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EWING SARCOMA; TREATMENT, PROGNOSIS AND LATE EFFECTS

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Ewing sarcoma; treatment, prognosis and late effects

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"You must look at facts, because they look at you"

-Winston Churchill

This thesis was written in a rather turbulent time

During which I wondered why bother my mind

By writing a silly little scientific book

In which very few will actually look

But at least it gave my mind some ease

To think of something else than a viral disease

To my beautiful wife **Helena**, who supported me in every possible way throughout the hours in which my attention was merely focused on this thesis. You always knew when I said that I was almost done, that I was nowhere near the end, yet I was met with nothing but kindness, love and humor-thank you.

ABSTRACT

Ewing sarcoma (ES) is a rare and aggressive childhood/adolescent malignancy which relies on a multidisciplinary treatment approach for cure. The overall survival rate for this young patient group has hardly improved over the last 30 years despite large multinational treatment trials. Thus, there are important research questions to be answered with regards to systemic treatment. However, this thesis is about local treatment. Local treatment is indisputably important for overall survival, yet we have not reached a consensus for which local treatment regime is best for the individual patient. Therefore, local treatment is always a matter of debate on multidisciplinary meetings in sarcoma centers around the world. In the national multidisciplinary sarcoma conferences hosted at the Karolinska University Hospital every fortnight, every ES case in the country is brought up for discussion and there is an unproportioned amount of time spent on each case compared to other sarcomas. Due to the rare nature of this disease, randomized trials on local treatment are unlikely to occur and local treatment relies on case series and studies hampered by low number of patients. This thesis aims to shed light on the following questions: (1) which is the optimal local treatment strategy for pelvic ES; (2) how are ES of the spine treated in Scandinavia and what is the neurologic and oncologic outcome; (3) what is the true risk for subsequent primary neoplasms (SPNs) among ES survivors and (4) what is the effect of local treatment on local failure, long term mortality and morbidity?

Study 1 evaluates different local treatment options in treatment of pelvic ES and whether local control of sacral ES can be achieved with radiotherapy alone. Innominate bone ES were in most cases treated surgically and sacral bone tumors were largely treated with radiotherapy (RT) alone. We found that sacral site was an independently favorable site as regards disease-free survival compared with the innominate bones. Furthermore, good local control could be obtained with RT alone for tumors located in the sacrum.

Study 2 investigated if the same good outcome found among ES of the sacrum could be seen among patients with ES of the mobile spine. Additionally, the neurologic deficits at time of diagnosis and at end of follow-up for ES of the mobile spine were examined in relation to local treatment. Only 24 patients with sufficient data were found eligible for the analysis. Neurologic deficits at presentation were common which often led to emergency decompression before having a histopathologic diagnosis. Emergency decompression was associated with a higher rate of local failure. Most patients recovered neurologically regardless of local treatment, perhaps because of prompt initiation of systemic treatment leading to tumor shrinkage and less pressure on the spinal cord. Disease-free survival was relatively favorable, but perhaps not as good as that of sacral ES. As was the case with sacral ES, the majority of patient were treated with RT exclusively which reflects the difficulties in performing "en bloc" surgery in this site. Local control was excellent for the few patients with tumors manageable by surgery.

Study 3 investigated the risk for secondary malignancies among Ewing- and osteosarcoma (OS) survivors in a population-based nationwide cohort. ES survivors had double the risk of OS survivors, and a four times higher risk than the general population of acquiring an SPN. The excess risk was largely driven by breast cancer and female genital malignancies for ES as well as OS survivors, and not as much by secondary sarcoma as anticipated. Due to the high background breast cancer incidence in the general population, the excess breast cancer risk among ES survivors translates to 127 extra breast cancers per 10 000 person years. The excess cancer risk remained elevated with increasing follow-up and over calendar time.

Study 4 evaluated the role of surgery and RT in relation to surgical margin and local control. Secondarily, the effect of local treatment on long-term mortality, occurrence of SPN and hospitalization was studied. Local treatment had a significant effect not only on local failure, but also on overall survival. Surgical treatment gave superior local control compared with definitive RT. The lowest local failure rate was achieved if surgery was performed with a wide surgical margin. Nonetheless, RT also played a significant role with regards to local control since marginally resected tumors treated with adjuvant RT achieved an equal local control rate to that of a wide margin. This is a key finding because accomplishing a wide surgical margin is rarely possible for tumors located in the pelvis or spine, sites which comprise a third of all ES.

In conclusion, tumor site is an important prognostic factor in ES. Pelvic and spinal sites pose a specific challenge since surgery of the primary tumor is less often performed due to the morbidity associated with surgery in these sites. Hence, more patients will receive definitive RT. Definitive RT seems to achieve good local control for tumors of the sacrum. Nevertheless, the long-term results of definitive RT are unknown. For tumors located in sites other than the sacrum, surgery is superior to definitive RT in achieving local control and improving survival. Moreover, best local control is achieved when surgery is carried out with wide margins, in which case RT does not improve outcome. Importantly, radiotherapy improves local control for marginally resected tumors.

The long-term excess risks for SPNs are mainly driven by breast cancer among females. Unfortunately, the risks remain high in recent treatment periods. Moreover, the excess risk persists with extended follow-up, indicating the need for lifelong surveillance and tailored follow-up. However, the outcome after local recurrence is dismal. The benefit of administering RT when indicated must therefore not be overshadowed by the risk for treatment related cancer.

LIST OF SCIENTIFIC PAPERS

I. Hesla AC, Tsagozis P, Jebsen N, Zaikova O, Bauer H, Brosjo O. **Improved Prognosis for Patients with Ewing Sarcoma in the Sacrum Compared with the Innominate Bones: The Scandinavian Sarcoma Group Experience**. *The Journal of bone and joint surgery* American volume 2016; 98(3): 199-210; doi 10.2106/JBJS.O.00362.

II. Hesla AC, Bruland OS, Jebsen N, Styring E, Eriksson S, Tsagozis P. **Ewing sarcoma of the mobile spine; predictive factors for survival, neurological function and local control. A Scandinavian sarcoma group study with a mean follow-up of 12 years**. *Journal of bone oncology* 2019; 14: 100216; doi 10.1016/j.jbo.2018.100216.

III. Hesla AC, Discacciati A, Tsagkozis P, Smedby KE. **Subsequent primary neoplasms among bone sarcoma survivors; increased risks remain after 30 years of follow-up and in the latest treatment era, a nationwide population-based study.** *British journal of cancer* 2020; doi 10.1038/s41416-020-0748-3.

IV. Hesla AC, Smedby KE, Tsagkozis P. **Local treatment, local control and long-term morbidity in a paediatric nationwide cohort of Ewing sarcoma patients**. *In manuscript*

Additional publications on the same topic

Chen Y, Hesla AC, Lin Y, Ghaderi M, Liu M, Yang C, Zhang Y, Tsagkozis P, Larsson O, Haglund F. **Transcriptome profiling of Ewing sarcomas-treatment resistance pathways and IGF-dependency.** *Molecular Oncology* 2020, March 2.

CONTENTS

LIST OF ABBREVIATIONS

ES	Ewing sarcoma
OS	Osteosarcoma
SIR	Standardized Incidence Ratio
AER	Absolute Excess Risk
SPN	Subsequent Primary Neoplasm
RT	Radiotherapy
CNS	Central Nervous System
ICD	International Classification of Diseases
SSG	Scandinavian Sarcoma Group
BCCSS	British Childhood Cancer Survival Study
CCSS	Childhood Cancer Survivor Study
SEER	Surveillance Epidemiology End-Result
GPOH	German Paediatric Oncology and Haematology Group
COG	Children´s Oncology Group
CESS	Cooperative Ewing´s Sarcoma Study
EICESS	European Intergroup Cooperative Ewing's Sarcoma Study
CCLG	United Kingdom Children's Cancer and Leukaemia Group
BRCA	Breast Cancer Susceptibility Protein
RCC	Regional Cancercentrum
CWS	Cooperative Weichteilsarkomstudiengruppe
SBCR	Swedish Childhood Cancer Registry
UPS	Undiffrentiated Pleomorphic Sarcoma
MRI	Magnetic Resonance Imaging
CT	Computed Tomography
ALiCCS	Adult Life After Childhood Cancer in Scandinavia
SALUB	Svenska Arbetsgruppen för LångtidsUppföljning efter Barncancer
INFORM	Idividualized Therapy For Relapsed Malignancies in Childhood
rECCur	International Rabdomized Controlled Trial of Chemotherapy for the Treatment of Recurrent and Primary Refractory Ewing Sarcoma

1 INTRODUCTION

Background:

Ewing sarcoma (ES), first described as diffuse endothelioma of bone by James Ewing in 1921, is an aggressive form of sarcoma formerly referred to as Ewing's family of tumors (ESFT)[1]. EFTS was previously differentiated into classic Ewing's sarcoma, Askin tumor (Ewing sarcoma of the chest wall), and peripheral primitive neuroectodermal tumor (PNET). The term PNET and Askins tumor is no longer in use, and the group of tumors are now simply classified as Ewing sarcomas[2]. Before chemotherapy was introduced in the 1970s, around 90 % of Ewing sarcoma patients died[3]. Today, around 65-75 % of patients without detectable metastatic disease at time of diagnosis will survive. Nevertheless, the improvement in survival has plateaued out and the prognosis for the 1/4 of patients who present with metastatic disease is still dismal[4].

Epidemiology

Sarcomas are a group of heterogeneous aggressive malignancies that arise in tissues of mesenchymal origin, including muscle, fat, cartilage and bone. The incidence in Europe is 5-6 /100 000 per year[5]. Soft tissue and bone sarcomas account for less than 1% and 0.2% of all malignant tumors respectively[6,7].

Ewing sarcoma is the second most common bone sarcoma after osteosarcoma among children and adolescents, with an annual incidence of 1-3 persons per million[8,9]. There is a slight male predominance and the incidence is much higher among Caucasians than among Afroamericans and Asians[10]. Around a quarter of ES arise in soft tissue rather than in bone. A quarter of all skeletal ES occurs in the pelvis, and around 20 % occurs in the femur[11-13]. If arising in the long bones, the tumor is typically located in the diaphysis. The most prevailing metastatic site are the lungs (50%) followed by bone (25%) and bone marrow (20%)[11,14]. Seventy percent of the patients are younger than 20 years of age, with a median age at diagnosis between 14 and 17 years[11,13,15].

Clinical presentation and diagnosis

Typically, the patients present with pain and swelling, and it is not uncommon with a history of trauma around the onset of diagnosis. The duration of symptoms prior to the first medical visit is over 6 months[16]. Patients occasionally have systemic changes such as fever or weight loss. A plain x-ray will usually lead to a high suspicion of a primary bone malignancy. Furthermore, an MRI of the tumor including the whole compartment is performed to determine the extent of bone and soft tissue involvement. Frequently, there will be a significant soft tissue component of which relation to the vessels and nerves is of central importance when planning for biopsy and local treatment.

Definitive diagnosis is established through biopsy, either fine-needle, core-needle or open. Morphologically, ES appears as an undifferentiated small round blue-cell malignancy. Mitotic index is low. The surface antigen MIC2 (CD99) can be found in over 90% of tumors, and ES cells ordinarily stain positive for periodic acid–Schiff (PAS) and vimentin[17]. Molecular diagnosis is of particular importance in the diagnosis of ES since 85 % of Ewing sarcomas carry a specific t(11;22) translocation resulting in an EWS-FLI1 fusion transcript. The remaining 15 % of cases that do not have the EWS-FLI1 fusion gene, will have the EWS gene fused to another member of the ETS family of genes, such as ERG, ETV1, or E1AF[14]. Molecular analysis using fluorescent in-situ hybridization (FISH) or reverse transcript PCR (RT-PCR) to detect the fusion gene, has been a routine part of the diagnosis of ES in Scandinavia since the end of the nineties.

Computer tomography of the chest is performed as part of the staging procedure in order to screen for lung metastases. Detection of bone metastases is traditionally done with bone scintigraphy. However, [18F]fluorodeoxyglucose positron emission tomography (FDG-PET) or a fusion PET-CT is becoming a routine part of staging procedures in many centers. Curiously, the proportion of patients presenting with clinically evident metastasis has stayed unaltered at around 25 % despite the improved sensitivity of imaging techniques in recent years[17].

Systemic treatment

Combination chemotherapy has significantly enhanced the survival rates of patients with localized disease from 10% to around 75%[14,18]. However, it has had little effect on patients who present with metastasis. For this group of patients, overall survival at 5 years is less than 30%. Furthermore, for patients who suffer from relapse, the 5-year event-free survival rate is only 10%[19,20]. The standard treatment algorithm today is neoadjuvant multi-agent chemotherapy for at least 12 weeks followed by local treatment which consists of surgery or radiotherapy or a combination of the two. Thereafter, maintenance chemotherapy is given for a period of time ranging from 25-37weeks. The duration and type of chemotherapy given during the maintenance period depends on the tumor response to chemotherapy and the risk profile of the patient. Most patients are included in multinational studies comparing different maintenance treatments. The standard induction chemotherapy treatment in Europe today consists of 6 cycles of VIDE (vincristine, ifosfamide, doxorubicin and etoposide), whereas in North America VDC-IE (vincristine, doxorubicin, cyclophosphamide-ifosfamide and etoposide) given at a compressed interval is the standard. A recently closed trial (Euro-Ewing 2012) aims to compare which of these regimens is the better. Furthermore, the use of high dose chemotherapy and stem cell rescue has shown a favorable effect in some studies, while other studies have questioned its value. Therefore, no consensus on its role has been reached yet[21-23].

Local treatment

It is well known that ES is a radiosensitive tumor. Initially, surgical treatment was therefore confined to expandable bones, but as surgical techniques evolved, surgical treatment indications extended. Growing endoprostheses, allografts and vascularized autografts have all improved functional outcome and enabled limb sparing surgery. Despite advancements in surgical treatment, complications such as post-operative infection, endoprosthetic loosening and bone healing difficulties are common in this young and active patient group.

Radiotherapy on the other hand has fewer early complications, but serious late effects are a problem, becoming increasingly evident as the number of long-term survivors is increasing. The long-term side effects of radiotherapy include growth impairment, insufficiency fractures and most importantly a significantly increased risk for secondary malignancies.

Available data favor surgery over definitive radiotherapy in the local treatment of ES[24-33]. The indications for post-operative radiotherapy are debatable, but most would agree for its indication in the setting of an intralesional, or perhaps a marginal surgical margin[26,33].

The current recommendation regarding local treatment of ES is therefore surgical resection with a wide margin. Surgical treatment or radiotherapy is rarely a matter of debate when the primary tumor is located in the extremities. However, given the fact that a third of all ES are located in the pelvis and spine, one can certainly imagine that that the issue of the best primary local treatment is quite often debated at multidisciplinary tumor meetings around the world[13]. The discussion usually comes down to whether the tumor can be excised with a clear (wide or marginal) margin, preferably a wide one, without significant morbidity. Since almost all tumors can be resected with a clear margin regardless of location if the associated morbidity and loss of function is ignored, the definition of an acceptable surgical morbidity is debatable and individualized. A pelvic tumor in proximity to the acetabulum can in most circumstances be excised and reconstructed with an acceptable risk for complications and a good functional outcome. Most specialists would probably opt for surgery in such a case, but how about a pelvic tumor that would require a hindquarter amputation; is that an acceptable surgical outcome that would outweigh the benefits of surgery over radiation treatment? What is the actual benefit of surgery over RT as regards to local control?

Custom made pelvic endoprosthesis, the use of large allografts, autografts and excision and extracorporeal irradiation or cryotherapy and re-implantation are all evolving techniques which have improved the arsenal of reconstructive options in recent years. Therefore, the idea of what is an operable tumor and what is not may have shifted over time. Furthermore, the negative long-term effects of the alternative local treatment; definite radiotherapy, are becoming evident as the number of long-term survivors is increasing. This has led to different local treatment strategies in North America and Europe, with more restricted use of RT in North America.

A randomized study comparing radiotherapy and surgery does not seem feasible[33]. Therefore, there is a demand for good retrospective studies examining the oncological outcome as well as the late effects with regards to local treatment.

2 AIMS

The overall aim of this thesis was to study the role of local treatment on local failure and late morbidity. Ultimately, the purpose was to understand which parameters the most important when choosing the most appropriate local treatment for the individual patient. The specific aims were:

-to study if sacral ES are treated differently and whether they have a worse oncologic outcome compared with ES elsewhere in the bony pelvis.

-to study the clinical presentation of spinal ES and how local treatment affects the oncologic outcome.

-to evaluate the role of surgery, radiotherapy (RT) or the combined treatment on the risk for local failure and disease relapse.

-to delineate the importance of surgical margin with regards to local control.

-to investigate the long-term risk and time trends for subsequent primary neoplasms (SPN) among ES and osteosarcoma (OS) survivors.

3 MATERIALS AND METHODS

This thesis is based on registries and review of individual medical records. The first two papers were based on the Scandinavian Sarcoma Registry (SSG); the third paper was based on the Swedish Cancer Register. In the last paper, the cohort was comprised of ES patients identified in the Swedish Pediatric Cancer Registry, which was linked with the SSG registry, the Swedish Cancer Register, the Death Cause Register and the National Patient Register.

3.1 THE REGISTRIES

The Scandinavian Sarcoma registry, which strangely enough contains data on patients from Sweden, Norway and Finland, was established in 1986. It was initially started by orthopedic sarcoma surgeons and later extended to include data provided by visceral sarcoma surgeons. The registry is therefore very surgically oriented with robust data, prospectively collected on parameters believed to be important for a surgeon such as surgical margin, tumor location, tumor size, type of reconstruction performed after excision and above all on local recurrence. Information on chemotherapy and radiotherapy is not as sound and there are no data on complications and side effects. Therefore, many individual medical records had to be reviewed in study I and for all cases in study II. The data in the registry were found to be very reliable with regards to local recurrence and metastasis when compared with the medical charts that were reviewed. The disadvantage of the registry is that the completeness of the registry is not as good. Pediatric sarcoma doctors report to their "own" registry, the Swedish Pediatric Cancer Registry, some of the pediatric sarcoma cases are therefore unknown to the SSG registry. The SSG registry has been administered by the Scandinavian Sarcoma Group up to 2018, when the Regional Cancer Centers (RCC) included the sarcoma register to a common platform for cancer quality registers (INCA) run by the National Board of Health and Welfare. There is therefore no longer a Scandinavian registry, instead independent national registries. Close collaboration between the Scandinavian countries still exists, but the exchange of data between Scandinavian countries has become increasingly troublesome after the European General Data Protection Regulation (GDPR) was introduced.

The Swedish Pediatric Cancer Registry was initiated by pediatric oncologists in the 1970-ies, which saw the need for gathering treatment information on leukemia patients in order to evaluate which treatments were working[34]. The registry expanded to other childhood malignancies and became nationwide including basically all pediatric malignancies occurring in Sweden since 1982. The completeness of the registry is good, but it contains only pediatric patients and follow-up is lost after the patients turn 18 and are transferred to adult oncology departments. Because it has always been run by pediatricians, the registry is more oncologic oriented with more detailed data on chemotherapy than on variables such as type of reconstruction after surgical excision. Nevertheless, the registry contains very detailed information on treatment, which of course is essential for evaluating complications and side effects caused by the treatment. The registry does not include structured information on late-effects, but late- effects are often commented on in the registry indicating that pediatric oncologists have always shown a strong interest for this issue, an interest that has become a

very important research area in recent years. The problem with registering late-effects lies in the above mentioned fact, that pediatric patients are transferred to adult health care after the age of 18. Some patients are even lost to regularly follow-up and turn up in other medical institutions with complications related their primary treatment. The complications may be unknown to the treating physician because they range broadly across disciplines, such as cardiovascular, endocrinology or musculoskeletal. The registry is currently administered by the Karolinska Institute, and there is ongoing work aimed at transferring data to a national database for all care and research on cancer.

