren op het moment van diagnose zijn de leeftijd van de patiënt, het tumorvolume, de locatie van de primaire tumor en de aanwezigheid van metastasen. Op basis van deze factoren zijn 5 risicocategorieën (A tot E) geïdentificeerd met een 5-jaaars overleving (95% betrouwbaarheidsinterval) van 88% (86-94), 69% (64-74), 57% (50-64), 51% (42-60) en 28% (22-34) respectievelijk. Figuur 1 toont een stroomdiagram dat gebruikt kan worden om patiënten gemakkelijk in te delen in risicocategorie A tot en met E.



Figuur 1 - Stroomdiagram om patiënten op het moment van diagnose gemakkelijk te stratificeren naar risicocategorie A tot E.

De C-index van het predictiemodel, een maat voor het discriminerende vermogen, is 0.70, wat duidt op een goed discriminerend vermogen van het model. Vervolgens hebben we de associaties tussen prognostische factoren op het moment van de

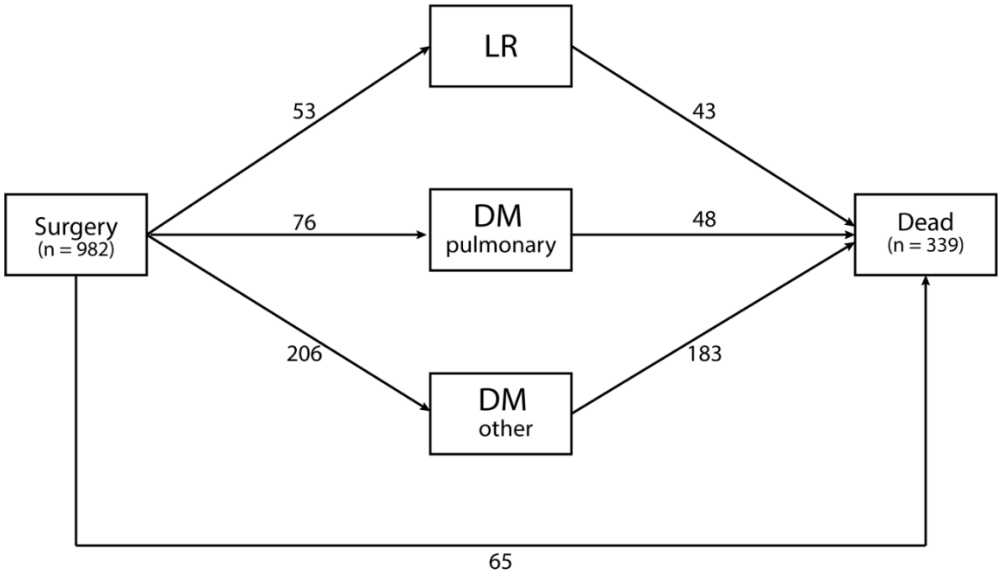
operatie en overleving onderzocht. De onafhankelijke prognostische factoren op het moment van operatie zijn: de leeftijd van de patiënt, het tumorvolume, de aanwezigheid van metastasen en de histologische respons. Vervolgens hebben we een *Cox proportional hazards* model met de variabelen histologische respons en risicocategorie vanaf het moment van operatie gemaakt. Figuur 2 laat een stroomdiagram zien waarmee patiënten op het moment van operatie gestratificeerd kunnen worden op basis van het Cox model. In de categorieën A en B steeg de 5-jaars overleving tot respectievelijk 92% (87-97) en 79% (71-87) voor 100% necrose en daalde deze tot 76% (67-85) en 62% (55-69) voor <100 % necrose. In de categorieën C tot E steeg de 5-jaars overleving tot 65% (55-75), 65% (52-78) en 52% (38-66) respectievelijk voor ≥90% necrose en daalde deze tot 38% (22-54), 11% (0-26) en 7% (0-19) respectievelijk voor <90% necrose.



Figuur 2 - Stroomdiagram voor stratificatie van Ewing sarcoom patiënten ten tijde van de operatie met behulp van de Kaplan Meier-methode.

Het door ons ontwikkelde model is gebaseerd op een groot cohort van 1314 Ewing sarcoom patiënten met uniformiteit in diagnostiek en behandeling en beschikbaarheid van alle relevante prognostische factoren. De ontwikkelde stroomdiagrammen zijn eenvoudig te gebruiken en gebaseerd op toegankelijke variabelen. Bovendien geven de 13 ontwikkelde prognostische groepen een gedetailleerd inzicht in de verwachte overleving en kunnen ze helpen bij het verfijnen van de individuele behandeling. Het relatief eenvoudige en klinisch gemakkelijk te gebruiken karakter van het model draagt sterk bij aan de bruikbaarheid. Bovendien bied het model nieuwe inzichten in hoe overleving verandert tijdens de behandeling. De informatie verkregen na de operatie levert informatie voor een tweede belangrijk moment van multidisciplinaire besluitvorming op. Na operatie is de histologische respons een sterke additionele prognostische factor voor overleving in elke risicocategorie.

