Individual clinical advanced decision-making and risk evaluation for Ewing sarcoma

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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.

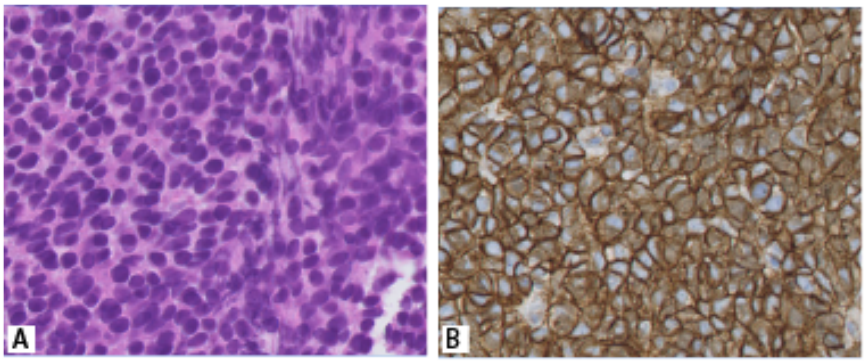

Figure 1 – Microscopic images of Ewing sarcoma
A) Small uniform cells with scanty cytoplasm and round hyperchromatic nuclei (HE x 400). B) Characteristic CD99 immunoreactivity of the cell membranes (HE x 400).

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.

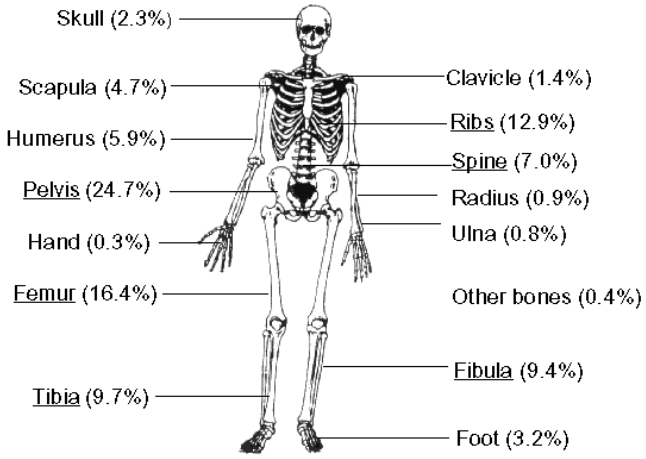

Figure 2 – Distribution of primary tumor sites in Ewing sarcoma (19)

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)