The Swedish Cancer Register is a nationwide population-based registry founded in 1958[35]. The methods of registration in the Register are very well described in numerous publications and the completeness of the Register is over 94%[36]. It is mandatory by every health care provider to report on every new cancer case. Registration relies on dual reporting, as the pathologist as well as the treating physician has to report new cases. Even malignancies diagnosed at autopsy are registered. There is a very strong organization involved in confirming and correcting the Register, which is run by the Swedish National Board of Health and Welfare through six regional oncologic centers throughout Sweden. Coding of a new malignancy in the Register is based on internationally accepted rules for classification of cancer established by the *International Association of Cancer Registries (IACR)* and *International Agency for Research on Cancer (IARC).* A few benign tumors such as meningioma and other benign tumors of the central nervous system are also classified in the Swedish Cancer Register, due to their potentially aggressive clinical course. Notifications to the registry are also done for papillomas of the lower urinary tract, due to the known difficulty of ascertaining the malignant potential of lower urinary tract tumors. Skin cancers reported to the registry include malignant melanoma and squamous cell carcinoma exclusively; basal cell carcinomas are not registered. Tumors in the Swedish Cancer Register are all classified according to tumor site, as stated by the International Classification of Diseases, seventh edition (ICD-7). Codes reported in newer versions of the classification system (ICD-8, ICD-9 and ICD-10) are automatically recoded into ICD-7 codes. The histological tumor type is recorded according to ICD-O/2 and ICD-O/3 since 1993 and 2005, respectively. For the whole period from 1958 to the present, the codes are also available as historic histology codes (WHO/HS/CANC/24.1). The strength of the registry is also its weakness; only a few patient parameters are registered such as patient age, gender and place of residence. Medical data includes site, morphology, tumor stage, date of diagnosis, reporting institution and identification number for each specimen. Follow-up is limited to the death date, cause of death and date of migration. Data on treatment are completely lacking.

The National Patient Register was started in 1960, initially including only 16 percent of patients treated in somatic in-patient care, but all in-patients treated in psychiatric care. Since 1984 the Ministry of Health and Welfare made it compulsory by all councils to participate, and since 1987 it includes all in-patient care in Sweden, public and private. Data reported from the different councils are updated monthly and underreporting is very low. The Register contains data on age, gender, place of residence, date of admission and discharge, length of

hospital stay, where the patient was admitted from and where the patient was discharged to. The medical data includes the main diagnosis corresponding to an ICD code, additional diagnosis and surgical procedures. Quality controls on the submitted data are performed regularly, and if too much data are erroneous or missing, caregivers are asked to complete or correct them[37].

The Death Cause Register is also administered by the National Board of Health and Welfare. The Register contains date of death, the main cause of death and the illness leading to death for all deaths occurring in Sweden since 1961. The register is updated annually[38].

3.1.1 Patients

The cohort analyzed in paper 1 was extracted from the Scandinavian Sarcoma Group registry. It included only ES patients diagnosed with a primary tumor in the bony pelvis between 1986 and 2011. The bony pelvis consists of two bones: the sacrum and the hip bone/innominate bone. The latter consisting of three bones: the ilium, ischium and the pubic bones. The cohort comprised patients from Sweden, Norway and Finland. One hundred and twenty-one patients were identified in the registry. Three patients were excluded because of loss to follow-up; all with primary tumors in the innominate bone. One patient with a tumor in the sacrum was excluded due to insufficient information on the primary treatment. In the final analysis there were 88 and 29 patients in the innominate- and sacrum group respectively. Medical records were reviewed in detail if the data of interest could not be extracted from the registry.

The Scandinavian Sarcoma Group registry was also used for identifying the cohort in paper 2. All patients with a primary ES tumor located in the mobile spine (from the 1st cervical to the 5th lumbar vertebra) diagnosed between 1986 and 2012, and with a minimum follow-up of 2 years were included. One patient was excluded because the spinal tumor was not believed to be a primary tumor, but rather a metastasis. In the final analysis there were 24 patients. All medical records were reviewed in order to obtain the relevant clinical data.

The cohort studied for paper 3 consisted of ES and osteosarcoma patients identified in the Swedish Cancer Register from 1958 through December 31st 2015. Only patients with tumors of the extremities, trunk or chest wall were included. Time at risk started at time of primary bone sarcoma diagnosis and continued until the occurrence of a new primary neoplasm or until death or end of follow-up. Four individuals had identical morphology codes for the primary bone sarcoma and the subsequent primary neoplasm. Three of these patients, had a primary OS and were diagnosed with a subsequently occurring OS within 24 months. One patient with a primary ES suffered from a subsequent tumor classified as a new ES. These 4 patients were thus either misclassified or considered as having synchronous or metachronous OS/ES. Synchronous (at time of diagnosis) or metachronous (separated in time) OS, defined as 2 or more skeletal lesions occurring without the presence of lung metastasis, is observed among patients with predisposing syndromes. It constitutes a controversial issue because it is unknown if synchronous or metachronous lesions are clonally unrelated tumors or skeletal

metastasis from the primary OS. The 4 patients identified in this cohort were excluded due to this uncertainty. In the final analysis, there were 1779 patients; 1201 OS and 578 ES patients.

Paper 4 included 229 patients with a Ewing family of tumor were identified in the Swedish Child Cancer Register from January 1st 1982 until June 1st 2017. All ES patients (not restricted to extremities, trunk or torso) were analyzed. Therefore, the cohort included patients with tumors in the head and neck region, which are treated by ear nose throat tumor surgeons, as well. The register classifies tumors according the WHO-2005 *International Classification of Childhood Cancer (ICCC-3)*, which is a slightly different classification system than the previously described registries. Indeed, the ICCC-3 is also based on ICD-O-3 histology and ICD-O-2/3. The Swedish Cancer Register was linked with the SSG registry, the Cancer Register, the Death Cause Register and the Patient Register, a linkage that is possible due to each individual's unique personal identification number. Linkage with the latter registers was not possible for 26 of the patients identified in the Swedish Cancer Register. For some, the reason was that the patients had emigrated to Sweden and either had a temporary personal identification number or had changed their personal identification number, for others it was unknown why the individuals were not registered in the other registers. Nevertheless, data on these individuals only registered in the Swedish Cancer register were good; hence they were not excluded from the analysis. Furthermore, latest time of follow-up in the Cancer Register and Patient Register was December 31st 2015, while latest follow-up in the Scandinavian Sarcoma registry was February 18th 2015. Consequently, the analysis requiring data from the latter registers does not include the 26 patients identified exclusively in the Swedish Child Cancer Register, thus the latest time of follow-up in this analysis is December 31st 2015.

3.2 ETHICAL ASPECTS

The Nuremberg code is the basic guideline for the legislation linked to ethics in Sweden. All medical research involving human subjects follow the ethical principles stated in the declaration of Helsinki. All registries used in this thesis were approved by the Swedish Data Inspection Board and follow the Swedish legislation, which includes the Swedish Personal Data Act, the Swedish Patient Data Act and, since May 2018, the European General Data Protection Regulation. Patients are informed that registration in a national registry will take place, and that they have the right to decline. The Regional Ethical Board waved the requirement for a signed informed consent from the individuals studied in this thesis, because studies using the National Quality Registers do not require a signed informed consent according to Swedish legislation. The benefit of such an opt-out system for National Quality Registers in Sweden is uniformly believed to outweigh the requirement of a signed consent.

The studies in this thesis were approved by the Regional Ethical Board I Stockholm (Dnr: 2013/933-31/4 (Study I and II) and Dnr: 2016/953-32 (Study III and IV)).

3.3 DIAGNOSIS AND TREATMENT

3.3.1 Diagnosis

The diagnosis of ES is based on morphology consistent with a CD-99 positive undifferentiated small round blue-cell tumor and a molecular translocation analysis demonstrating the EWSR fusion transcript. The fusion transcript, which is considered mandatory for the diagnosis to be made, has been in routine use in Scandinavia since 1999. Prior to 1999, the diagnosis was established based on morphology alone. However, almost all ES patients in Scandinavia have been included in different international trials, which require the histology to be peer-reviewed by an expert musculoskeletal tumor pathology board. Any patient with histology consistent with ES, Askin´s tumor or pNET (primitive neuroectodermal tumor) of bone and soft tissue, was included in this analysis.

3.3.2 Systemic treatment

Because the ES patients studied in this thesis in large were enrolled in different ongoing trials, they received chemotherapy protocols mainly depending on which study they were enrolled in. The patients in the cohorts of study I and II received chemotherapy according to Scandinavian or collaborative Scandinavian-Italian protocols. Patients diagnosed between 1984 and 1990 were treated according to the SSG IV protocol, from 1990 to 1999 with the SSG IX protocol and from 1999 up to current day with the SSG/Italian Sarcoma Group (ISG) protocol. Before these study periods ES patients were treated solely with surgery, RT or the combination of both.

ES patients treated in pediatric centers in Sweden were primarily enrolled in,- or at least treated according to, the protocols in pan European studies such as the Euro-E.W.I.N.G-99, Euro Ewing 2012 or the joint European-North American Ewing 2008. Unlike the ES patients of cohorts I and II, the cohort in study IV was treated according to the pediatric Ewing protocols above. The chemotherapy administered across the different protocols use the same drugs in different constellations; Vincristine, Ifosfamide, Doxorubicin, Etoposide, Cyclophosphamide and Actinomycin D. High dose treatment and stem cell rescue or autologous bone transplantation was included in the EuroEwing trials as well as the SSG/ISG trials.

3.3.3 Local treatment

For non-metastatic ES, the SSG/ISG protocols recommended surgery with wide margins after induction chemotherapy. The SSG/ISG III and IV protocols, which was the basis for most patients in study I and II, were very specific. Excision was recommended for all tumors located in bones that do not require reconstruction after excision; clavicle, rib, scapula, iliac wing, pubic rami, but also for tumors in the pelvis and long bones that require reconstruction such as the humerus, distal radius, proximal ulna, femur and tibia. Amputation was recommended when the functional deficit caused by radiotherapy would be greater than that after amputation, typically that would include the following two situations: children under the

age of 8 with a tumor involving a major growth plate where radiation would cause a severe limb length discrepancy or a patient with a pathological fracture. For children under the age of 10 with lesions of the proximal and distal femur, rotation plasty was advocated[39].

If surgery resulted in inadequate margins (intralesional or marginal), regardless of chemotherapy response, the recommendation was to administer RT in full doses of 42 or 54 Gy. Radiotherapy alone was reserved for tumors, which because of site or size exclude the possibility of surgery with adequate margins. Radiotherapy was administered in a hyper fractionated and accelerated fashion in order to shorten treatment time and to achieve the maximum effect with the least long-term side effect by superimposing chemotherapy and RT. RT was administered at the beginning of the consolidation treatment. The EuroEwing protocols are less detailed with regards to recommendations on local treatment.

3.3.4 Surgical margin

Surgical margin was based on Enneking´s classification. Intralesional margin was defined as a procedure resulting in the tumor being transected or opened during surgery. The definition of a marginal margin was when the closest margin was outside the tumor, but close to the tumor and transecting the reactive zone. A surgical procedure resulting in a wide margin was achieved when there was a cuff of healthy tissue surrounding the specimen and covering the reactive zone around the tumor. A radical margin was consisted with the whole tumor bearing compartment being excised[39].

3.3.5 Follow-up

Investigation performed after end of treatment included a set of mandatory examinations; complete physical examination, complete blood count, serum creatinine, GFR, electrolytes, LDH, ALP and liver transaminases. The radiologic requirements during follow-up were x-ray of the chest and the entire involved bone, CT and MRI scans over the entire involved bone (except for patients who had undergone reconstruction with metal implants) and cardiac ultrasound.

Follow-up for pediatric patients was done in the pediatric oncology department every 3 months for the first 3 years, thereafter at 4 month intervals for another 2 years and thereafter biannually up to 10 years. Children were transferred to the adult oncology clinic when they turned 18; otherwise regularly follow-ups were terminated 10 years after end of treatment. However, in recent treatment periods, pediatric patients in Sweden have been followed even past 10 years in so called late-effect clinics.

At each follow-up the following investigations were done; physical examination, chest X-ray, blood count, transaminases, ALP, LDH and serum creatinine. Glomerular filtration rate (GFR) every 6 months, cardiac ultrasound at 3 months, 6 months and then every 3 years after treatment. ECG was recommended every 3 years after treatment as well as a sperm count for males. The radiologic examinations performed at each follow-up were x-rays of the entire involved bone and of the chest. A CT of the chest was recommended if lung metastases were

suspected on the chest x-ray and a bone scan was recommended if bone metastases were suspected.

3.3.6 Statistics

In study I and II, the categorical parameters were studied for statistical significance by the use of the chi-square test. The continuous variables were analyzed using the student-t test. The statistical tests for significance were two sided and a p-value <0.05 was considered significant. The method of Kaplan-Meier was used to investigate the rate of local recurrence, relapse (local or distant) or death. The time from diagnosis until occurrence of any of the events mentioned above or end of follow-up was used for the Kaplan-Meier estimates. The log-rank test was used to test the statistical significance of each variable with regards to the outcomes studied. Variables considered to be statistical significant for each end point were entered into a Cox proportional hazards model, and if they contributed significantly to the fit of the model, they were included in the analysis. A fixed covariate model was assumed because none of the variables were believed to be time-dependent.

In study III, standardized incidence ratios and absolute excess risks were used to investigate the risk for SPNs in the study population compared to the general population. Incidence rates in the study group were matched with regards to age-, sex- and calendar year of diagnosis-with the population based incidence rates retrieved from the National Board of Health and Welfare in order to calculate the standardized incidence rates (SIRs). The number of expected cancer cases was calculated by multiplying the Swedish incidence rates by the total person-time at risk for each stratum in the cohort. The standardized incidence ratios were calculated by dividing the observed number of cases with the expected number of cases. Overall and SPN-specific incidence ratios were estimated and stratified by sex, age at diagnosis (0-9, 10-19 and >20 year intervals), follow-up (0-5, 5-30 and >30 year intervals), calendar year at diagnosis (1958-1979, 1980-1999 and 2000-2015) and site of primary tumor. The rationale for dividing age at diagnosis into the intervals as stated above was that we assumed that the risk for certain SPNs was higher the younger the patients were at the time of treatment (e.g. that the risk for RT- or chemotherapy related sarcoma would be greatest if administered to the growing child). The age group 10-19 years was chosen to include all girls treated during puberty, a period in which the breast tissue proliferate the most. We could have divided the age groups even more, but this would have been at the expense of less power as the number of patients in each group would have been smaller. Age at time of diagnosis had been reported in earlier studies as an important host-related risk factor[40,41]. The intervals chosen to subgroup patients according to calendar year at diagnosis were based on time periods corresponding to major changes in treatment. Nineteen fifty eight to 1979 corresponds to the pre chemotherapy- or first generation chemotherapy era, 1980-1999 is the era in which combination chemotherapy was introduced for ES as well as for OS patients. In the modern treatment era (2000-2015), perhaps more restricted use of RT due to the increased awareness of late effects would be reflected in a lower risk for SPNs.

The absolute excess risk (AER) was calculated by subtracting the expected number of SPNs from the observed number of SPNs divided by person-years at risk multiplied by 10 000. The Confidence Intervals (CIs) were calculated assuming that the number of observed cases of SPN followed a Poisson distribution. We also estimated the overall cumulative SPN incidence in an analysis limited to our cohort. Because patients with a history of malignancy prior to their ES or OS diagnosis were believed to represent a subgroup with an inherent increased cancer risk, we also did an analysis excluding this patient group to see if this altered the results found in the initial analysis.

In study IV, tests for statistical differences at baseline between the local treatment groups were done with the use of the chi square- and the Independent samples Kruskal-Wallis test for categorical and continuous variables respectively. The method of Kaplan-Meier was used to estimate the local recurrence- and overall survival rates for the different exposures studied. Time at risk started at time of diagnosis and ended at time of an event or end of follow-up. Differences in hospital admittance and hospital stay between the treatment groups were tested with the Independent samples Mann-Whitney U-test. All tests were two sided and the significance level was set to $p<0.05$.

4 RESULTS

4.1 DISEASE FAILURE IN PELVIC EWING SARCOMA DEPENDS ON WHETHER THE TUMOR IS LOCATED IN THE SACRUM OR INNOMINATE BONE (HIP BONE) (STUDY I)

Study I was conducted after a clinical observation by a senior colleague who had noticed a seemingly low local and distant failure rate in patients with sacral ES. Patients with ES elsewhere in the bony pelvis were studied as a control group. Bear in mind that ES of the pelvis is indisputably associated with a poor prognosis.

Patient demographics were similar for patients with tumors located in the sacrum and in the innominate bone. However, tumor characteristics differed with regards to tumor size. Measured in the largest diameter, ES of the innominate bone were in average 2 cm larger than sacral ES ($p<0.05$).

As predicted, we found that patients with tumors of the innominate bone received more aggressive local treated than patients with tumors of the sacrum. Only 17% of patients with sacral ES had surgery or surgery with radiotherapy, whereas 56% of patients with tumors located in the innominate bone underwent surgery +/- radiotherapy. Four patients in the innominate bone group and one patient in the sacrum group received only systemic therapy.

Twenty-five patients of surgically treated patients received adjuvant radiotherapy. Fourteen of these patients received post-operative and 8 patients received pre-operative radiotherapy. For 3 patients, it was unknown how RT was administered. Of patients who underwent post-operative radiotherapy, only two had a wide surgical margin, the remaining had intralesional or marginal margins.

Of the 49 surgically treated patients in the innominate bone group, a clear surgical margin was achieved for 42 (86%) of the patients. Only 1/5 surgically treated patient in the sacral site group had a wide surgical margin.

For patients with a minimum follow-up of 2 years, there was only 1(4%) local failure in the sacrum group and 9 (11%) local failures in the innominate bone group. The median time to local failure was 2 years.