Het optreden van een lokaal recidief en metastase op afstand en een slechte overleving bij patiënten met metastatische ziekte vormen een grote zorg bij de behandeling van Ewing sarcoom. De associaties tussen de lokale behandelingsmodaliteit, het optreden van een lokaal recidief, metastase op afstand en overlijden zijn nog niet duidelijk vastgesteld. In **hoofdstuk 4** hebben we een diepgaande analyse van de evolutie van de ziekte uitgevoerd door middel van de ontwikkeling van een *multistate* model. Multistate modellen bestuderen de evolutie van de ziekte en includeren daarbij het optreden van intermediaire gebeurtenissen die zich voordoen na chirurgie, zoals een lokaal recidief en metastase op afstand, . Dit geeft nuttige inzichten in hun relatie met het eindpuntoverlijden. De ziekte-evolutie is retrospectief geanalyseerd in een cohort van 982 patiënten met Ewing-sarcoom die een operatie hebben ondergaan na chemotherapie. Een multistate model (Figuur 3) met start situatie chirurgie, intermediaire gebeurtenissen zoals [lokaal recidief (LR), pulmonale metastase (DMpulm), andere metastasen (met of zonder lokaal recidief) DM ± LR (DMother)] en de uiteindelijke gebeurtenis overlijden is ontwikkeld.



Figuur 3 - Multistate model voor Ewing sarcoom

Het effect van diverse risicofactoren op intermediaire gebeurtenissen wordt bepaald met behulp van Cox-modellen. Marginale of intralesionale chirurgische marges zijn een belangrijke risicofactor voor de transitie van chirurgie naar LR en wanneer een patiënt een LR ontwikkelt is de kans op overlijden hoger in het geval van een vroeg LR (binnen 0-24 maanden). De tijd tot recidief kan in deze situaties als het meest relevant worden beschouwd. De histologische respons is een sterke prognostische factor voor de transitie van chirurgie naar metastase op afstand en overlijden.

Wanneer een patiënt nieuwe metastase op afstand ontwikkelt (pulmonaal, bot, anders of gecombineerd), verliest de histologische respons zijn relevantie als risicofactor, aangezien het optreden van metastase op afstand *an sich* de overleving sterker beïnvloedt. Een primaire tumor gelokaliseerd in het bekken is een belangrijke risicofactor voor de transitie van operatie naar LR. Eerdere longmetastase zijn een risicofactor voor de overgang naar nieuwe longmetastasen, echter zodra een patiënt nieuwe longmetastasen ontwikkelt, is de aanwezigheid van (een) eerdere longmetastase(n) niet langer een prognostische overlevingsfactor. Eerdere long- of bot / andere metastasen zijn een risicofactor voor de transitie naar nieuwe bot / andere metastase met of zonder LR. Bij het bereiken van de DMother-toestand blijven alleen eerdere bot / andere metastasen van prognostische waarde voor overleving. Met deze studie hebben we de kennis uitgebreid over het effect van prognostische factoren voor intermediaire gebeurtenissen en de uiteindelijke kan op overlijden bij een Ewing sarcoom. We hebben aangetoond dat prognostische factoren verschillende effecten hebben op verschillende transities en dat de impact op de volgende transitie afhangt van de situatie waarin een patiënt verkeert. Naast de eerdere gebeurtenissen is ook het tijdselement van het groot belang voor de besluitvorming. Het optreden van een lokaal recidief binnen 2 jaar of van metastase op afstand met of zonder daaropvolgende lokaal recidief heeft een significant effect op de overlevingskansen, en ondanks onze inspanningen als artsen sterven bijna alle patiënten in deze situatie aan progressieve ziekte. De balans tussen de toxiciteit van intensieve behandelingen en de kwaliteit van leven tijdens de resterende levensduur van deze patiënten dient daarom zorgvuldig overwogen te worden. Radiotherapie lijkt beschermend voor LR vooral in het bekken / axiaal skelet.

Het aantal patiënten dat behandeld moet worden met chirurgie en radiotherapie (NNT) om een enkele LR te voorkomen, is 72 voor alle tumorlocalities samen. Daarentegen is de NNT voor extremiteitstumoren 80 en de NNT voor bekkentumoren 10. Dit brengt de vraag met zich mee wat de waarde is van radiotherapie bij patiënten met een extremiteit Ewing sarcoom: een individuele patiënt met een extremiteit Ewing sarcoom zou kunnen profiteren echter zijn er slechts weinigen die deze potentieel toxische behandeling echt nodig hebben, vooral kijkende naar het opgroeiende kind. Radiotherapie wordt geassocieerd met een aanzienlijk risico op secundaire radiotherapie-geïnduceerde maligniteiten, groeiverstoring en postoperatieve complicaties van chirurgische reconstructies. Indicaties voor radiotherapie moeten verder worden onderzocht, bij voorkeur in een prospectieve gerandomiseerde setting.