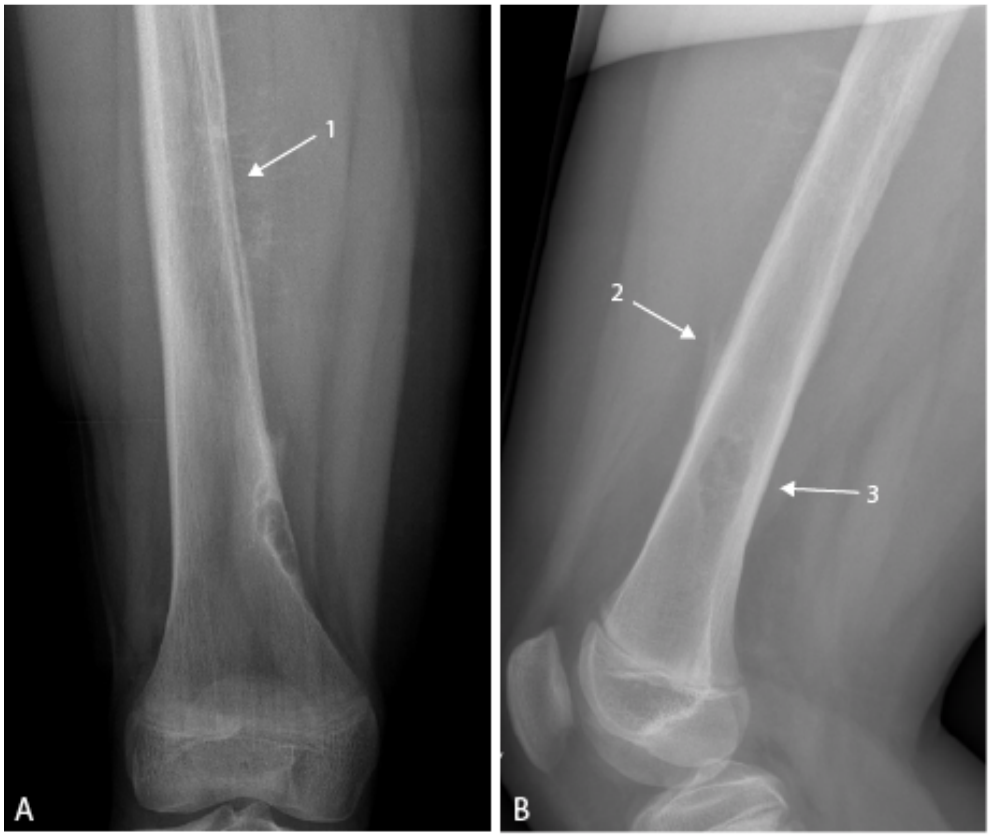

Figure 3 – Radiograph of Ewing sarcoma
A) Anterior-posterior image of the right femur showing widespread cortex destruction with a hair-on-end periosteal reaction (arrow 1). B) Lateral image of the right femur that shows a Codman triangle (arrow 2) and moth-eaten, permeative destruction of the bone (arrow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

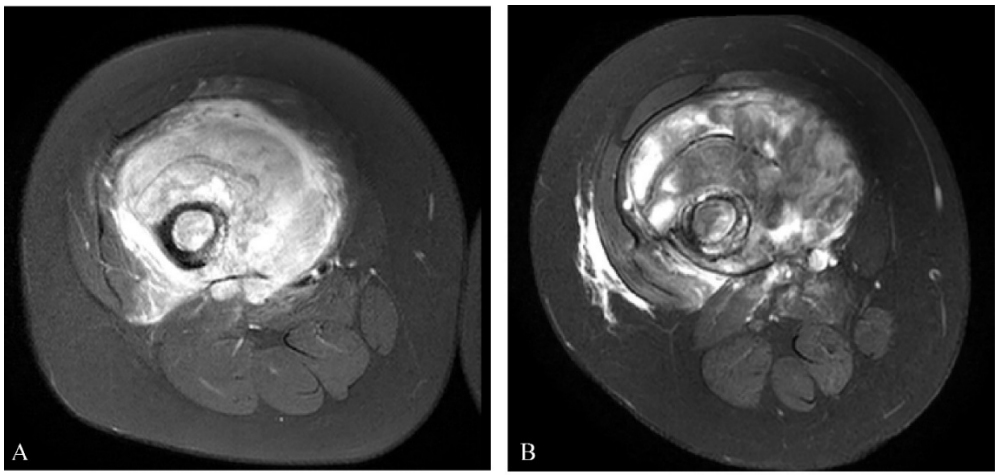

Figure 4 – Local imaging by MRI of Ewing sarcoma
A 12-year-old boy with Ewing sarcoma of the right thigh. Axial fat-suppressed T2-weigthed images at diagnosis (A) and after 6 cycles of VIDE chemotherapy (B) show a lesion in the right thigh with a circumferential soft-tissue mass that shows a high signal.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