Disease-free survival was significantly better for patients with tumors located in the sacrum compared with patients with tumors located in the innominate bones (P<0.05).
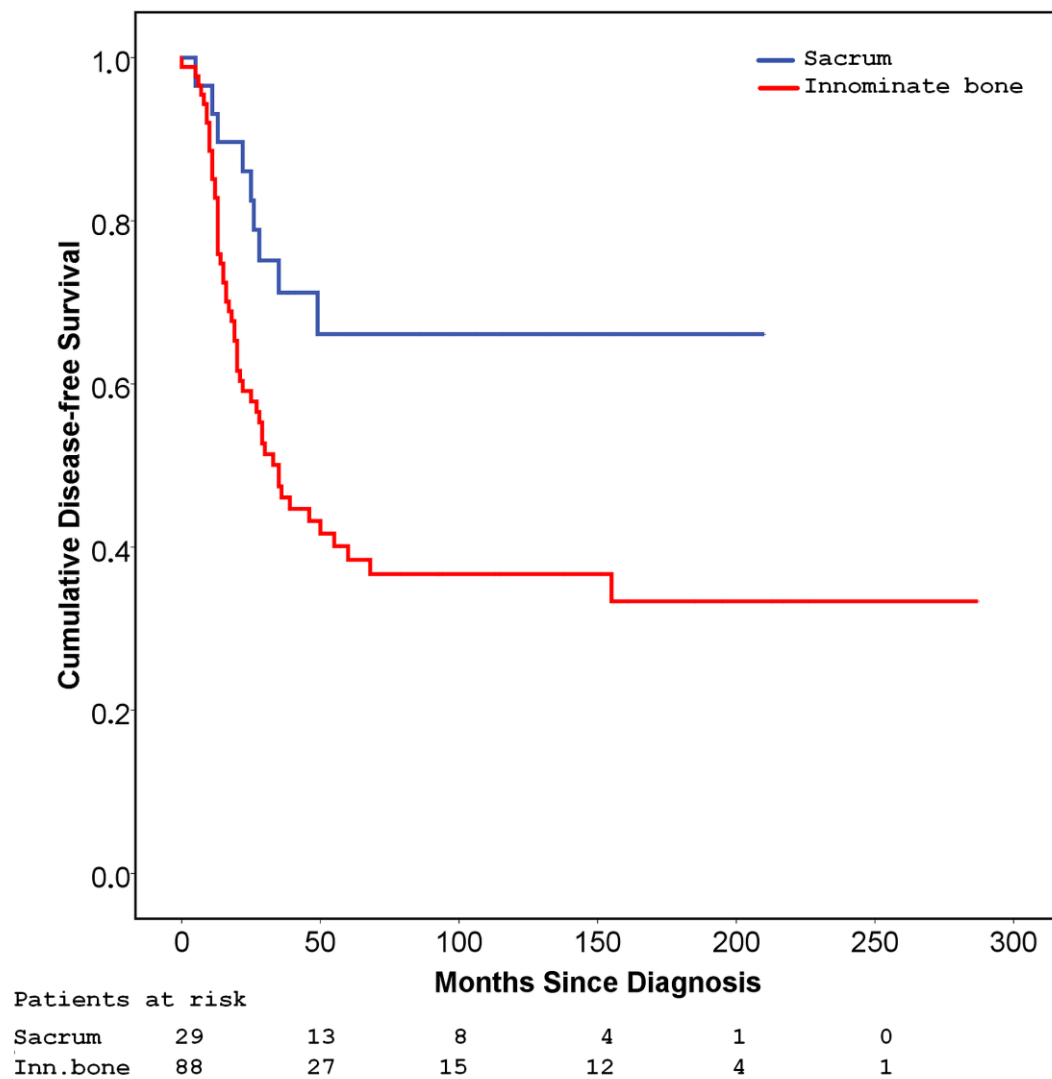

Fig.1 Disease-free survival analysis of 117 pelvic Ewing sarcomas showing cumulative 5-year disease-free survival rates of 66% and 40% for sacral and innominate bone tumors respectively. Log rank p=0.01. With permission from JB&JS.

The overall median time to event was 16 months (range 155 months).

In the univariate analysis of tumor characteristic- or treatment related parameters being associated with the occurrence of any event (local failure, distal failure or death), the absence of metastasis at diagnosis, tumor size < 8 cm and sacral tumor site were significantly favorable clinical parameters (surgical treatment showed a trend for being a positive prognostic factor, p=0.07). The cut-off for large and small tumors of 8 cm was based on previous studies and staging systems, in which a large tumor (> 8 cm) places the patient in a different stage, associated with a higher risk for disease failure. A tumor diameter of 8 cm corresponds to a tumor volume of slightly more than 200 ml. In the multivariate cox regression analysis, tumor size was no longer a significant prognostic factor for disease-free survival. Sacral site on the contrary, remained a significantly favorable prognostic factor (Table 1).

Table 1. **Multivariate Disease-Free Survival Analysis**			
Variable	HR	95 % CI	P-value
Site (Sacrum)	0.34	0.14 to 0.84	0.02
Tumor Size (continous)	1.02	0.96 to 1.10	0.50
Metastasis at time of diagnosis	2.68	1.42 to 5.08	0.002
Abbreviations: HR=hazard ratio, CI=Confidence Interval			

Regarding overall survival, there was a trend for an improved survival for patients with tumors in a sacral site compared with innominate bone site (overall survival rate of 65 % and 49% for sacral and innominate bone tumors respectively, p=0.08). Inferior overall survival was observed for patients with metastasis at diagnosis, tumor size > 8 cm, positive surgical margin and local recurrence. In the multivariate analysis, only metastasis at diagnosis (HR, 2.04, 95% CI, 1.04-4.01) and local recurrence (HR, 2.68; 95% CI, 1.09-6.6) significantly affected overall survival, although surgical margin was omitted from the analysis due to the low number of patients treated in the group of patients with sacral tumor site.

The role of postoperative radiotherapy in correlation to surgical margin is demonstrated in Fig 2 and Fig 3. Supplementing surgery with radiotherapy for patients with marginal or intralesional surgical margin significantly improved the overall survival rate to a rate equal to that of patients with a wide surgical margin.
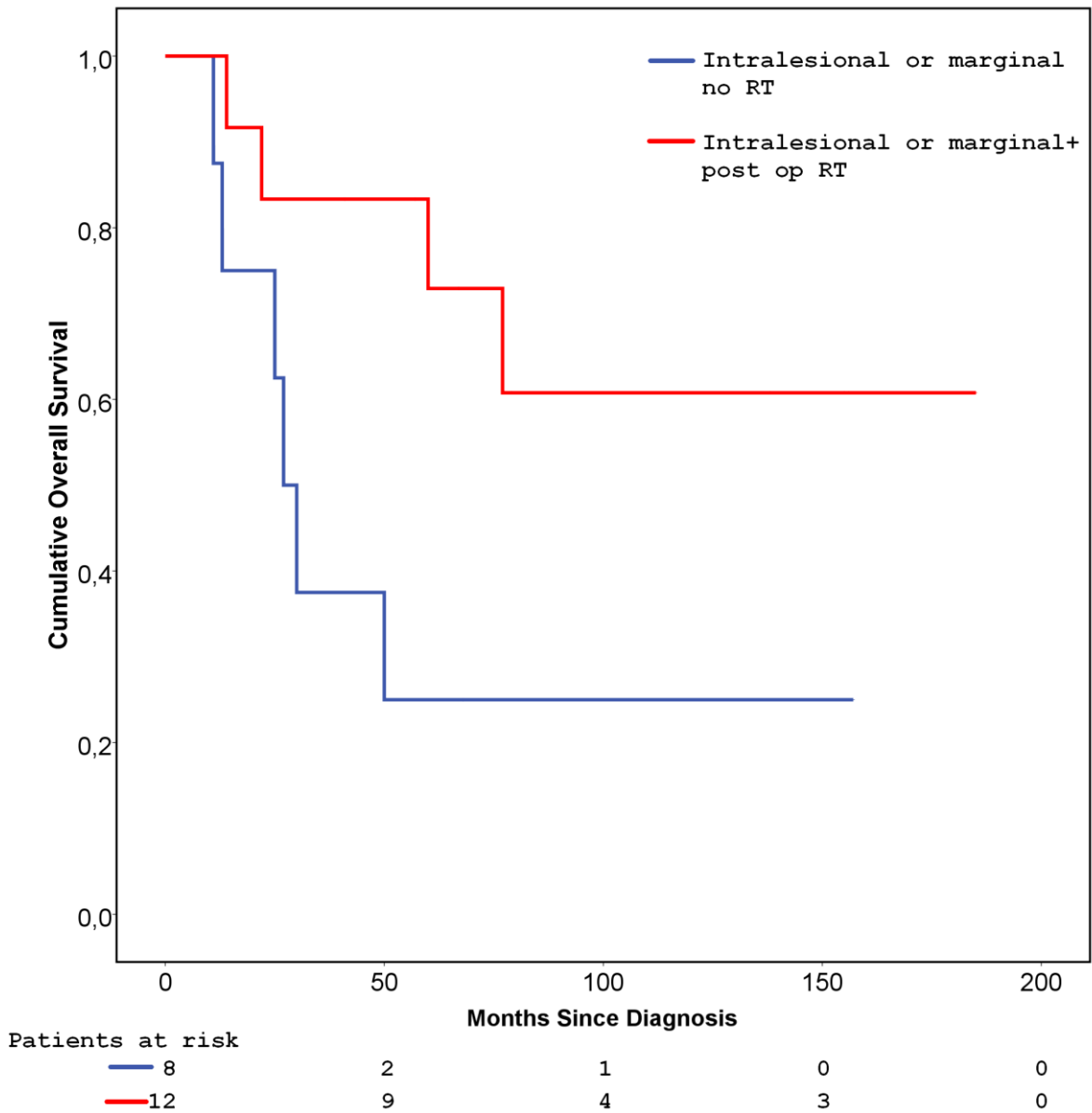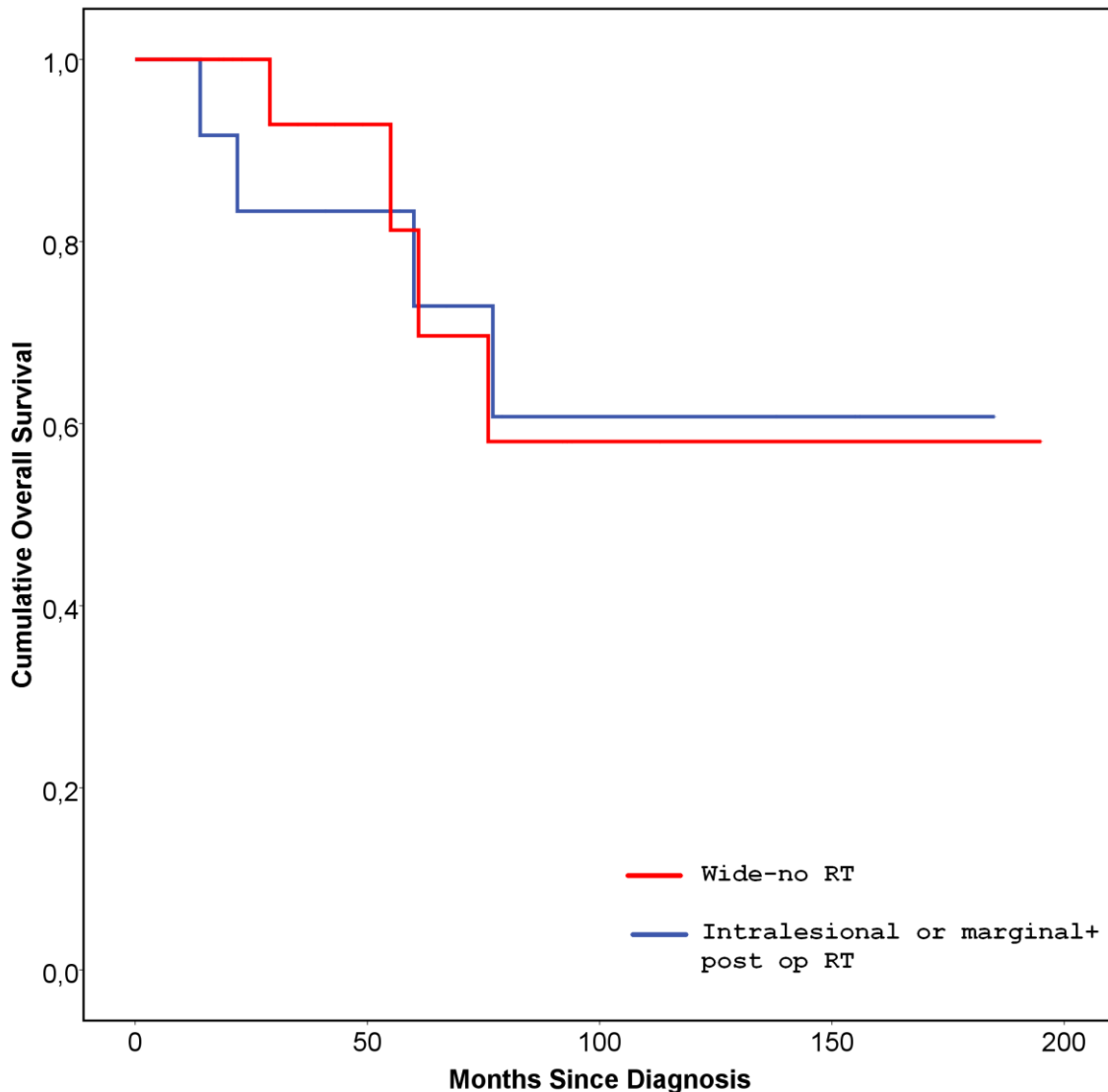
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Fig 2 showing the cumulative overall survival of patients with intralesional or marginal margin without radiation treatment compared to patients with intralesional or marginal margin with post-operative radiation treatment. Log rank p=0.04. With permission from JB&JS.

Fig 3 showing the cumulative overall survival of patients with wide margin without radiation treatment compared to patients with intralesional or marginal margin with post op radiation treatment. Log rank p=0.91. With permission from JB&JS.

Metastasis at presentation was common among patients with tumors in the sacrum (41%) as well as among patients with tumors in the innominate bone (38%), although as many as 33% were alive 5 years after diagnosis.

4.1.1 Conclusion

Radiotherapy alone seems to give good local control in patients with ES of the sacrum. For patients with tumors elsewhere in the bony pelvis, surgery should be the treatment of choice, and if a wide surgical margin is not achieved, radiotherapy should be administered. Sacral site itself appears to be a favorable prognostic factor compared with other pelvic sites, for reasons that cannot be explained by this study.

4.2 DISEASE FAILURE AND NEUROLOGIC SYMPTOMS AMONG PATIENTS WITH EWING SARCOMA OF THE MOBILE SPINE (STUDYII)

Based on the results of study I where we demonstrated that sacral site (fixed spine) was a positive prognostic factor and that radiotherapy alone provided good local control, we conducted study II to investigate if the same favorable local control rate and overall survival rate could be shown among patients with tumors in the mobile spine. Twenty-four patients were included in this study.

As was observed among patients with ES of the fixed spine (sacrum) in study I, the majority (18/24) of patients with ES of the mobile spine were also treated with definitive RT. The median radiation dose among patients treated with radiotherapy alone was 51.5 Gy. Six patients underwent surgery, of which 4 also received RT post-operatively. Three out of 6 patients had clear resection margins. Nineteen out of 23 patients presented with neurologic symptoms of which 9 patients were treated with urgent decompressive surgery without excision of the tumor. Two patients underwent urgent laminectomy and simultaneous tumor excision, while 2 patients received chemotherapy prior to a complete tumor excision and laminectomy.

There were 5 local recurrences, one of which occurred as long as 10 years after diagnosis. The local recurrence rate was thus 27% at 10 years (in comparison, for innominate bone- and sacral tumors, the 10-year local recurrence rate was 15% and 5 % respectively). None of the 6 patients treated surgically experienced a local recurrence; however there was no statistical difference in local recurrence rate between patients treated with definitive radiotherapy or surgery (p=0.12). Only treatment era had a significant impact on local failure as there were no local failures from1999-present. However, performing urgent decompressive surgery showed a tendency to increase the local failure rate (p=0.06).

The 5 year disease-free survival rate was 54% (the disease-free survival rates for patients with tumors in the innominate bone and sacrum were 40% and 65%, respectively). The overall survival rate was 63% (65% and 49% for sacral and innominate bone tumors respectively). Treatment era (p=0.02) and performing decompressive surgery (p=0.05) had a significant effect on disease failure in the univariate analysis, although neither (p=0.06 for decompressive surgery) were significant in the multivariate analysis.

Excisional surgery (p=0.05) and local failure (p=0.01) had a significant effect on overall survival in the univariate, but not in the multivariate analysis.

Neurologic deficits due to spinal cord compression/injury were classified according to the Frankel scale (Fig 4).

Fig 4

Frankel scale	
A	Absent motor and sensory function
B	No motor function, but some sensation below level of lesion
C	Sensation present. Some motor function without practical application (grade 2-3/5)
D	Sensation present. Useful motor function below level of lesion (grade 4/5)
E	Normal sensation and motor function

Of the 19 patients that presented with neurologic deficits, the majority was Frankel grade D. Four out of 6 and 9/13 patients who were treated with radiotherapy and surgery respectively had complete neurologic recovery at latest follow-up (Frankel grade E). Among patients with neurologic sequelae, all were able to walk (Frankel grade D).

Serious surgical late complications requiring revision surgery occurred in 5/13 patients, 3 of which were due to kyphotic deformities. Five late complications were related to chemotherapy, and 1 patient with a C6 tumor treated with radiotherapy suffered severe esophageal strictures requiring repeated esophageal dilatation and a percutaneous endoscopic gastrostomy.

4.2.1 Conclusion

Patients with ES located to the mobile spine are generally treated with definitive radiotherapy which results in a relatively high local failure rate. Neurologic symptoms at presentation are common, which often leads to urgent spinal decompression and contamination of tumor in the surgical wound. We saw a tendency for a higher failure rate among patients treated with urgent spinal decompression. As neurologic recovery was good regardless of whether or not spinal decompression was performed, urgent spinal decompression without excision of the tumor should be avoided if possible. If laminectomy is performed, posterior stabilization should be considered to minimize the risk for later spinal gibbus deformity. Furthermore,

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improved local control was seen in the recent treatment era, perhaps due to improved systemic treatment.

4.3 RISK PATTERNS FOR SUBSEQUENT PRIMARY NEOPLASMS AMONG BONE SARCOMA SURVIVORS (STUDY III)

The risk for secondary cancer or subsequent primary neoplasms (SPNs) has shown great variation in previous uncontrolled studies. The rationale for the current study was to assess the overall risk for SPNs among bone sarcoma (Ewing and osteosarcoma) survivors and to delineate the risk pattern, e.g. which specific SPN are survivors at risk for; which age group are at highest risk; has the risk been reduced in recent treatment eras and for how long does the increased risk persist in comparison to the risk in the general population

From 1958 until December 31st 2015, 115 SPNs were observed among 104 individuals previously diagnosed with an ES or osteosarcoma. Sixteen patients had more than 1 SPN. Eighty six patients were diagnosed with a malignancy prior to the bone sarcoma diagnosis. This was much more common among osteosarcoma patients (7%) than among ES patients (1%) (p=0.001).