Het tweede deel van dit proefschrift richt zich op pre-operatieve en intra-operatieve beeldvormingstechnieken. In **hoofdstuk 5** vergelijken we retrospectief de diagnostische accuratesse van 18F-FDG PET-CT in vergelijking met met MRI voor detectie van skeletmetastasen in bij de patiënt met een Ewing sarcoom.

Nauwkeurige detectie en lokalisatie van alle metastasen in Ewing sarcoom is erg belangrijk, omdat indien al deze locaties behandeld worden curatie soms mogelijk is. Om het niveau van discrepantie tussen MRI en 18F-FDG PET-CT te bepalen bij de detectie van skeletmetastasen, hebben we 20 patiënten geïncludeerd die gediagnosticeerd zijn met Ewing sarcoom tussen 2000 en 2017 en waarbij een 18F-FDG PET-CT en MRI verricht zijn binnen een bereik van 4 weken. In totaal werden 112 botlaesies gezien bij 13 patiënten, 107 maligne en vijf benigne. Bij zeven patiënten waren geen metastasen zichtbaar op 18F-FDG PET-CT en/of MRI. Eenenveertig skeletmetastasen (39%) gedetecteerd met MRI toonden geen verhoogde 18F-FDG-opname op PET-CT (fout-negatief). Botlaesies waren vaker fout-negatief op 18F-FDG PET-CT als hematopoietische beenmergactiviteit aanwezig was tijdens of na (neo) -adjuvante behandeling of wanneer de laesie kleiner was dan 10 mm. We toonden aan dat voorzichtigheid is geboden bij het gebruik van 18F-FDG PET-CT voor het diagnosticeren van skeletmetastasen van het Ewing sarcoom. Slecht contrast tussen metastasen en actief hematopoietisch beenmerg, chemotherapeutische behandeling en / of kleine omvang vermindert de diagnostische opbrengst van 18F-FDG PET-CT aanzienlijk, maar niet van MRI.

Intra-operatief onderscheid tussen gezond en tumorweefsel is van groot belang maar tegelijkertijd een uitdaging, vooral na chemotherapie en op complexe anatomische locaties. Intraoperatieve navigatietechnieken zijn ontwikkeld om de chirurgische nauwkeurigheid met betrekking tot de chirurgische marges tijdens de operatie te verbeteren, waardoor minder complicaties ontstaan en toch de overlevingskansen behouden blijven. Ongeveer 25% van de Ewing sarcomen ontstaat in het bekken. Resecties in het bekken en sacrum zijn uitdagend vanwege de anatomische en chirurgische complexiteit. Computer geassisteerde chirurgie kan helpen bij het verhogen van de chirurgische nauwkeurigheid. In **hoofdstuk 6** hebben we de chirurgische marges van genavigeerde bekken- en sacrale primaire botsarcoom resecties vergeleken met niet-genavigeerde resecties. Zesendertig patiënten met bekken- of sacrale sarcomen behandeld met intraoperatieve navigatie werden retrospectief vergeleken met een historisch cohort van 34 patiënten die resectie ondergingen zonder navigatie. Adequate botmarges worden vaker gevonden bij patiënten in de genavigeerde groep dan in de niet-navigatiegroep (29 van 36 patiënten (81%) versus 17 van 34 (50%); odds ratio, 4,14 (95% betrouwbaarheidsinterval, 1,43-12,01); p = 0.007). Er werd geen verschil gevonden met betrekking tot de weke delen marges tussen de navigatie- en niet-navigatiegroep. Intraoperatieve navigatietechnieken verbeteren ons vermogen om negatieve botmarges te bereiken tijdens resecties bij patiënten met bekken- en sacrale primaire botsarcomen. Het verkrijgen van adequate weke delen marges blijft een uitdaging, en deze marges lijken niet te verbeteren door gebruik van navigatie. Nabij infrarood (NIR) fluorescentie geleide chirurgie (FGS) kan de oplossing zijn om de marges van weke delen te verbeteren. NIR FGS draagt bij aan het visualiseren