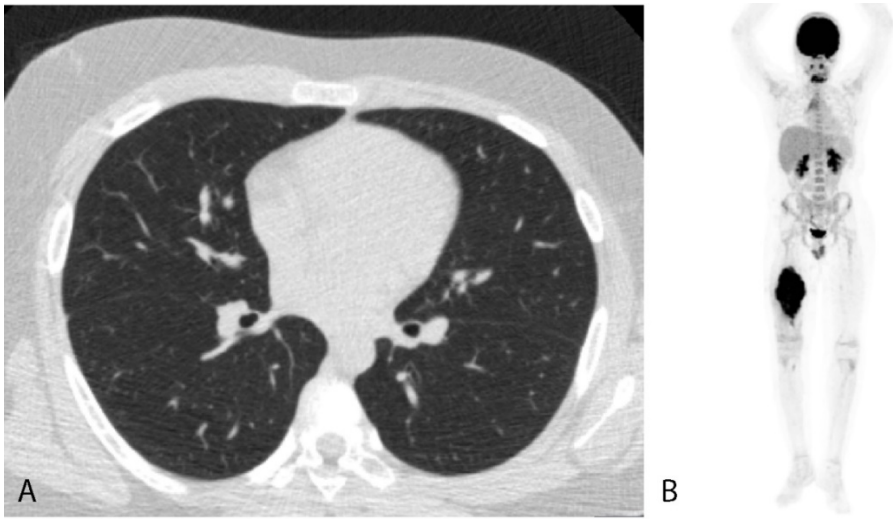

Figure 5 – Whole body staging in Ewing sarcoma
A 12-year-old boy with Ewing sarcoma of the right thigh. A) Chest-CT showing clear lung fields without nodules, consolidations or lymphadenopathy. There is no sign of pulmonary metastasis. B) Whole body FDG-PET/CT showing high FDG-uptake at the right thigh. 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.

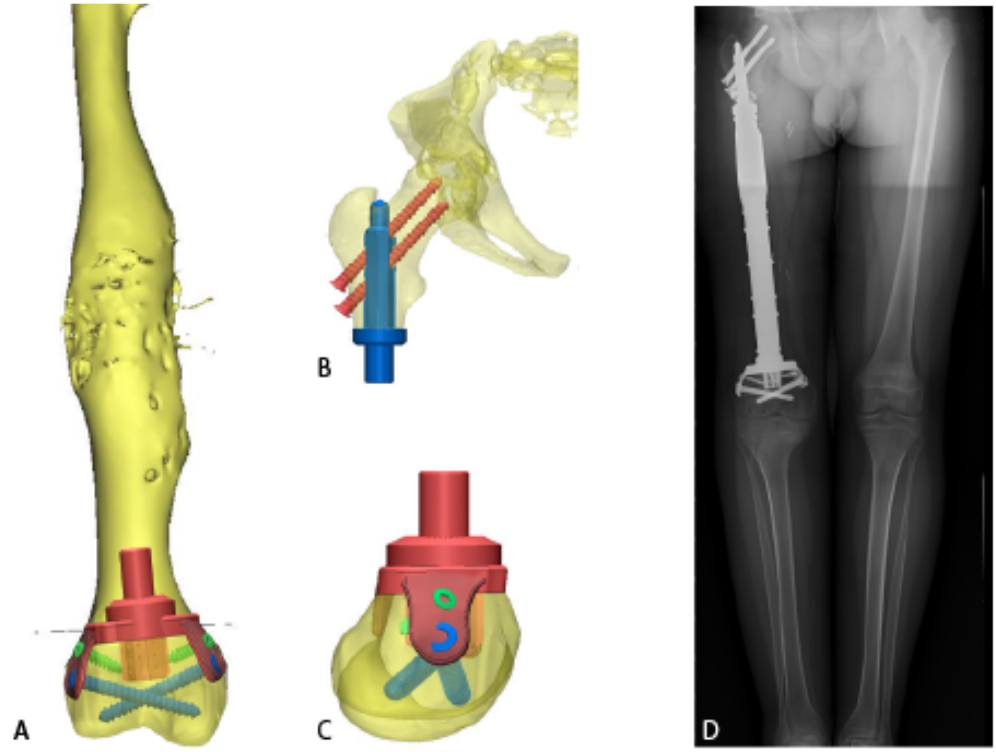

Figure 6 – Reconstruction of the femur with 3D printed custom made implant.
Hip- and knee-sparing custom made 3D printed endoprosthesis. A+C) Fitting of the knee-sparing computer-aided design (CAD) model on 3D reconstruction showing the planning of the screw placement. B) Fitting of the hip-sparing CAD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