ES survivors were more than four times as likely to experience a SPN than the general population and twice as likely as OS survivors to experience a SPN (SIR 4.2; 95% CI 2.8–6.1 and SIR 1.9; 95% CI, 1.5–2.4, for Ewing- and osteosarcoma survivors respectively). For ES survivors, a more than 7-fold risk increase was observed in the age group 10-19 years at age of diagnosis (95% CI, 4.2–11.6). Among OS survivors, the highest risk was observed in the youngest patient group (0-9 years of age) who also showed a nearly 7-fold risk increase compared with the general population (95% CI, 1.8–17.2) (Fig 5).
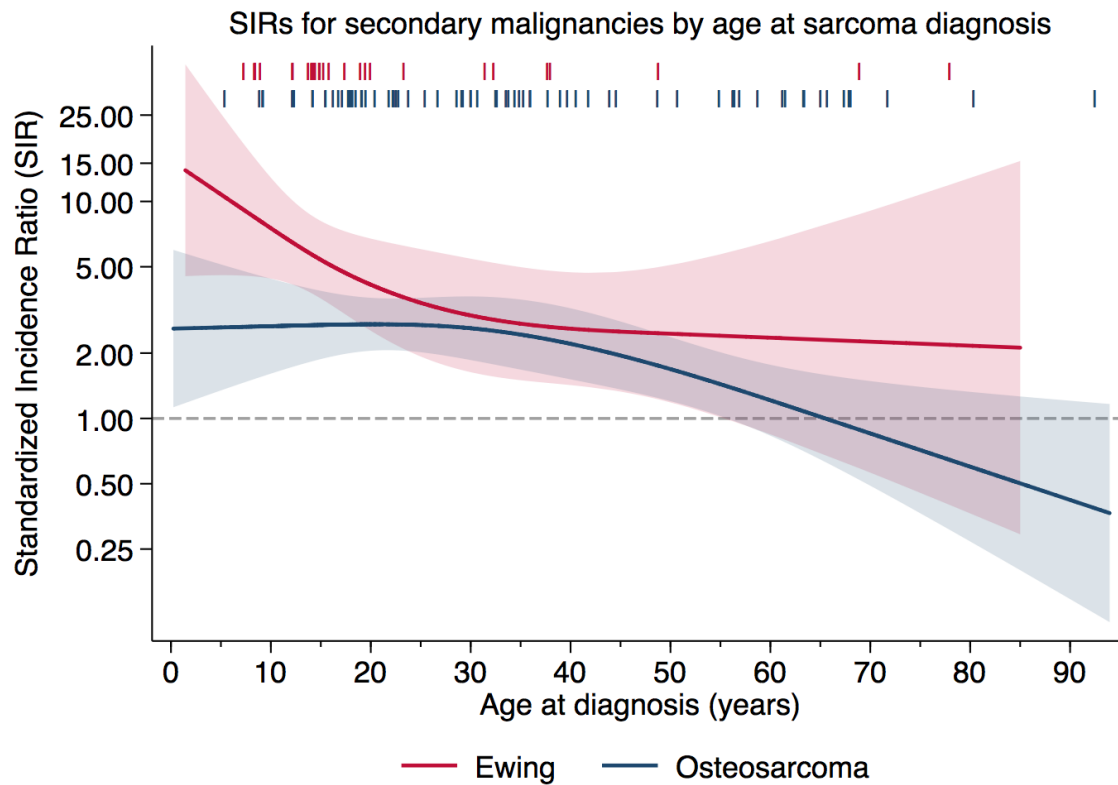
Fig 5. showing SIRs by age at diagnosis for ES and OS survivors.

The highest risk with regards to anatomic site was observed among ES survivors with a primary tumor in the pelvis who demonstrated a nearly 8-fold increased risk compared with the general population (95% CI, 2.1–19.4). By treatment era, the risk remained the same for patients treated in the first and last treatment era (SIR $_{1958-1979}$ 2.0; 95% CI, 1.5–2.6 and SIR $_{2000-2015}$ 2.0; 95%CI, 1.1–3.5) (Fig 6). The 30 year cumulative SPN risk was 7% and 9% for OS and ES survivors respectively.
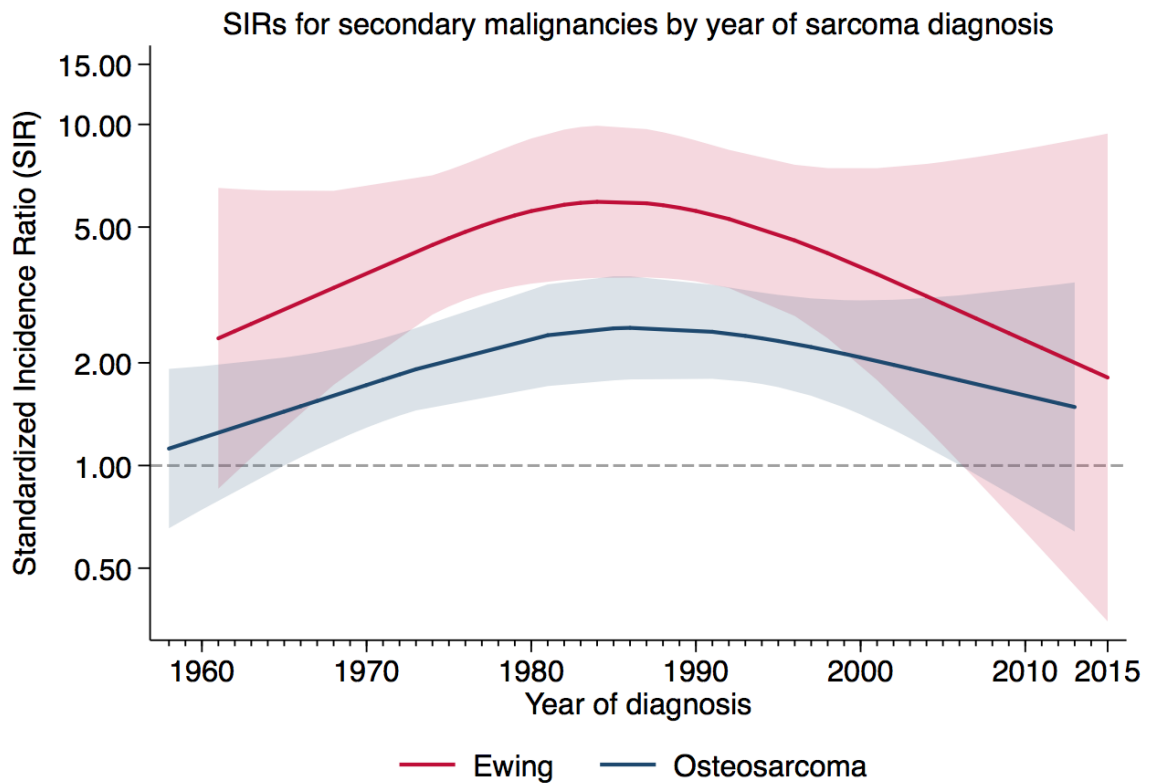
Fig 6 showing SIRs for SPN by year of diagnosis.

Ewing sarcoma survivors were nearly 5 times as likely to develop a breast cancer compared with the general population and OS survivors more than twice as likely, making breast cancer the largest driver of excess cancer risk among ES as well as OS survivors (95% CI, 1.7–10.2 and 1.1–3.6 for ES and OS survivors respectively). The overall increased risk for breast cancer remained higher than expected for survivors with more than 30 years of follow-up (SIR 2.6; 95% CI, 1.0–5.7). Due to the high background breast cancer incidence in the general population, the elevated risk translates to an excess of 13 breast cancers for every 1000 person-years of follow-up. By anatomic site, 12 times more SPNs than expected were observed among ES survivors with a central but not pelvic location of the primary tumor (95% CI, 2.4–34.0). Overall, survivors with a central location of the primary tumor had a 7-fold higher breast cancer risk, while survivors with a tumor in the extremities had a 2-fold higher breast cancer risk than expected (95% CI, 2.1–15.2 and 1.1–3.6).

Second to breast cancer, female genital malignancies contributed the most to the excess cancer risk observed with an AER of 9.7/ 10 000 person years (95% CI, 2.4–21.5). Subsequent CNS tumor- and skin cancer risk was only higher than expected among OS survivors (SIR 6.4; 95%CI, 3.2–11.4 and SIR 2.5; 95% CI, 1.3–4.3). Genitourinary malignancies contributed significantly to the excess risk if the primary bone sarcoma was located in the pelvis (AER 22.3; 95 CI, 1.5–72.7). The risk for digestive tract malignancies was not elevated for neither OS nor ES survivors compared with the general population (SIR $_{overall}$ 0.7; 95% CI, 0.3–1.5).

By specific SPN, the highest overall risk was observed for bone sarcoma and soft tissue sarcoma, which was nearly 14- respective 21-fold higher than for the general population (95% CI, 1.7–49.7 and 8.9–40.5 for bone and soft tissue sarcoma respectively). However, due to the low incidence rate in the general population, the absolute excess risk was only 1.1 and 4.5 / 10 000 person-years for bone and soft tissue sarcoma respectively (95% CI, 0.1–4.2 and 1.8–9.1). The highest SIR for soft tissue sarcoma was observed among ES survivors, who had a 67 times higher risk than that of the general population (95% CI, 21.9–157, AER 9.8; 95% CI 3.1–23.1).

The risk for hematological malignancies were higher than expected among ES as well as OS survivors (SIR 5.5; 95% CI, 1.5–14.4 and SIR 1.6; 95% CI, 0.6–3.5). Hematological malignancies were together with CNS, the only malignancies that demonstrated the highest SIR within 5 years after diagnosis (SIR 6.1; 95% CI, 2.2–13.3 and SIR 6.3; 95% CI, 1.3–18.3 for hematological and CNS respectively).

The median time from OS or ES diagnosis to SPN was as follows; breast cancer; 24 years (range 1-49 years), soft tissue sarcoma; 18 years (range 1-44 years), skin cancer; 15 years (range 2-46 years), CNS tumors; 17 years (0-49 years), genitourinary malignancies; 22 years (0-48 years), female genital malignancies; 18 years (range 0-40 years), hematological malignancies; 5 years (range 0-31 years).

4.3.1 Conclusion

Ewing- and osteosarcoma survivors have elevated cancer risks compared with the general population attributed to specific cancer types. The risk in relation to the population remains elevated even past 30 years of follow-up. Female ES patients are at high risk for breast cancer, but the excess cancer risk was also driven in large by female genital malignancies, a finding that may indicate a role of BRCA-associated phenotype among a subset of patients. The increased risk for OS and ES patients was also high in recent treatment eras, indicating the need for prolonged surveillance among these patients, even with modern treatment regimes.

4.4 THE ROLE OF LOCAL TREATMENT ON LOCAL FAILURE AND LATE EFFECTS IN A PEDIATRIC EWING SARCOMA COHORT (STUDY IV)

This study had two main aims; first we asked the same research questions regarding reasons for disease failure as posed in study I and II, albeit with a different cohort .Additionally, we included patients with ES not only confined to the spine and pelvis, but with any ES location (including head and neck ES). Secondarily, since we were totally lacking treatment variables in study III, we sought to investigate the effect of treatment on late effects such as hospitalization, SPNs and death unrelated to disease failure.

Of the 229 patients in the cohort, we had complete treatment details on 205 patients. In this cohort, only18/205 (17%) patients were treated locally with radiotherapy alone. A surprisingly large percentage of patients (n=97) were treated exclusively with surgery (47%).

Patients treated with surgery alone had less often metastasis at diagnosis (10%) compared to patients treated with RT alone (32%) or surgery with RT (31%). Tumor size was similar across all treatment groups. Local failures were observed in 37(16%) of the patients. The local failure rate at 5 years was 28% for patients treated with RT alone and 11% for patients treated with surgery alone (P<0.05). The local recurrence-free survival rate was only 47% at 20 years for patients treated with RT alone.

Among surgically treated patients, 65% had a wide resection margin. Better local control was achieved if a wide surgical margin was obtained compared to a marginal one (the 5-year local recurrence-free survival rates were 90% and 69% for patients with wide and marginal margins respectively (p<0.05).

Nevertheless, there was no difference in local recurrence-free survival rate among patients who had a wide margin and patients with a marginal margin who also received RT (p=0.27) (Fig 7). For patients with a marginal margin without RT, the 5 year local recurrence-free survival rate was only 58% compared to 91% for patients with a wide surgical margin (p=0.02) (Fig 8).

Adding RT to patients with a wide surgical margin did not improve the local control rate (p=0.33) (Fig 9). Moreover, there was no difference in local control rate for patients with intralesional margins with or without RT (Fig 10).

Fig 7

Fig 7 Five year local recurrence-free survival rates of 91%, 79% and 82% for patients with wide margin- no RT, marginal margin with RT, and intralesional margins with RT, respectively (p=0.27).

Fig 8

Fig 8 Five year local recurrence-free survival rates of 91%, 58% and 86% for patients with wide margin-no RT, marginal margin-no RT and intralesional margin-no RT respectively (p=0.013).

Fig 9

Fig 9 Five year local recurrence-free survival rates of 87% and 91% for patients with wide margin with RT and wide margin-no RT respectively (p=0.33).
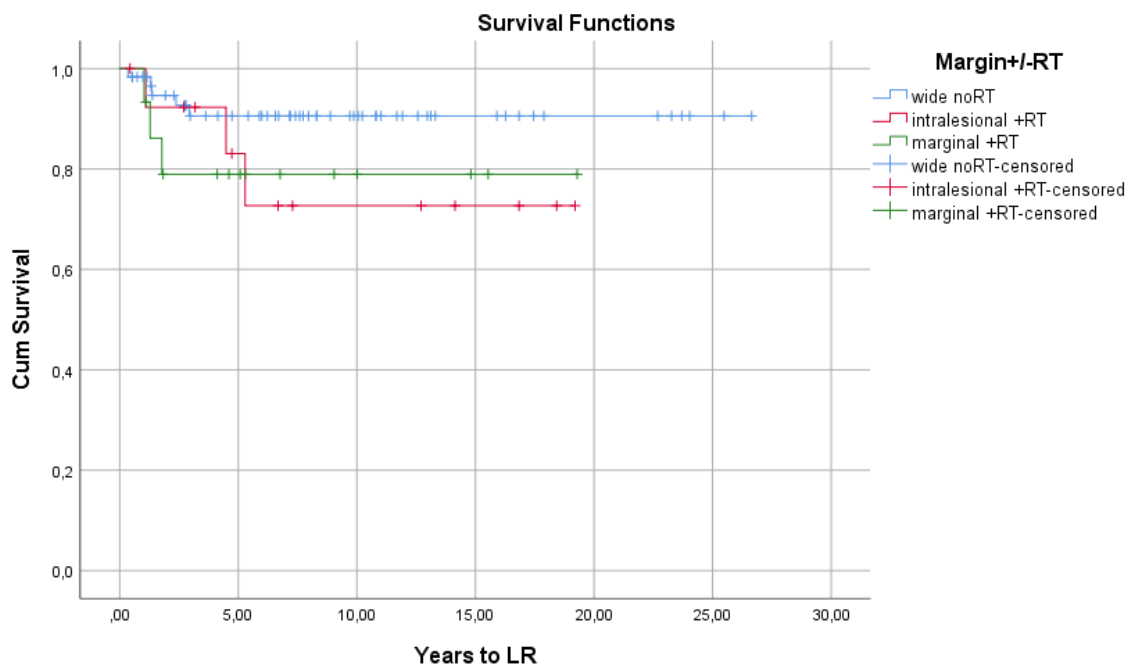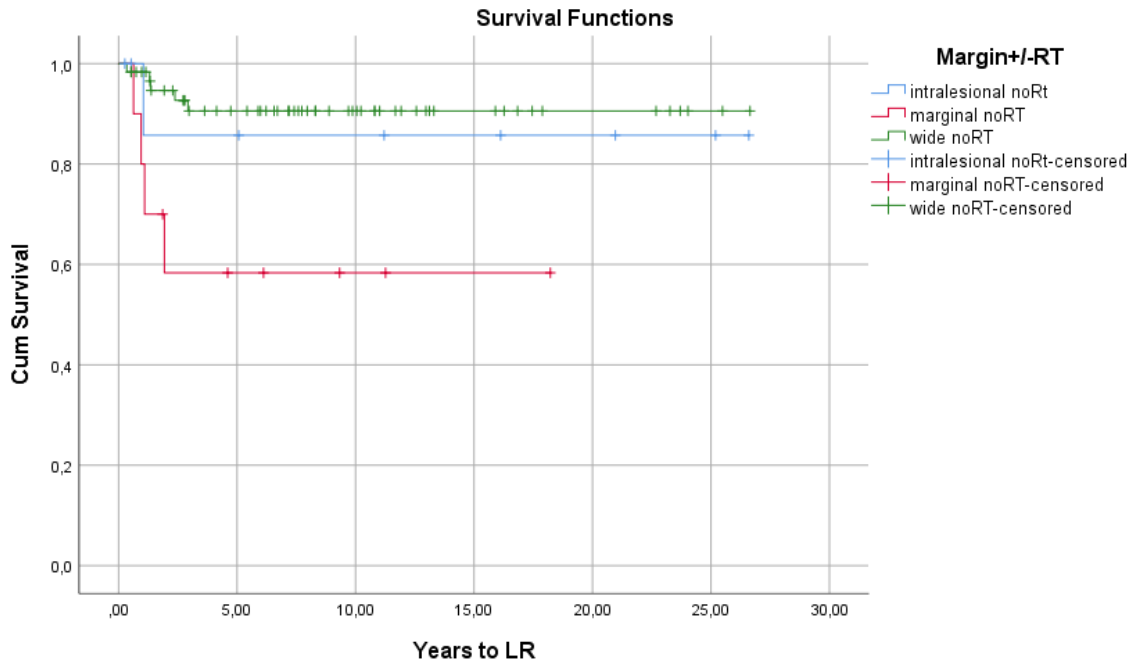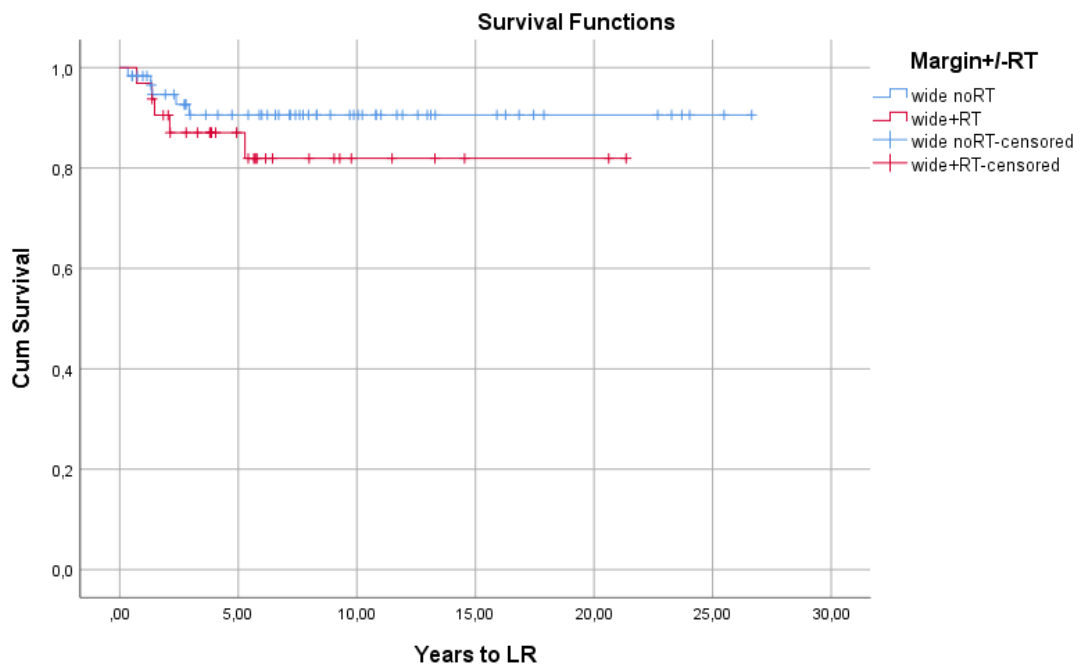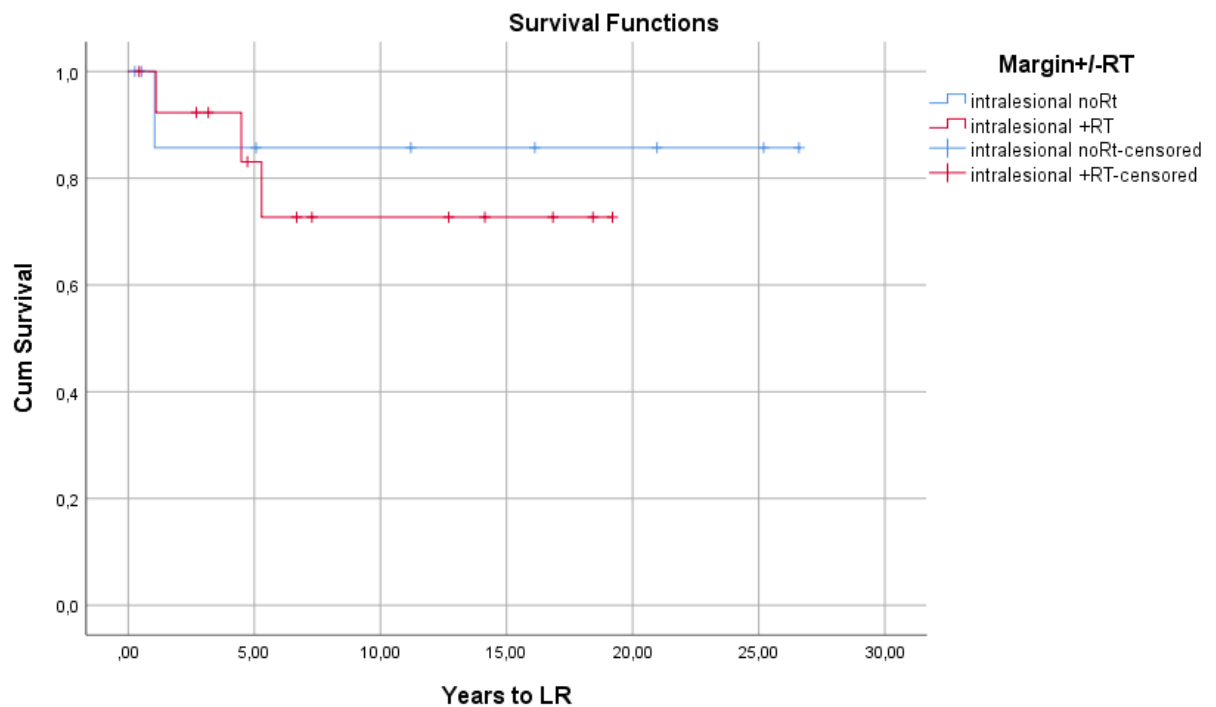
Fig 10