van tumorgrenzen intra-operatief, waardoor de kans op volledige resectie en daarmee dus de overleving wordt vergroot. Het maakt gebruik van membraanreceptoren die tot overexpressie worden gebracht op tumor of tumor-geassocieerde cellen om tumoren te visualiseren. Bij het definiëren van een potentiele membraanreceptor voor zijn de volgende kenmerken van belang: extracellulaire lokalisatie, het expressiepatroon, ratio tussen expressie in de tumor en het gezonde weefsel, het percentage positieve tumoren en succesvol gebruik van de receptor in *in vivo* studies. **Hoofdstuk 7** geeft een overzicht van mogelijke tumorspecifieke receptoren in Ewing sarcoom geschikt voor NIR FGS. In Ewing sarcoom is er een groot aantal tumorspecifieke receptoren met een overexpressie. Met het gebruik van een scoresysteem hebben we CD99, LINGO-1, C-kit, NOTCH-receptor, CxCR4, NPY-receptor Y1, Claudin-1 en Occludin geïdentificeerd als de meest interessante Ewing sarcoom specifieke receptoren geschikt voor gebruik bij NIR-fluorescentie geleide chirurgie. Verder immunohistochemisch en cellijn-gebaseerd onderzoek van deze potentiële receptoren is nodig om de meest optimale kandidaat te selecteren. Met deze studie hebben we de eerste stappen gezet om deze veelbelovende techniek te introduceren voor het optimaliseren van orthopedische oncologie .

Appendices

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Pediatrics III, Hematology/Oncology, West German Cancer Center, German Cancer Research Center	Uta Dirksen, MD PhD Andreas Ranft, PhD

List of publications

Bosma SE, van Driel PB, Hogendoorn PC, Dijkstra PS, Sier CF. *Introducing fluorescence guided surgery into orthopedic oncology: A systematic review of candidate protein targets for Ewing sarcoma.* J Surg Oncol. 2018 Nov;118(6):906-914.

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S.E. Bosma, D. Vriens, H. Gelderblom, M.A.J. van de Sande, P.D.S. Dijkstra, J.L. Bloem. *^{18}F-FDG PET-CT versus MRI for detection of skeletal metastasis in Ewing sarcoma.* Skeletal Radiol. 2019 Apr 23.

S.E. Bosma, A.H.G. Cleven, P.D.S. Dijkstra**.** *Can Navigation Improve the Ability to Achieve Tumor-free Margins in Pelvic and Sacral Primary Bone Sarcoma Resections? A Historically Controlled Study.* Clin Orthop Relat Res. 2019 Jul;477(7):1548-1559.

S.E. Bosma & C. Lancia, A.J. Rueten-Budde, A. Ranft, H. Gelderblom, M. Fiocco, M.A.J. van de Sande, P.D.S. Dijkstra, U. Dirksen. *Easy-to-use clinical tool for survival estimation in Ewing sarcoma at diagnosis and after surgery.* Sci Rep. 2019 Jul 29;9(1):11000.

S.E. Bosma & A.J. Rueten-Budde, C. Lancia, , A. Ranft, A.D. Krol, H. Gelderblom, M.A.J. van de Sande, P.D.S. Dijkstra, U. Dirksen, M. Fiocco *Individual risk evaluation of local recurrence and distant metastasis in Ewing sarcoma: A multistate model.* Pediatr Blood Cancer. 2019 Aug 6

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Curriculum Vitae

Sarah Elise Bosma was born on September 29th in Leeuwarden, the Netherlands. She graduated from the OSG Sevenwolden in Heerenveen in 2010 and started medical school at University of Groningen that same year. Before starting her clinical rotations she did 2 months of healthcare volunteering in Nepal and Cambodia and worked 4 months as a ski- and snowboard instructor in Ischgl, Austria. During her studies she participated in the student trauma team (a research team investigating intestinal trauma in type-A traumapatients) of University Medical Hospital Groningen and was a member of several student boards, including the IFMSA Standing Committee on Sexual & Reproductive Health incl. HIV/AIDS.

After her clinical rotations she did her scientific research internship on Computer Assisted Surgery and Patient specific instrumentations in orthopedic oncology under supervision of Dr. P.C. Jutte (UMCG) and Dr. K.C. Wong at Prince of Wales hospital in Hong Kong.

After graduating from medical school in 2017 she started her PhD on Ewing sarcoma at the LUMC, Leiden under supervision of prof. dr. P.D.S. Dijkstra and prof. dr. H. Gelderblom. During her PhD she set up a collaboration with Prof. dr. U. Dirksen from the EWING study group in Essen, Germany. She was assigned a travel scholarship from the European Ewing Concertium to cover her travel expenses,that way getting access to a large and homogenous database of Ewing sarcoma patients. This dataset forms the base of this thesis. During her PhD research she presented her work at multiple national and international scientific meetings.

In the last 2 years she developed a great love for cycling. She participated twice in the Tourmalet. A charity event of Team Westland to raise money for (local) projects involving cancer and cancer research, including the research involved in the thesis.

Sarah lives together with Mathijs de Groot in Utrecht. After traveling through South-America and Asia for 5 months she will now start working as a surgery resident.