Fig 10 Five year local recurrence-free survival rates of 83% and 86% for patients with intralesional margin with RT and intralesional margin-no RT respectively (p=0.66).

The 10 year overall survival rate for patient was 23% and 66% for patients with and without a local relapse respectively (p<0.05).

Eight subsequent primary neoplasms occurred in 7/229 individuals (Table 2).

Table 2 Subsequent neoplasms

Patient no	Second neoplasm	Time to second neoplasm	Primary tumor location	Local treatment
1	Acute myeloid leukemia	5 years	Pelvis	RT
2	Parathyroid adenoma	25 years	Rib	Surgery
3	Cervical intraepithelial neoplasia (CIN)	6 years	Femur	RT
4	Cervical intraepithelial neoplasia (CIN)	7 years	Rib	Surgery
5	Osteosarcoma	5 years	Foot	RT
6	Bilateral breast malignancies	32 years	Unknown	Surgery
7	Cervical intraepithelial neoplasia (CIN)	22 years	Femur	Surgery

Of the 13 late deaths that occurred, only 2 were caused by other reasons than disease relapse.

Forty out of 99 patients without relapse of disease were admitted to hospital after 5 years of follow-up. There was no difference in hospital admittance across type of local treatment (Fig 11). The median number of admittances was 3 and the median length of hospital stay was 8.5 days.

Fig

Admitted to hospital after 5 years of follow-up
(not including patients with disease relapse)
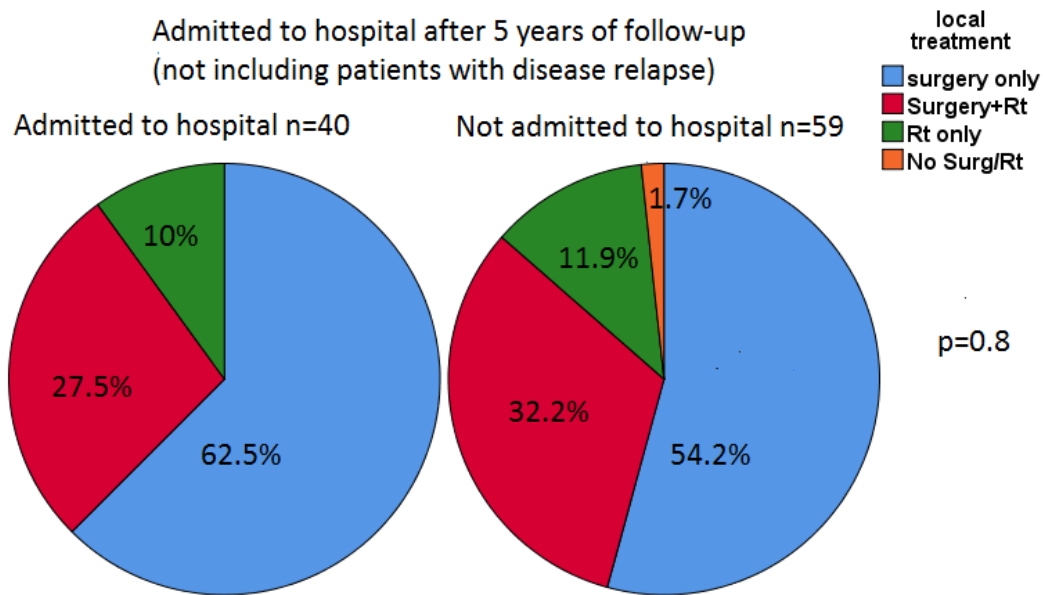
Fig 11 Hospital admission (yes/no) not related to disease relapse after 5 years of follow-up.

4.4.1 Conclusion

This study supports aggressive local treatment with surgery striving for wide margins in order to achieve local control which is essential for improved survival. If only marginal margin is obtained, adjuvant RT should be given. This study could not prove any benefit of adding RT for patients with a wide or intralesional margin. Subsequent primary neoplasms were few and unrelated to local treatment. Late mortality unrelated to disease relapse was low. However, treatment related morbidity, assessed by investigating hospital admission after 5 years of follow-up, was common, affecting nearly half of all ES survivors.

5 DISCUSSION

Many cancer patients, particularly children, have seen the benefit of recent advances in systemic treatment as well as radiation and surgical treatment. Unfortunately, the standard of care has not changed notably in the last decades for patients diagnosed with Ewing sarcoma, where conventional chemotherapy and local treatment with surgery and/or radiotherapy is still the mainstay of treatment. Fusion-derived antigens and CD 99 or IGF1R expression are potential targets that could be approached by cancer vaccines or chimeric antigen receptor T-cell therapy (CAR-T). Nevertheless, studies investigating the possible role of such treatments, as well as the use of immune checkpoint inhibitors have not been promising[42]. For patients with non-metastatic ES the prognosis with conventional treatment is good; however, for patients with metastatic disease overall survival is dismal. Important research questions which need to be answered are: which novel systemic treatment is most promising and should be included in future multinational trials for patients with metastatic disease or for poor responders to chemotherapy? What is the optimal combination and timing of current chemotherapy? What is the role of whole lung radiation for patients with lung metastasis? Which local treatment strategy, surgery, RT or the combination of both results in best local control and what are the late effects of clinical importance related to treatment modality? In the four studies presented in this thesis, we aimed to shed light on the latter question.

Local treatment in ES is controversial[31,43-45]. Some studies have questioned the contribution of local failure to overall disease failure, while other studies highlight its importance[12,29,46,47]. In study I, II and IV which are based on 3 different cohorts, local control was pivotal for overall survival, with a 5 years overall survival rate between 20% and 30 % for patients with local failure.

For a patient with metastatic disease at presentation, definitive RT may seem like the best local treatment option, but if the patient responds well to chemotherapy with remission of the metastatic disease, surgery may be the preferred treatment. Metastasis, tumor size and site, patient age, national and institutional practice and patient preference are all important factors affecting the choice of treatment but also disease relapse. Therefore, these factors must be accounted for in all studies on local treatment with regards to local and distant relapse..

In study 1, the clinical observation of a sacral tumor localization being a favorable prognostic factor was confirmed in terms of disease-free survival in a Scandinavian cohort. We adjusted for tumor size which was the most likely known confounder and still found sacral site to be an independent favorable prognostic factor. Tumor size is universally believed to be a negative prognostic factor, although many have questioned its relevance[4,27,31]. In contrary to what was seen for ES of the non-sacral pelvis and spine as well as for the whole cohort in study IV, local control using RT alone was excellent for ES of the sacrum. The favorable prognosis associated with sacral site is difficult to explain since there are no other studies to confirm or dispute this finding. Another observation that was puzzling was the high percentage of metastasis at diagnosis (41% compared with 38% in the innominate bone) found in the group of patients with sacral ES. This may indicate that inherent biologic factors

assigned to sacral site play a role in metastasis and response to treatment. A theory would be that the tumors of sacral site are supposedly well vascularized leading to early metastasis, perhaps by direct tumor ingrowth into the prominent vessels of the presacral venous plexus. If metastasis is driven by direct tumor seeding into the circulatory system instead of for instance hypoxia, which is a known underlying mechanism of metastasis, this could also explain the better response to chemotherapy. In other words, a sacral tumor may metastasize early but respond better to chemotherapy. The good local control in sacral ES using exclusively radiotherapy could also be interpreted in the same context; a well vascularized tumor may respond better to radiation treatment than for example a necrotic, poorly vascularized tumor of the ilium. The significance of necrosis in relation to survival has been well demonstrated in a paper from 1986 examining the histopathology of 286 untreated cases of ES[48].

This theory has clear limitations, for one there is no data to confirm the positive prognostic value of sacral site found in our study. Therefore, our findings must be validated in other cohorts. Secondly, there are no publications to support that the presence of necrosis or other differences in tumor microenvironment can be attributed to site. Indeed, some studies have shown that subsets of ES with specific genetic mutations affecting the p53, p16INK4, p14ARF and MDM2 genes are associated with poor response to chemotherapy and thereby poor prognosis[49-52]. Whether these findings can be related to site needs to be elucidated.

Another limitation to this study which may play a significant role is the duration of follow-up. We have seen in studies I, II and IV that recurrences may occur later than within the classical 5-year follow-up period, some recurrences can occur even as late as 10 years after diagnosis (Study II). Theoretically, definitive RT as performed in the majority of patients with sacral ES, may not prevent, but just delay the onset of local recurrences. Although the mean follow-up time in study I is 5.4 years (including patients dying due to disease), the duration of follow-up raises some concern since it may be too short to draw strong conclusions. It is therefore necessary reevaluate this cohort in the future to see if our findings stand true with extended follow-up.

Local treatment was not statistically significant as a prognostic factor for overall survival, although 8 patients in the innominate bone group and none of the patients in the sacrum group had a relapse, but were still alive at the end of the study period. Therefore, it is likely that a difference in OS would be seen with a longer follow-up.

There were not enough local recurrences for a meaningful analysis with regards to local treatment. Nonetheless, there was an indication (p=0.07) that local treatment affected disease-free survival. This may indicate an underreporting of local recurrences or that distant relapse and death are competing events for local recurrences. Although, not significant in the overall survival analysis, local treatment is likely to be biased as patients with favorable disease characteristics are generally treated surgically.

For patients that were treated surgically, we were not able to prove that margin was a prognostic factor for local or distal relapse in study I (in contrary to study IV). Although,

overall survival was significantly affected by surgical margin, we did not include surgical margin in the multivariate analysis, simply because we chose to include the covariate local recurrence, which also reflects patients treated without surgery. We chose to dichotomize surgical margin into R0 and R1 resections instead of into wide, marginal and intralesional. This was done because in many studies, R0 and R1 classification is the preferred way to describe a surgical margin, perhaps because separation between marginal and wide margin is difficult and inconsistent. Furthermore, the nomenclature clear (R0) and contaminated (R1) margin, is commonly used in clinical practice in many parts of the world. Having only a dichotomous (R0 and R1) variable for margin requires less participants to show an effect, but it is also problematic because a lot of the R0 margins were marginal and therefore received RT.

The more detailed margin description of wide, marginal or intralesional was used to show the effect of administering RT to surgically treated patients without adequate surgical margins. This could only be demonstrated in the overall survival analysis were there was a significant benefit of adding RT to patients with intralesional or marginal resection status. Even this result must be interpreted with caution; why were patients with intralesional or marginal margins not treated with adjuvant RT? Perhaps they had disseminated disease and a poor prognosis which precluded local treatment. The number of patients was too low to adjust for metastasis at diagnosis. Nonetheless, in study IV we were able to show the importance of surgical margin on local control, which supports the findings in study I.

Because of the anatomic and structural similarities between the sacral vertebrae and the mobile spine vertebrae we anticipated that some of the factors usually subject to bias such as tumor size and choice of local treatment would be eliminated when comparing the two sites. This was the case for tumor size which was on average 8 cm for both sites. A slightly higher proportion of the ES located in the mobile spine were treated surgically (6/24) compared to the sacrum (5/29). Interestingly, all surgically treated patients in the mobile spine cohort were treated in one institution, none relapsed, and all patients were alive at end of follow-up. Two other studies, each with 6 surgically treated mobile spine ES, published results with 100% local control[53,54]. The comparison between the two sites is very limited by the lack of power making it unjustified to give the results any merit. It is also worth mentioning that local control in the mobile spine was not as good as in the sacrum. Overall survival for tumors of the mobile spine seemed to be intermediate compared with the innominate bone and the sacrum. The limited number of other studies comparing fixed and mobile spine ES have showed contradictory results[55-57]. Two studies with only 7 respective 13 sacral ES showed inferior disease-free survival for sacral site[58,59]. However, three other studies, two of which had smaller cohorts, and one larger than ours could not prove a difference with regards to site: disease-free survival at 5 years was between 35% and 45%[56,57]. The local recurrence rate found in study II was comparable to what has been presented by others[53,59-61]

Even though the low numbers in the study limits analysis of primary treatment, it was worth noting that emergency decompression tended to negatively affect local control. This concern

has also been raised by others[55,61,62]. It is reasonable to assume that a laminectomy, which usually has to traverse through the tumor, increases the risk for local relapse. Even though most patients in our study presented with neurologic deficits in the present study, most recovered regardless of treatment mode. This may reflect the effect that chemotherapy usually has on ES, causing a volume reduction quite rapidly after start of induction treatment. However, even this needs wary interpretation. It is possible that the patients who underwent urgent decompression had more severe neurologic compromise than patients who did not undergo spinal decompression, and that the good results observed in this group would not have been as good if decompression had not been performed. It is possible that post-operative radiotherapy can compensate for the increased risk of performing urgent laminectomy, in which case the procedure is justified. Another point is that for a patient presenting with neurologic symptoms and a spinal tumor of unknown etiology, an emergency decompression will quickly give tissue for histopathology. At some centers, a frozen section can be done during surgery, thus allowing prompt diagnosis and start of systemic treatment.

Also the risk for local relapse may be overestimated (or underestimated) as the number of recurrences (5 patients) was low making statistical analysis unreliable.

The complications delineated among spinal ES patients in study I are predominantly surgical complications, some perhaps are worsened by the combination of surgery and radiotherapy. They are of interest, because some are avoidable. In particular, if an emergency decompressive procedure is performed without posterior stabilization, there is a risk that a sagittal deformity (usually gibbus) will occur in the spinal column. Therefore, authors have advocated that posterior stabilization should be performed[63]. Five gibbus deformities developed in our cohort, some of which would have been avoided with posterior stabilization. The downside of posterior stabilization, which is usually done by the use of titanium rods and screws, is that the surgical wound becomes bigger necessitating a larger field of post-operative radiotherapy. The risk for surgical site infection increases manifold, a complication that may delay chemotherapy. Also, the presence of hardware in the spine impedes the use of magnetic resonance imaging (MRI) to surveil the surgical site for local recurrences. The less sensitive computer tomography (CT) can be used, but even this modality is troubled by hardware. Good artefact reducing software have reached the market but monitoring for local relapses is still by far easier in a patient without spinal hardware. Moreover, titanium or stainless-steel rods and screws are not compatible with the use of emerging radiation techniques such as proton- or carbon ion therapy. Another point that should discourage spinal stabilization is that many patients will not live long enough to develop spinal deformities. Indeed, most patients that underwent posterior decompression without posterior stabilization did not develop a spinal deformity. Posterior stabilization in this group of patients would be overtreatment.

The risk of treatment-related secondary malignancies is a variable that is attracting increased interest in the decision-making regarding the use of radiotherapy, one reason being that before the era of multi agent chemotherapy many of these patients never survived long

enough for the secondary malignancies to occur. Now that more childhood and young adulthood cancer patients survive and are starting to reach an age in which cancer is more common in the general population, we are observing an increasing number of secondary cancers. Although secondary cancer is seen after treatment of all childhood malignancy, lymphoma and ES survivors belong to the group of patients which have shown the highest risks[40]. Subsequent malignancies after treatment of cancers occurring mainly in adults are also a concern, but the age distribution in adult cancer makes the risk in adults less of an issue. Some subsequent tumors can be a serious concern even though they are not malignant, an example being benign brain tumors such as meningioma, which are commonly registered in cancer registries. Subsequent primary neoplasm (SPN) is therefore a term frequently used when reporting on secondary neoplasms, as it encompasses the few benign tumors that may have more of a malignant course.

Genetic factors as well as treatment related factors such as chemotherapy and radiotherapy are all associated with the risk of developing SPNs among bone sarcoma survivors[64-66]. The genetic factors associated with a general increased cancer risk are concentrated to osteosarcoma patients. No known inheritable factors are coupled to the increased risk seen in ES. The chemotherapeutic agents used in treatment of ES and OS, particularly alkylating agents, but also anthracyclines are known to be carcinogenic, increasing the risk for mainly for hematological malignancies but also for solid tumors such as breast cancer and OS[67-71]. Even treatment with platinum-based agents, which is used in treatment of OS, increases the risk for secondary malignancies[72]. Radiotherapy, is the only modifiable risk factor[9,40,73].

The literature search performed ahead of study III showed a wide range in risk estimations calculated for SPN among ES patients. The studies which were uncontrolled and not population based, showed cumulative incidence rates varying from 5% at 10 years to 35% at 10 years [74-79]. There are two well-documented large pediatric cohorts, the North American Childhood Cancer Survivor Study (CCSS) and the British Childhood Cancer Survivor Study (BCCSS), both of which have yielded numerous studies on the risk for SPN among childhood cancer survivors.[73,80]. The cumulative incidences of SPN among ES survivors in these cohorts were 9% and 10 % at 30 years, which correlates strikingly well with the results in study III (9% at 30 years). The BCCSS and CCSS are pivotal studies due to the size, extensive longitudinal follow up and detailed treatment information. Both studies have their limitations: treatment details were by no means complete (75% and 83% completeness respectively) and one third of all eligible patients in the CCSS cohort were lost to follow-up or refused to participate. Furthermore, the study period for the BCCSS cohort was only up to 1991, and in the CCSS cohort up to 1999, thus the results do not reflect the current trends which may have changed substantially. Lastly, the BCCSS cohort only included patients younger than 15 years of age. However, these studies raise two important questions which have been addressed in study III: for how long does the risk remain elevated compared to the general population, and what are the risks for patients treated after 1991 and 1999 respectively? The BCCSS claimed that the risk for SPN among bone cancer survivors was no higher than for the general population after 30 years of follow-up. The study also assigned a significant cause

of the excess cancer risk to bone sarcoma, even more than to breast cancer. In study III we saw a tendency for a decline in risk over time, which nonetheless remained elevated past 30 years of follow-up. This was mainly driven by the excess breast cancer risk among ES survivors. In contrary to the BCCSS results, we also found that excess risk due to secondary bone cancer among ES and OS survivors constituted a rather small proportion. A likely cause for the contradictory results found in the BCCSS cohort and in study III is the low number of observed and expected cases in the BCCSS cohort. In the latter only 5 SPNs were reported past 30 years of follow-up, compared to the 21 cases observed in study III. Two of the 5 cases of SPN in the BCCSS cohort were breast cancer and none were due to bone sarcoma. In the whole BCCSS cohort there were 13 subsequent bone sarcomas, thus more than observed in study III. It is therefore reasonable to believe that with longer follow-up and more survivors entering the +30 years of follow-up group, we would see more SPNs, as observed in study III. The differences observed in risk attributed to subsequent bone sarcoma among primary OS and ES patients may be due to two reasons; either the BCCSS cohort, basically reflecting the whole of Great Britain, has been subject to more extensive radiotherapy than the Swedish cohort, or the interpretation of what is a subsequent bone sarcoma differs between the studies. The latter does represent a difficult issue and may be the cause of differences seen in other cohorts; how do you know that a subsequently occurring bone lesion (-s) with a morphology consistent with OS is de facto a synchronous or metachronous OS and not a bone metastasis of the same clonal origin as the initial bone sarcoma? Even if the subsequent OS is clonally different, let's say with a histopathology in line with an undifferentiated pleomorphic sarcoma of bone, the lesion can be a metastasis that has dedifferentiated from the original OS rather than a radiation-related new bone sarcoma. The problem is only relevant among OS survivors, because the morphology of a subsequent bone sarcoma in an ES survivor is so different from the morphology in the primary ES that it simply cannot have derived from the original ES. Although unlikely, there may be situations of subsequent ES occurring long after the primary ES where the same problem occurs, and that the subsequently occurring ES is wrongly recorded as a SPN. For the situations in study III where the morphology codes of the original bone sarcoma were the same as the subsequently occurring bone sarcoma, the subsequently reported bone sarcoma was not recorded. It is not clear how they have handled this issue in the BCCSS study or most other studies, but it may reflect why the subsequent bone sarcoma risks are lower in study III than in many other studies[80-83]. The authors in the BCCSS discuss why lower SPN risk was observed after 25 years of follow-up and conclude that the excess risk among bone sarcoma survivors is caused by direct radiotherapy exposure, and with extended follow-up survivors reach an age in which other subsequent malignancies than bone sarcoma dominate, such as breast, digestive tract, genitourinary and lung carcinomas. These malignancies are according to the authors unlikely to be higher than expected among primary bone sarcoma survivors, for which an estimated 80% of the patients had a primary bone sarcoma in the limbs, accordingly unlikely to have received radiotherapy to the sites in which these malignancies arise. There are two main oppositions to this explanation; only 70% of ES in their cohort were located in the extremities. In study III, 43% of ES and 15% of OS developed in a central location, in which the malignancies discussed

above arise. In other studies 1/3 to 1/2 of ES are located in the axial skeleton[84-86]. Furthermore, tumors arising in the scapula are coded as an extremity location according to the WHO- ICD classification. ES patients with a primary tumor located in the scapula, or even in the proximal upper arm, may therefore well have received RT towards the breast tissue.

Another observation from study III was that the overall standardized incidence rates (SIRs) for SPNs among ES and OS survivors were in the lower range compared to the BCCSS and some other studies. Absolute excess risks (AERs) on the other hand, were in the higher range[80,82]. It is reasonable to believe that this is due to the BCCSS and other cohorts being younger, as SIRs in the lower age group in study III also had higher SIRs. The difference in AERs may reflect a higher background cancer incidence in the Swedish population. If the aim is to reduce the number of survivors developing SPNs, then the focus should be on breast and female genital malignancies, because the highest AERs were seen for these malignancies.

In study III we saw an increased risk for SPNs even in the latest treatment era (2000-2015) however; it was insignificantly lower than for the previous (1980-1999) time period. If this was more than a decreasing trend, did it reflect more restricted use of radiotherapy or perhaps that modern radiotherapy techniques are less likely to cause secondary malignancies? A lower risk could hardly be assigned to changes in chemotherapy as standard treatment has remained constant across the previous treatment eras. In North America, we know the use of radiotherapy for patients with childhood malignancies has been reduced, even so for ES patients[73,87]. The latest CCSS report showed a lower overall cumulative incidence for subsequent malignant neoplasms among patients treated the latest (1990-1999) study period, a phenomenon assigned to changes in the use of radiotherapy. In the subgroup analysis, no decrease was seen for OS or ES survivors, which by all means could be a result of insufficient power. Anyway, temporal trends will often misinterpret changes in the latest time eras simply because the follow-up time is shorter. As follow-up time increases so will the number of patients developing SPNs. It is therefore likely that we will continue to see an increasing number of SPNs among bone sarcoma survivors in the future.

The lack of treatment details is the major limitation in study III. It was one of the reasons that OS patients were included as a comparative group. Chemotherapy-related differences between ES and OS are small and do not need to be accounted for, but as a proportion of OS patients will harbor genetic changes predisposing for subsequent malignancies, higher risks could be foreseen in this group. We did not exclude patients with hereditary retinoblastoma, who have a known high risk for OS, still ES showed double the risk compared to OS. Hence, a large part of the excess cancer risk in ES must be attributed to RT. Moreover, we found higher risks for centrally (axial skeleton) located tumors, a risk that was attributed to female genital malignancies, and urogenital malignancies. The risk for subsequent breast cancer was only elevated for centrally, but not pelvic primary bone tumors. These are sites in which surgery with wide margins is very challenging, and most survivors will have received RT. These findings point towards RT being a significant contributor to the excess risk for ES patients. Overall, OS and ES survivors did not show an elevated risk for digestive tract

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malignancies in study III. This is perhaps somewhat unexpected. The bowels are likely to have been exposed to radiotherapy in ES survivors with tumors in the pelvis and lower spine. The BCCSS group found a high AER for colorectal cancer among childhood survivors who had reached the age of 40 and undergone direct abdominopelvic radiation[88]. The risk was literally comparable to that of individuals with a genetic predisposition to colorectal cancer, a patient group in which screening with colonoscopy is recommended. Regarding specific childhood malignancies, the study presented the risks for bone sarcoma as one entity, for which the risk was not significantly elevated. Once again, the results must not be over interpreted and could be a result of low numbers. In study III we did actually see an increase in digestive tract malignancies for ES survivors, although insignificant and concealed in the overall analysis by OS survivors who showed a lower risk. Even in the BCCSS, there was an insignificantly higher risk among bone sarcoma survivors that might increase further as more patients reach the age in which this cancer type is more common. However, we can conclude that digestive tract malignancies did not constitute a major contributor to the excess cancer risk observed in study III or in the BCCSS.

The pattern of SPNs also indicates that there are different etiologies driving the excess cancer risk for OS and ES survivors. ES survivors displayed an elevated risk for the development of all solid tumors except for CNS and skin cancer. OS survivors on the other hand did not show elevated risks for genitourinary and digestive tract malignancies, but the risk was elevated for other solid tumors. One could expect that the skin cancer risk would be elevated among ES survivors if RT was a main risk contributor, otherwise the risk for solid tumors according to these findings indicates that RT plays a major role among ES survivors, while a genetic susceptibility dominates the enhanced risk observed among OS patients. The hematologic malignancies, which were elevated among OS and ES survivors, tended to occur early in the follow-up period, and were most likely caused by the chemotherapeutic agents that are used in treatment of both ES and OS. Indeed, ES patients appeared to be at a higher risk for hematologic malignancies than OS patients.

The significant contribution of female genital malignancies to the excess cancer risk observed among ES and OS survivors is a novel finding that needs to be confirmed. Together with breast sarcoma, we found the two malignancies contributing to nearly 60% of all absolute excess risk among ES survivors. Germline mutations in the BRCA1 or BRCA2 genes are typically seen among subsets of breast and ovarian cancer patients; however, tumor mutations in BRCA pathways are seen in a variety of other malignancies. Our finding raised the theory of a BRCA like phenotype existing among a subset of ES or OS patients. The literature investigating BRCA traits among bone sarcoma survivors was nonetheless scarce[89,90].

The absolute excess risk for cancer seen among bone sarcoma survivors is relatively small and deserves to be put into critical perspective. Even though the risk for a subsequent sarcoma (bone or soft tissue) to occur in a patient surviving ES is 115 time that of the general population, the AER is actually just14 extra sarcomas / 10 000 person years. On the contrary, due to the high cancer rate in the adult general population, the excess risk for breast cancer

among ES survivors followed past 30 years after diagnosis amounts to nearly 130 / 10 000 years.

ES and OS survivors are not only at risk for secondary cancers, but also for a wide range of other serious medical conditions[91]. Much work is therefore being done into systematically following these children and young adults long after they have reached the adult world and are considered cured from their primary cancer. Surveilling for secondary cancers is one such important measure included in long-term follow-up clinics / late effect-clinics. In Sweden, such clinics are satisfactorily evolving, owing to the work done by the six regional cancer centers (RCC) in collaboration with the Swedish working group for follow-up after pediatric cancer (SALUB). There are existing guidelines for caregivers, not only working in the field of oncology, which is important as treatment-related morbidity involves a broad range of organs. Evidence for screening after childhood cancer is perhaps best described for survivors of lymphoma. Bone sarcoma survivors are a group of patients who also may benefit from similar screening guidelines[92].

The aims in study IV were based on the results of study I, II and III. The lack of treatment data in study III warranted a new study with a cohort where detailed treatment information was available. Morbidity and treatment-related mortality were also of interest as these parameters were lacking in study III. Initially, the idea was to classify all complications into surgical, radiation-related or as a result of chemotherapy. However, it proved very difficult to ascertain which complication or type of reoperation was related to which treatment. Many times, it was a combination of different treatments. Therefore, we decided to use late hospital admittance/hospital stay as an indicator of morbidity. Cause of mortality was also recorded so that mortality due to subsequently occurring cancers or other serious side-effects such as heart failure would reflect the severity of treatment-related complications.

Also, due to the relatively low numbers in study I and II, another aim in study IV was to evaluate whether choice of local treatment and surgical margin had an effect on local failure. Local failure is a better outcome in evaluating local treatment than disease-free survival or overall survival, but it requires a larger cohort due to the relatively low local failure rates, which varied from 5% to 20% in study I and II. The Swedish child cancer registry satisfied those criteria since it encompassed good data on treatment and follow-up.

One of the main findings in study IV, that radiotherapy improves local control in patients with marginal resection status, was a confirmation of what was anticipated in study I. In study I, the beneficial role of surgery + RT was only demonstrated in the overall-survival analysis. One could argue that this could be a result of selection bias. Depicting the same positive effect of RT in a local recurrence analysis, which is far less likely to be a result of selection bias, confirms the importance of RT in marginally resected tumors. Also concluded from study IV; local control was not improved by adding radiation treatment to patients with a wide margin. The same observation was made in study I, although only in the overall survival analysis. Why patients with an intralesional margin did not benefit from RT is speculative; patients with intralesional margin had poorer overall survival than patients with wide margin.

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They also had more distant relapses than patients with marginal margin. Perhaps, patients with intralesional margin after surgery had fewer local recurrences due to death as a competing risk. Moreover, there may be an underreporting of local relapses among patients suffering synchronous distant and local relapses. Even though there are difficulties associated with defining a marginal respective wide or even intralesional margin, study I and IV clearly show the benefit of discriminating marginal from wide margins as compared with an R0/R1 description.

Even though most would advocate surgical treatment over RT, there is still a question to whether definitive RT is inferior to surgery in achieving local control[29,32,93-96]. In one of the cooperative Ewing sarcoma studies (CESS), a better local control was seen for surgically treated patients compared with patients treated with definitive RT, but no difference was seen in distant relapse, thereby questioning the significance of local control[97]. A very interesting report was made on the EICESS randomized trial undertaken as a collaborative approach by the German Paediatric Oncology and Haematology Group (GPOH) and the Children's Cancer Leukemia Group (CCLG) of UK. The aim was to investigate chemotherapy options for ES. They found an over 60% survival for the entire group at 5 years, but this concealed a14% inferior survival rate for patients in the CCLG cohort. Given the baseline characteristics and systemic treatment were the same, this was peculiar. The analysis revealed that the difference in overall survival was attributed to an inferior local control in the British cohort where local treatment was clearly less aggressive. The CPOH cohort was more often (66% vs 24%) treated with combined modalities (surgery+RT) and less often with definitive RT. GPOH patients also received local therapy earlier; 43% and 9% in the GPOH and the CCLG group respectively, received local treatment (generally pre-operative RT) within 12 weeks after start of induction chemotherapy[46].

The results from study I, II and IV support the importance of achieving local control. Sacral ES excluded; study I showed a strong trend for a better disease-free survival for surgery or surgery+RT compared with RT alone. In study IV, better local control was achieved with surgery or combination treatment than with definitive RT. The key message from these studies and the latter EICESS study is that local control is pivotal, and aggressive local treatment, with combined surgery and RT if a wide margin is not achieved, is essential for survival.

There were few late deaths (only two) in study IV due to other reasons than relapse of the primary disease. The conclusion drawn from this study is that much longer follow-up is needed to assess mortality related to primary treatment. Another point made is that disease relapse is the major cause of mortality for much longer than 5 years after diagnosis. Therefore, a larger cohort with more patients surviving past at least 20 years of follow-up is required to investigate mortality caused by treatment.

The same conclusion applies to the analysis of secondary malignancies in relation to local treatment. Typical treatment associated SPNs, such as an acute leukemia and a radiation-related osteosarcoma, developed 5 years after diagnosis. Nonetheless, a much longer follow-

up, or a larger cohort would have been necessary to assess the treatment related risk for SPNs.

Almost half of the patients surviving past 5 years, and without a disease relapse, were admitted to hospital and hospitalized for a median of almost 9 days. There seemed to be an over representation of surgically treated patients subject to late (+ 5 years) admission to hospital, but this was not significant. Charts were reviewed to investigate the cause of admission, but the causes varied significantly regarding severity and type ranging from reconstruction of surgical scars to organ transplantation. Typical causes were deformity surgery due to leg length discrepancies, amputations, neurologic sequelae, pathological fractures, late infections after surgery, renal and cardiac failures etc. As stated earlier, categorizing complications in relation to treatment is challenging because there is no clear definition of what is regarded as a serious complication and what is a more expected reason for admission, for example revision of implant due to mechanical wear. The study showed that many patients were admitted to the hospital long after end of treatment, but worth noting was that the majority were not admitted to hospital after the first 5 years of follow-up. Hospital admission was also studied by the BCCSS group which showed that bone sarcoma survivors were twice as likely as the general population to be hospitalized as an inpatient than the general population. No difference in the need for late hospitalization was seen between OS and ES patients[80].

6 FUTURE PERSPECTIVES

One lesson learned during the work in this thesis, obvious to the epidemiologist, is that it is important to have a good research question, but without enough power to detect a difference, the question cannot be answered. A struggle with power was obvious throughout most of the studies in this thesis. However, the author was in good company as lack of power was evident across most scientific literature on the topics of this thesis. Therefore, results drawn in many studies were often uncontrolled or made upon biased comparisons, subject to type I and II errors or over-interpretation of the effect of a certain exposure. Another phenomenon, commonly observed in large scale studies on childhood cancer survivors, was that OS and ES patients were pooled together and presented as "bone sarcoma". This thesis and other studies show that OS and ES are two very different entities with different inherent risks and risk patterns for secondary malignancies. The cost of presenting the two different bone tumors as one entity in order to gain power is that key results may be concealed.

Given the rare nature of the disease, it would have been beneficial if the author would have used the available registries in all of the Nordic countries. Good registries with similar variables exist throughout our countries, and a collaborative effort would have yielded a better result.

It´s clear that a significant number of bone sarcoma survivors face serious long-term complications. An ensuing goal would be to continue the work started in study IV; to identify the modifiable treatment related late-affects. Furthermore, retrospective and prospective

studies investigating patient related outcomes and functional outcomes in relation to local treatment should be within reach. In the Nordic countries, where population-based registries are similar and well administered, it should be feasible to agree on a way of recording and classifying complications and late-affects so that they can be properly addressed. Much good work has already been done through SALUB, which administer a registry recording late complications after treatment for childhood cancer. The Adult Life after Childhood Cancer in Scandinavia study (ALiCCS) is another promising large-scale collaborative study that will give important information on late effects. Collaborating with these research groups would be a natural next step for the author.

There is much work yet to be done in delineating the risks associated with administering radiotherapy and chemotherapy for ES. A next study would preferably be a case control study, in which patients with SPNs in the Nordic countries would be analyzed through national registries and compared with primary ES and OS patients not developing SPNs. Exposures at interest would be RT and chemotherapy. The same could be done for other complications examining surgery as an exposure.

A low hanging fruit would be to investigate the role of early or pre-operatively administered RT. It is an approach that has been advocated in the Nordic countries and never been evaluated. The results from the GPOH cohort show that the early RT treatment approach may be beneficial with regards to local control.

However, retrospective studies on local control and late effects related to traditional photon or electron RT may have less relevance in the future as emerging treatments such as proton or carbon ion treatment are being used more often in the treatment of ES. Such treatments should be considered in the coming clinical multinational trials.

Another future perspective would be to include translational research into epidemiological studies. If tissue samples would have been available for study I, a difference in survival with regards to site could have been investigated simultaneously on a basic biology level. The same applies for the theory about BRCA like traits existing in OS and ES patients raised in study III. Consequently, the lesson learned is that the work up prior to starting a study is essential and significant effort should be done to engage basic scientists early so that the clinical data can be used on a molecular level. To promote translational research for a common disease, like for example breast cancer, is perhaps less of a problem as most basic researchers have access to tumor tissue. The lack of improvements in outcome over the last 30 years for ES emphasizes the need to share tissue and clinical data for this rare disease.

Perhaps reaching out to potential partners working with other rare cancers would be a way to achieve results faster. It may solve many problems concerned with funding, regulatory bodies and with the industry. It may also enable ES patients to participate in studies which are not restricted to specific cancer types. The INFORM study is one such registry-based study where tumor tissue is harvested aiming to offer individualized treatment for children with recurrent disease regardless of primary type of malignancy.

The author would also like to see an accentuated focus on local treatment when planning for multinational clinical trials. Much attention in pan European studies like Euro Ewing has been exclusively on the timing and combination of different chemotherapy regimens. Questions regarding local treatment have been completely lacking and assigned to the individual treatment centers. An increased engagement by sarcoma surgeons in these clinical trials is longed for.

Low accrual in the multinational studies that have been undertaken in Europe is also a problem. This was evident in the Euro Ewing 2012 trial which closed in May 2019. It is also evident in the rEECur study conducted by the Euro Ewing consortium. The study investigating 4 different treatment arms (bio specimens are collected) for recurrent ES, was started in 2015 and has only accrued half of the 525 patients needed and until recently no patients from the authors' institution. The study has wide inclusion criteria and almost every patient with a recurrent ES in the country should be given the opportunity to participate. To improve accrual, national sarcoma centers, or even better Nordic collaborations like Scandinavian Sarcoma Group, should take a leading role. Low accrual in clinical trials is a waste of human and economic resources and most importantly it leads to a failure in improving clinical practice. Fortunately, there are hopes on the horizon. Initiatives to promote clinical research, as currently being done by the EU funded studies of the Euro Ewing consortium, are admirable and will answer important research questions.

7 CONCLUSIONS

Based on the papers in this thesis, the following conclusions can be drawn:

- Ewing sarcoma should be treated surgically if possible, if wide margins is not achieved, the addition of radiotherapy is crucial for local control.
- Administering radiotherapy in the setting of a wide surgical margin for Ewing sarcoma is not indicated, the role of radiotherapy for intralesionally resected tumors is unknown.
- Sacral site seems to be favorable prognostic factor compared with other pelvic sites.
- Good local control of sacrally located Ewing sarcoma may be achieved with definitive radiotherapy.
- Ewing sarcoma of the mobile spine is associated with a high local failure rate and surgery should be the preferred treatment of choice even in this difficult anatomic location.
- The high local failure rate may be related to less aggressive treatment (definitive RT) or to emergency decompressive surgery being performed in an emergency setting.
- Neurologic recovery is excellent for the vast majority of patients presenting with neurologic symptoms, perhaps owing to the prompt response to chemotherapy more than to spinal decompression.
- The excess risk for subsequent primary neoplasms among osteosarcoma and Ewing sarcoma survivors has different etiologies and is driven by different cancer types

although breast cancer and female genital malignancies are the main drivers of excess risk for osteosarcoma as well as Ewing sarcoma survivors.

- The cancer risk remains elevated compared with the general population past 30 years of follow-up and for patients treated in the latest treatment era. This indicates the need for prolonged cancer surveillence in this patient group
- The risk for SPNs in Ewing sarcoma survivors are moderately elavated and should not preclude the use of radiotherapy in a situation of marginal surgical margin.

8 SAMMANFATTNING PÅ SVENSKA

Ewing sarkom (ES) är en sällsynt och aggressiv malign tumör som drabbar främst barn och unga vuxna. Sjukdomen drabbar årligen ca 2.9 per million barn. Ewing sarkom är i pediatriska populationer det näst vanligaste skelettsarkomet efter osteosarkom och utgör tillsammans med osteosarkom 5% av alla maligniteter hos barn och ungdomar[8,14]. Multimodal behandling med kombinationscytostatika, kirurgi och strålbehandling är avgörande för prognosen. Överlevnaden över 5 år har dock stagnerat på mellan 60% och 70% sedan 30 år tillbaka[12,26,39,98-100]. Stora multinationella studier i syfte att optimera den bästa kombinationen och timingen av cytostatika har varit en besvikelse och har inte förbättrat överlevnaden för den 1/5 av patienterna som presenterar med metastatisk sjukdom[14]. Det ligger därför stora utmaningar i att förbättra den systemiska behandlingen av ES.

Denna avhandling handlar dock om lokalbehandling av Ewing sarkom och den har två delar. Den första delen handlar om lokal behandling och lokal tumörkontroll i förhållande till kirurgisk marginal och anatomisk lokalisation. Den andra delen handlar om sena komplikationer hos långtidsöverlevare och främst risken att drabbas av sekundära maligniteter.

Lokal behandling utgör närmast alltid ett diskussionsämne på multidisciplinära sarkomkonferenser. Orsaken är att många tumörer (35% till 45%) uppstår i eller i anslutning till axiala skelettet, dvs anatomiska områden som ligger centralt. Ur en kirurgisk synvinkel, är lokalisationen problematisk då kirurgisk excision med sedvanliga krav på kirurgisk marginal skulle leda till betydande funktionsnedsättningar. Strålbehandling, vilket historiskt sett haft en viktig roll i behandlingen av ES, är också behäftad med svårigheter när tumören är lokaliserad till axiala skelettet. Det kan vara svårt att komma upp i terapeutisk stråldos, och att uppnå önskat strålfält på grund av tumörens närhet till vitala strukturer. Strålbehandlingens negativa effekter på skelettet utgör också ett signifikant problem då den kan leda till betydande tillväxtstörningar och strålrelaterade frakturer. Dessutom utgör den ökade risken för strålrelaterade sekundära maligniteter ett uttalat bekymmer som allt mer

framkommer i diskussion. Kontrovers föreligger dessutom avseende hur stor betydelse valet av lokalbehandling har för lokal tumörkontroll och för överlevnaden.

Målet med de fyra arbetena var att studera följande: vilken lokalbehandling som ger bäst lokal tumörkontroll för ES lokaliserad till bäckenet (arbete I); hur stor del av patienter med ES i kotpelaren som har neurologiska symptom vid diagnos, och vilken betydelse neurologiska symptom har för lokal tumörkontroll (arbete II); vad risken är för sekundära maligniteter (SPN) bland överlevare efter behandling för ES och OS, och vilken typ av malignitet driver riskökningen (arbete III); vilken betydelse kirurgisk marginal har för lokal tumörkontroll och vilken betydelse lokalt återfall har för överlevnad (arbete I och IV).

ES utgående från sakrum är särskilt utmanande. I arbete I studerades en kohort bestående av 117 patienter med bäcken ES, Där kunde vi visa att sakrala ES till stor del behandlas med enbart strålbehandling (definitiv strålbehandling) med goda resultat mätt i lokal tumörkontroll, sjukdomsfri överlevnad och total överlevnad. Vi noterade att sakral lokalisation i bäckenskelettet var en oberoende prognostiskt gynnsam faktor jämfört med övriga bäckenben (os ilium, os ischium och os pubis). Sakral lokalisation var förknippad med bättre sjukdomsfri överlevnad trots att en hög andel (41%) av patienterna med sakrala ES hade metastaser vid diagnos. Kirurgisk behandling tenderade dock att ge en bättre sjukdomsfri överlevnad för patienter med tumörer som inte var lokaliserad till sakrum. Slutligen kunde vi påvisa att postoperativ strålbehandling ledde till signifikant bättre överlevnad vid kirurgi med marginell eller intralesionell kirurgisk marginal.

Sakrum är en del av kotpelaren och har följjaktligen fler anatomiska likheter med övriga kotpelaren än med bäckenbenen. I arbete II undersöktes huruvida ES lokaliserad till kotpelaren hade motsvarande god prognos som observerats för ES i sakrum i arbete I. Vi undersökte också förekomsten av neurologiska symptom vid diagnos, hur de neurologiska symptomen behandlades och vilken effekt behandlingen hade på lokalt återfall. Studien som utgjordes av 24 patienter med tumörer i kotpelaren, visade att denna patientgrupp hade en relativt god prognos med en sjukdomsfri överlevnad i mellanskiktet jämfört med patienter med ES lokaliserad till sakrum och övriga bäckenet. Dock noterades en hög frekvens av lokala recidiv bland patienterna, vilka till stor del behandlades med definitiv strålbehandling framför kirurgi. Många patienter hade neurologiska symptom vid diagnos och genomgick därför akut dekompression av ryggmärgen/nervrötter innan diagnos. De allra flesta patienterna återhämtade sig dock neurologiskt oberoende av huruvida de genomgick akut dekompression eller inte. Akut dekompression tenderade att vara kopplat till högre risk för lokalt återfall. Lägre risk för lokalt återfall kunde kopplas till den senaste behandlingseran, vilket kan indikera att förbättrad systemisk behandling var orsaken till förbättrad lokal kontroll.

Arbete III genomfördes för att undersöka risken för sekundära maligniteter bland överlevare efter behandling för Ewing- och osteosarkom i en nationell populationsbaserad kohort. Studien, som baserades på 1779 OS och ES patienter, visade att risken för sekundära maligniteter var dubbel så hög för ES- som för OS- patienter och 4 gånger högre än för

normalbefolkningen. ES och OS överlevare drabbades av olika typer av sekundära maligniteter, men för båda grupperna drevs riskökningen till stor del av bröstcancer och gynekologiska maligniteter. Kombinationen av den höga riskökningen för bröstcancer och gynekologiska maligniteter kan indikera att BRCA liknande fenotyper förekommer hos en del av patienter med skelettsarkom. Den ökade cancerrisken kvarstår efter 30 års uppföljning samt även för patienter som behandlats under den senaste behandlingseran.

I arbete IV undersöktes en populationsbaserad pediatrisk ES kohort bestående av 229 patienter ur Svenska Barncancerregistret. Kirurgi, kirurgisk marginal och strålbehandling utvärderades i förhållande till lokal tumörkontroll. Vi studerade även lokalbehandlingens sena effekter på sekundära maligniteter och behov av slutenvård. Kirurgisk excision gav betydligt bättre lokal kontroll och var associerad med bättre överlevnad än enbart strålbehandling. Bäst lokalkontroll observerades vid kirurgisk excision med vida marginaler. Strålbehandling förbättrade inte lokal kontroll vid kirurgi med vida marginaler. Strålbehandling spelade dock en viktig roll för att åstadkomma lokal tumörkontroll vid kirurgi med marginell marginal. Sena, icke återfalls relaterade komplikationer vilka resulterade i sjukhusinläggning efter 5 års uppföljning, var ungefär lika vanligt förekommande oavsett typ av lokalbehandling.

Sammanfattningsvis utgör anatomisk lokalisation en viktig prognostisk faktor för patienter med Ewing sarkom. Kirurgisk marginal med vida marginaler ger i regel överlägsen lokal tumörkontroll jämfört med definitiv strålbehandling. Adjuvant strålbehandling i samband med kirurgi med marginell marginal ger dock lika god lokal tumörkontroll. Definitiv strålbehandling förefaller ge adekvat lokal tumörkontroll enbart vid ES i sakrum.

Patienter som överlever ES eller OS har en måttligt ökat risk för drabbas av sekundära maligniteter. Riskökningen drivs till största delen av bröstcancer och gynekologiska maligniteter, men skiljer sig i övrigt åt mellan ES och OS vilket indikerar ett behov av skräddarsydd långvarig uppföljning. Risken för strålrelaterade komplikationer så som sekundär cancer får dock inte överskugga fördelarna med att ge strålbehandling för att förhindra lokalt återfall, då utfallet är dystert vid lokalt återfall i ES.

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10 REFERENCES

1. Ewing J. Classics in oncology. Diffuse endothelioma of bone. James Ewing. Proceedings of the New York Pathological Society, 1921. *CA: a cancer journal for clinicians* 1972; **22**(2): 95-98.

2. Doyle LA. Sarcoma classification: An update based on the 2013 World Health Organization Classification of Tumors of Soft Tissue and Bone. *Cancer* 2014; **120**(12): 1763-1774; doi 10.1002/cncr.28657.

3. Nesbit ME. Ewing's sarcoma. *CA: a cancer journal for clinicians* 1976; **26**(3): 174-180; doi 10.3322/canjclin.26.3.174.

4. Rodriguez-Galindo C, Navid F, Liu T, Billups CA, Rao BN, Krasin MJ. Prognostic factors for local and distant control in Ewing sarcoma family of tumors. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2008; **19**(4): 814-820; doi 10.1093/annonc/mdm521.

5. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD *et al.* Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. *European journal of cancer* 2013; **49**(3): 684-695; doi 10.1016/j.ejca.2012.09.011.

6. Gatta G, Capocaccia R, Stiller C, Kaatsch P, Berrino F, Terenziani M *et al.* Childhood cancer survival trends in Europe: a EUROCARE Working Group study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005; **23**(16): 3742-3751; doi 10.1200/JCO.2005.00.554.

7. Zahm SH, Fraumeni JF, Jr. The epidemiology of soft tissue sarcoma. *Seminars in oncology* 1997; **24**(5): 504-514.

8. Esiashvili N, Goodman M, Marcus RB, Jr. Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: Surveillance Epidemiology and End Results data. *Journal of pediatric hematology/oncology* 2008; **30**(6): 425-430; doi 10.1097/MPH.0b013e31816e22f3.

9. Ginsberg JP, Goodman P, Leisenring W, Ness KK, Meyers PA, Wolden SL *et al.* Long-term survivors of childhood Ewing sarcoma: report from the childhood cancer survivor study. *Journal of the National Cancer Institute* 2010; **102**(16): 1272-1283; doi 10.1093/jnci/djq278.

10. Worch J, Matthay KK, Neuhaus J, Goldsby R, DuBois SG. Ethnic and racial differences in patients with Ewing sarcoma. *Cancer* 2010; **116**(4): 983-988; doi 10.1002/cncr.24865.

11. Grier HE. The Ewing family of tumors. Ewing's sarcoma and primitive neuroectodermal tumors. *Pediatric clinics of North America* 1997; **44**(4): 991-1004.

12. Grier HE, Krailo MD, Tarbell NJ, Link MP, Fryer CJ, Pritchard DJ *et al.* Addition of ifosfamide and etoposide to standard chemotherapy for Ewing's sarcoma and primitive neuroectodermal tumor of bone. *The New England journal of medicine* 2003; **348**(8): 694-701; doi 10.1056/NEJMoa020890.

13. Cotterill SJ, Ahrens S, Paulussen M, Jurgens HF, Voute PA, Gadner H *et al.* Prognostic factors in Ewing's tumor of bone: analysis of 975 patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2000; **18**(17): 3108-3114.

14. Balamuth NJ, Womer RB. Ewing's sarcoma. *The Lancet Oncology* 2010; **11**(2): 184-192; doi 10.1016/S1470-2045(09)70286-4.

15. Hesla AC, Discacciati A, Tsagkozis P, Smedby KE. Subsequent primary neoplasms among bone sarcoma survivors; increased risks remain after 30 years of follow-up and in the latest treatment era, a nationwide population-based study. *British journal of cancer* 2020; doi 10.1038/s41416-020-0748-3.

16. Widhe B, Widhe T. Initial Symptoms and Clinical Features in Osteosarcoma and Ewing Sarcoma*. *The Journal of Bone & Joint Surgery* 2000; **82**(5): 667-667.

17. Potratz J, Dirksen U, Jurgens H, Craft A. Ewing sarcoma: clinical state-of-the-art. *Pediatric hematology and oncology* 2012; **29**(1): 1-11; doi 10.3109/08880018.2011.622034.

18. Rosen G, Wollner N, Tan C, Wu SJ, Hajdu SI, Cham W *et al.* Proceedings: Disease-free survival in children with Ewing's sarcoma treated with radiation therapy and adjuvant four-drug sequential chemotherapy. *Cancer* 1974; **33**(2): 384-393.

19. Leavey PJ, Mascarenhas L, Marina N, Chen Z, Krailo M, Miser J *et al.* Prognostic factors for patients with Ewing sarcoma (EWS) at first recurrence following multi-modality therapy: A report from the Children's Oncology Group. *Pediatric blood & cancer* 2008; **51**(3): 334-338; doi 10.1002/pbc.21618.

20. Bacci G, Ferrari S, Longhi A, Donati D, De Paolis M, Forni C *et al.* Therapy and survival after recurrence of Ewing's tumors: the Rizzoli experience in 195 patients treated with adjuvant and neoadjuvant chemotherapy from 1979 to 1997. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2003; **14**(11): 1654-1659.

21. Ladenstein R, Hartmann O, Pinkerton CR. The role of megatherapy with autologous bone marrow rescue in solid tumours of childhood. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 1993; **4 Suppl 1**: 45-58.

22. Meyers PA, Krailo MD, Ladanyi M, Chan KW, Sailer SL, Dickman PS *et al.* High-dose melphalan, etoposide, total-body irradiation, and autologous stem-cell reconstitution as consolidation therapy for high-risk Ewing's sarcoma does not improve prognosis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2001; **19**(11): 2812-2820.

23. Ferrari S, Sundby Hall K, Luksch R, Tienghi A, Wiebe T, Fagioli F *et al.* Nonmetastatic Ewing family tumors: high-dose chemotherapy with stem cell rescue in poor responder patients. Results of the Italian Sarcoma Group/Scandinavian Sarcoma Group III protocol. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2011; **22**(5): 1221-1227; doi 10.1093/annonc/mdq573.

24. Donati D, Yin J, Di Bella C, Colangeli M, Bacci G, Ferrari S *et al.* Local and distant control in non-metastatic pelvic Ewing's sarcoma patients. *Journal of surgical oncology* 2007; **96**(1): 19-25; doi 10.1002/jso.20752.

25. Donaldson SS, Torrey M, Link MP, Glicksman A, Gilula L, Laurie F *et al.* A multidisciplinary study investigating radiotherapy in Ewing's sarcoma: end results of POG #8346. Pediatric Oncology Group. *International journal of radiation oncology, biology, physics* 1998; **42**(1): 125-135.

26. Granowetter L, Womer R, Devidas M, Krailo M, Wang C, Bernstein M *et al.* Dose-intensified compared with standard chemotherapy for nonmetastatic Ewing sarcoma family of tumors: a Children's Oncology Group Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009; **27**(15): 2536-2541; doi 10.1200/JCO.2008.19.1478.

27. Krasin MJ, Rodriguez-Galindo C, Davidoff AM, Billups CA, Fuller CE, Neel MD *et al.* Efficacy of combined surgery and irradiation for localized Ewings sarcoma family of tumors. *Pediatric blood & cancer* 2004; **43**(3): 229-236; doi 10.1002/pbc.20095.

28. Craft AW, Cotterill SJ, Bullimore JA, Pearson D. Long-term results from the first UKCCSG Ewing's Tumour Study (ET-1). United Kingdom Children's Cancer Study Group (UKCCSG) and the Medical Research Council Bone Sarcoma Working Party. *European journal of cancer* 1997; **33**(7): 1061-1069.

29. DuBois SG, Krailo MD, Gebhardt MC, Donaldson SS, Marcus KJ, Dormans J *et al.* Comparative evaluation of local control strategies in localized Ewing sarcoma of bone: a report from the Children's Oncology Group. *Cancer* 2015; **121**(3): 467-475; doi 10.1002/cncr.29065.

30. Schuck A, Rube C, Konemann S, Rube CE, Ahrens S, Paulussen M *et al.* Postoperative radiotherapy in the treatment of Ewing tumors: influence of the interval between surgery and radiotherapy. *Strahlentherapie und Onkologie : Organ der Deutschen Rontgengesellschaft [et al]* 2002; **178**(1): 25-31.

31. Schuck A, Ahrens S, Paulussen M, Kuhlen M, Konemann S, Rube C *et al.* Local therapy in localized Ewing tumors: results of 1058 patients treated in the CESS 81, CESS 86, and EICESS 92 trials. *International journal of radiation oncology, biology, physics* 2003; **55**(1): 168-177.

32. Bacci G, Forni C, Longhi A, Ferrari S, Donati D, De Paolis M *et al.* Long-term outcome for patients with non-metastatic Ewing's sarcoma treated with adjuvant and neoadjuvant chemotherapies. 402 patients treated at Rizzoli between 1972 and 1992. *European journal of cancer* 2004; **40**(1): 73-83.

33. Gaspar N, Hawkins DS, Dirksen U, Lewis IJ, Ferrari S, Le Deley MC *et al.* Ewing Sarcoma: Current Management and Future Approaches Through Collaboration. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015; **33**(27): 3036-3046; doi 10.1200/JCO.2014.59.5256.

34. SBCR. http://cceg.ki.se/sbcr/.

35. Socialstyrelsen. http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer.

36. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. *Acta oncologica* 2009; **48**(1): 27-33; doi 10.1080/02841860802247664.

37. Register TSP. https://www.socialstyrelsen.se/en/statistics-and-data/registers/register-information/the-national-patient-register/.

38. Register TSDC. https://www.socialstyrelsen.se/statistik-och-data/register/alla-register/dodsorsaksregistret/.

39.   Elomaa I, Blomqvist C, Saeter G, Nilbert M, Monge OR, Wiebe T *et al.* Chemotherapy in Ewing's sarcoma. The Scandinavian Sarcoma Group experience. *Acta orthopaedica Scandinavica Supplementum* 1999; **285**: 69-73.

40.   Friedman DL, Whitton J, Leisenring W, Mertens AC, Hammond S, Stovall M *et al.* Subsequent neoplasms in 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. *Journal of the National Cancer Institute* 2010; **102**(14): 1083-1095; doi 10.1093/jnci/djq238.

41.   Henderson TO, Whitton J, Stovall M, Mertens AC, Mitby P, Friedman D *et al.* Secondary sarcomas in childhood cancer survivors: a report from the Childhood Cancer Survivor Study. *Journal of the National Cancer Institute* 2007; **99**(4): 300-308; doi 10.1093/jnci/djk052.

42.   Dyson KA, Stover BD, Grippin A, Mendez-Gomez HR, Lagmay J, Mitchell DA *et al.* Emerging trends in immunotherapy for pediatric sarcomas. *Journal of hematology & oncology* 2019; **12**(1): 78; doi 10.1186/s13045-019-0756-z.

43.   Indelicato DJ, Keole SR, Shahlaee AH, Shi W, Morris CG, Marcus RB, Jr. Definitive radiotherapy for ewing tumors of extremities and pelvis: long-term disease control, limb function, and treatment toxicity. *International journal of radiation oncology, biology, physics* 2008; **72**(3): 871-877; doi 10.1016/j.ijrobp.2008.02.023.

44.   Sailer SL, Harmon DC, Mankin HJ, Truman JT, Suit HD. Ewing's sarcoma: surgical resection as a prognostic factor. *International journal of radiation oncology, biology, physics* 1988; **15**(1): 43-52.

45.   La TH, Meyers PA, Wexler LH, Alektiar KM, Healey JH, Laquaglia MP *et al.* Radiation therapy for Ewing's sarcoma: results from Memorial Sloan-Kettering in the modern era. *International journal of radiation oncology, biology, physics* 2006; **64**(2): 544-550; doi 10.1016/j.ijrobp.2005.07.299.

46.   Whelan J, Hackshaw A, McTiernan A, Grimer R, Spooner D, Bate J *et al.* Survival is influenced by approaches to local treatment of Ewing sarcoma within an international randomised controlled trial: analysis of EICESS-92. *Clinical sarcoma research* 2018; **8**: 6; doi 10.1186/s13569-018-0093-y.

47.   Rodriguez-Galindo C, Billups CA, Kun LE, Rao BN, Pratt CB, Merchant TE *et al.* Survival after recurrence of Ewing tumors: the St Jude Children's Research Hospital experience, 1979-1999. *Cancer* 2002; **94**(2): 561-569; doi 10.1002/cncr.10192.

48.   Llombart-Bosch A, Contesso G, Henry-Amar M, Lacombe MJ, Oberlin O, Dubousset J *et al.* Histopathological predictive factors in Ewing's sarcoma of bone and clinicopathological correlations. A retrospective study of 261 cases. *Virchows Archiv A, Pathological anatomy and histopathology* 1986; **409**(5): 627-640; doi 10.1007/bf00713429.

49.   Huang HY, Illei PB, Zhao Z, Mazumdar M, Huvos AG, Healey JH *et al.* Ewing sarcomas with p53 mutation or p16/p14ARF homozygous deletion: a highly lethal subset associated with poor chemoresponse. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005; **23**(3): 548-558; doi 10.1200/JCO.2005.02.081.

50.   Tsuchiya T, Sekine K, Hinohara S, Namiki T, Nobori T, Kaneko Y. Analysis of the p16INK4, p14ARF, p15, TP53, and MDM2 genes and their prognostic implications in osteosarcoma and Ewing sarcoma. *Cancer genetics and cytogenetics* 2000; **120**(2): 91-98.

51.   de Alava E, Antonescu CR, Panizo A, Leung D, Meyers PA, Huvos AG *et al.* Prognostic impact of P53 status in Ewing sarcoma. *Cancer* 2000; **89**(4): 783-792.

52.   Wei G, Antonescu CR, de Alava E, Leung D, Huvos AG, Meyers PA *et al.* Prognostic impact of INK4A deletion in Ewing sarcoma. *Cancer* 2000; **89**(4): 793-799.

53.   Schuck A, Ahrens S, von Schorlemer I, Kuhlen M, Paulussen M, Hunold A *et al.* Radiotherapy in Ewing tumors of the vertebrae: treatment results and local relapse analysis of the CESS 81/86 and EICESS 92 trials. *International journal of radiation oncology, biology, physics* 2005; **63**(5): 1562-1567; doi 10.1016/j.ijrobp.2005.05.036.

54.   Indelicato DJ, Keole SR, Shahlaee AH, Morris CG, Gibbs CP, Jr., Scarborough MT *et al.* Spinal and paraspinal Ewing tumors. *International journal of radiation oncology, biology, physics* 2010; **76**(5): 1463-1471; doi 10.1016/j.ijrobp.2009.03.042.

55.   Ilaslan H, Sundaram M, Unni KK, Dekutoski MB. Primary Ewing's sarcoma of the vertebral column. *Skeletal radiology* 2004; **33**(9): 506-513; doi 10.1007/s00256-004-0810-x.

56.   Grubb MR, Currier BL, Pritchard DJ, Ebersold MJ. Primary Ewing's sarcoma of the spine. *Spine* 1994; **19**(3): 309-313.

57.   Venkateswaran L, Rodriguez-Galindo C, Merchant TE, Poquette CA, Rao BN, Pappo AS. Primary Ewing tumor of the vertebrae: clinical characteristics, prognostic factors, and outcome. *Medical and pediatric oncology* 2001; **37**(1): 30-35; doi 10.1002/mpo.1159.

58.   Pilepich MV, Vietti TJ, Nesbit ME, Tefft M, Kissane J, Burgert O *et al.* Ewing's sarcoma of the vertebral column. *International journal of radiation oncology, biology, physics* 1981; **7**(1): 27-31.

59.   Bacci G, Boriani S, Balladelli A, Barbieri E, Longhi A, Alberghini M *et al.* Treatment of nonmetastatic Ewing's sarcoma family tumors of the spine and sacrum: the experience from a single institution. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society* 2009; **18**(8): 1091-1095; doi 10.1007/s00586-009-0921-0.

60.   Marco RA, Gentry JB, Rhines LD, Lewis VO, Wolinski JP, Jaffe N *et al.* Ewing's sarcoma of the mobile spine. *Spine* 2005; **30**(7): 769-773.

61.   Vogin G, Helfre S, Glorion C, Mosseri V, Mascard E, Oberlin O *et al.* Local control and sequelae in localised Ewing tumours of the spine: a French retrospective study. *European journal of cancer* 2013; **49**(6): 1314-1323; doi 10.1016/j.ejca.2012.12.005.

62.   Charest-Morin R, Dirks MS, Patel S, Boriani S, Luzzati A, Fehlings MG *et al.* Ewing Sarcoma of the Spine: Prognostic Variables for Survival and Local Control in Surgically Treated Patients. *Spine* 2018; **43**(9): 622-629; doi 10.1097/BRS.0000000000002386.

63.   Tomita K, Kawahara N, Baba H, Tsuchiya H, Fujita T, Toribatake Y. Total en bloc spondylectomy. A new surgical technique for primary malignant vertebral tumors. *Spine* 1997; **22**(3): 324-333.

64.   Hisada M, Garber JE, Fung CY, Fraumeni JF, Jr., Li FP. Multiple primary cancers in families with Li-Fraumeni syndrome. *Journal of the National Cancer Institute* 1998; **90**(8): 606-611.

65. Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990; **250**(4985): 1233-1238.

66. McIntyre JF, Smith-Sorensen B, Friend SH, Kassell J, Borresen AL, Yan YX *et al.* Germline mutations of the p53 tumor suppressor gene in children with osteosarcoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1994; **12**(5): 925-930; doi 10.1200/JCO.1994.12.5.925.

67. Morton LM, Dores GM, Tucker MA, Kim CJ, Onel K, Gilbert ES *et al.* Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975-2008. *Blood* 2013; **121**(15): 2996-3004; doi 10.1182/blood-2012-08-448068.

68. Tucker MA, D'Angio GJ, Boice JD, Jr., Strong LC, Li FP, Stovall M *et al.* Bone sarcomas linked to radiotherapy and chemotherapy in children. *The New England journal of medicine* 1987; **317**(10): 588-593; doi 10.1056/NEJM198709033171002.

69. Hawkins MM, Wilson LM, Burton HS, Potok MH, Winter DL, Marsden HB *et al.* Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. *Journal of the National Cancer Institute* 1996; **88**(5): 270-278.

70. Xu Y, Wang H, Zhou S, Yu M, Wang X, Fu K *et al.* Risk of second malignant neoplasms after cyclophosphamide-based chemotherapy with or without radiotherapy for non-Hodgkin lymphoma. *Leukemia & lymphoma* 2013; **54**(7): 1396-1404; doi 10.3109/10428194.2012.743657.

71. Diamandidou E, Buzdar AU, Smith TL, Frye D, Witjaksono M, Hortobagyi GN. Treatment-related leukemia in breast cancer patients treated with fluorouracil-doxorubicin-cyclophosphamide combination adjuvant chemotherapy: the University of Texas M.D. Anderson Cancer Center experience. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1996; **14**(10): 2722-2730; doi 10.1200/JCO.1996.14.10.2722.

72. Travis LB, Holowaty EJ, Bergfeldt K, Lynch CF, Kohler BA, Wiklund T *et al.* Risk of leukemia after platinum-based chemotherapy for ovarian cancer. *The New England journal of medicine* 1999; **340**(5): 351-357; doi 10.1056/NEJM199902043400504.

73. Turcotte LM, Liu Q, Yasui Y, Arnold MA, Hammond S, Howell RM *et al.* Temporal Trends in Treatment and Subsequent Neoplasm Risk Among 5-Year Survivors of Childhood Cancer, 1970-2015. *Jama* 2017; **317**(8): 814-824; doi 10.1001/jama.2017.0693.

74. Dunst J, Ahrens S, Paulussen M, Rube C, Winkelmann W, Zoubek A *et al.* Second malignancies after treatment for Ewing's sarcoma: a report of the CESS-studies. *International journal of radiation oncology, biology, physics* 1998; **42**(2): 379-384.

75. Bacci G, Longhi A, Barbieri E, Ferrari S, Mercuri M, Briccoli A *et al.* Second malignancy in 597 patients with ewing sarcoma of bone treated at a single institution with adjuvant and neoadjuvant chemotherapy between 1972 and 1999. *Journal of pediatric hematology/oncology* 2005; **27**(10): 517-520.

76. McLean TW, Hertel C, Young ML, Marcus K, Schizer MA, Gebhardt M *et al.* Late events in pediatric patients with Ewing sarcoma/primitive neuroectodermal tumor of bone: the Dana-Farber Cancer Institute/Children's Hospital experience. *Journal of pediatric hematology/oncology* 1999; **21**(6): 486-493.

77. Sultan I, Rihani R, Hazin R, Rodriguez-Galindo C. Second malignancies in patients with Ewing Sarcoma Family of Tumors: A population-based study. *Acta oncologica* 2010; **49**(2): 237-244; doi 10.3109/02841860903253538.

78. Navid F, Billups C, Liu T, Krasin MJ, Rodriguez-Galindo C. Second cancers in patients with the Ewing sarcoma family of tumours. *European journal of cancer* 2008; **44**(7): 983-991; doi 10.1016/j.ejca.2008.02.027.

79. Kuttesch JF, Jr., Wexler LH, Marcus RB, Fairclough D, Weaver-McClure L, White M *et al.* Second malignancies after Ewing's sarcoma: radiation dose-dependency of secondary sarcomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1996; **14**(10): 2818-2825.

80. Fidler MM, Frobisher C, Guha J, Wong K, Kelly J, Winter DL *et al.* Long-term adverse outcomes in survivors of childhood bone sarcoma: the British Childhood Cancer Survivor Study. *British journal of cancer* 2015; **112**(12): 1857-1865; doi 10.1038/bjc.2015.159.

81. Inskip PD, Curtis RE. New malignancies following childhood cancer in the United States, 1973-2002. *International journal of cancer* 2007; **121**(10): 2233-2240; doi 10.1002/ijc.22827.

82. Goldsby R, Burke C, Nagarajan R, Zhou T, Chen Z, Marina N *et al.* Second solid malignancies among children, adolescents, and young adults diagnosed with malignant bone tumors after 1976: follow-up of a Children's Oncology Group cohort. *Cancer* 2008; **113**(9): 2597-2604; doi 10.1002/cncr.23860.

83. Ji J, Hemminki K. Incidence of multiple primary malignancies among patients with bone cancers in Sweden. *Journal of cancer research and clinical oncology* 2006; **132**(8): 529-535; doi 10.1007/s00432-006-0100-1.

84. Oberlin O, Deley MC, Bui BN, Gentet JC, Philip T, Terrier P *et al.* Prognostic factors in localized Ewing's tumours and peripheral neuroectodermal tumours: the third study of the French Society of Paediatric Oncology (EW88 study). *British journal of cancer* 2001; **85**(11): 1646-1654; doi 10.1054/bjoc.2001.2150.

85. Marina NM, Liu Q, Donaldson SS, Sklar CA, Armstrong GT, Oeffinger KC *et al.* Longitudinal follow-up of adult survivors of Ewing sarcoma: A report from the Childhood Cancer Survivor Study. *Cancer* 2017; **123**(13): 2551-2560; doi 10.1002/cncr.30627.

86. Arndt CA, Rose PS, Folpe AL, Laack NN. Common musculoskeletal tumors of childhood and adolescence. *Mayo Clinic proceedings* 2012; **87**(5): 475-487; doi 10.1016/j.mayocp.2012.01.015.

87. Arndt CA, Crist WM. Common musculoskeletal tumors of childhood and adolescence. *The New England journal of medicine* 1999; **341**(5): 342-352; doi 10.1056/NEJM199907293410507.

88. Reulen RC, Frobisher C, Winter DL, Kelly J, Lancashire ER, Stiller CA *et al.* Long-term risks of subsequent primary neoplasms among survivors of childhood cancer. *Jama* 2011; **305**(22): 2311-2319; doi 10.1001/jama.2011.747.

89. Kovac M, Blattmann C, Ribi S, Smida J, Mueller NS, Engert F *et al.* Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nature communications* 2015; **6**: 8940; doi 10.1038/ncomms9940.

90.   Lord CJ, Ashworth A. BRCAness revisited. *Nature reviews Cancer* 2016; **16**(2): 110-120; doi 10.1038/nrc.2015.21.

91.   Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT *et al.* Chronic health conditions in adult survivors of childhood cancer. *The New England journal of medicine* 2006; **355**(15): 1572-1582; doi 10.1056/NEJMsa060185.

92.   Holmqvist AS, Chen Y, Berano Teh J, Sun C, Birch JM, van den Bos C *et al.* Risk of solid subsequent malignant neoplasms after childhood Hodgkin lymphoma-Identification of high-risk populations to guide surveillance: A report from the Late Effects Study Group. *Cancer* 2019; **125**(8): 1373-1383; doi 10.1002/cncr.31807.

93.   Shankar AG, Pinkerton CR, Atra A, Ashley S, Lewis I, Spooner D *et al.* Local therapy and other factors influencing site of relapse in patients with localised Ewing's sarcoma. United Kingdom Children's Cancer Study Group (UKCCSG). *European journal of cancer* 1999; **35**(12): 1698-1704; doi 10.1016/s0959-8049(99)00144-6.

94.   Ahrens S, Hoffmann C, Jabar S, Braun-Munzinger G, Paulussen M, Dunst J *et al.* Evaluation of prognostic factors in a tumor volume-adapted treatment strategy for localized Ewing sarcoma of bone: the CESS 86 experience. Cooperative Ewing Sarcoma Study. *Medical and pediatric oncology* 1999; **32**(3): 186-195.

95.   Bacci G, Longhi A, Briccoli A, Bertoni F, Versari M, Picci P. The role of surgical margins in treatment of Ewing's sarcoma family tumors: experience of a single institution with 512 patients treated with adjuvant and neoadjuvant chemotherapy. *International journal of radiation oncology, biology, physics* 2006; **65**(3): 766-772; doi 10.1016/j.ijrobp.2006.01.019.

96.   Dunst J, Sauer R, Burgers JM, Hawliczek R, Kurten R, Winkelmann W *et al.* Radiation therapy as local treatment in Ewing's sarcoma. Results of the Cooperative Ewing's Sarcoma Studies CESS 81 and CESS 86. *Cancer* 1991; **67**(11): 2818-2825; doi 10.1002/1097-0142(19910601)67:11<2818::aid-cncr2820671118>3.0.co;2-y.

97.   Ozaki T, Hillmann A, Hoffmann C, Rube C, Blasius S, Dunst J *et al.* Significance of surgical margin on the prognosis of patients with Ewing's sarcoma. A report from the Cooperative Ewing's Sarcoma Study. *Cancer* 1996; **78**(4): 892-900; doi 10.1002/(SICI)1097-0142(19960815)78:4<892::AID-CNCR29>3.0.CO;2-P.

98.   Jurgens H, Bier V, Dunst J, Harms D, Jobke A, Kotz R *et al.* [The German Society of Pediatric Oncology Cooperative Ewing Sarcoma Studies CESS 81/86: report after 6 1/2 years]. *Klinische Padiatrie* 1988; **200**(3): 243-252; doi 10.1055/s-2008-1033716.

99.   Bacci G, Ferrari S, Bertoni F, Rimondini S, Longhi A, Bacchini P *et al.* Prognostic factors in nonmetastatic Ewing's sarcoma of bone treated with adjuvant chemotherapy: analysis of 359 patients at the Istituto Ortopedico Rizzoli. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2000; **18**(1): 4-11; doi 10.1200/JCO.2000.18.1.4.

100.   Womer RB, West DC, Krailo MD, Dickman PS, Pawel BR, Grier HE *et al.* Randomized controlled trial of interval-compressed chemotherapy for the treatment of localized Ewing sarcoma: a report from the Children's Oncology Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2012; **30**(33): 4148-4154; doi 10.1200/JCO.2011.41.5703.