Local Control of Ewing Sarcoma/peripheral Primitive Neuroectodermal Tumour using multimodality treatment approach in children and adolescents in Johannesburg: A retrospective review

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DECLARATION

I, Ncumisa Ndamase, declare that this thesis is my own work. It is being submitted for the degree of Master of Medicine in Radiation Oncology at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other university.

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Dr. Ncumisa Ndamase

20th day of February 2023 in Johannesburg

DEDICATION

This work is dedicated to my husband and children, who served as solid support system throughout my training and countless compromises that they have endured until the end. It is with gratitude I walked this journey with you all.

Publications and presentations
This work has not been published or presented at the conference.

ABSTRACT

Background

Ewing sarcoma and peripheral primitive neuroectodermal tumours (ES/pPNET) are aggressive bone and soft tissue malignancies that are most common in children and adolescents. The use of a multimodality treatment approach improves outcome especially following advancement in systemic chemotherapy. Local treatment strategies for local disease control continues to show promise in improving overall survival and local control rates in patients with localized disease.

Objective

The objective of this study was to determine the effectiveness of treatment in ES/pPNET by comparing surgery and radiotherapy in overall disease and event free survival.

Materials and Methods

This was a retrospective analysis of medical records of ES/pPNET patients treated in a public academic institution in Johannesburg. Records of fifty-four (54) patients with localized tumours treated from January 2000 to December 2015 were included in the study. Five-year overall survival and event-free survival as well as incidence of recurrence were estimated using the Kaplan-Meier for the analyzed cohorts. Cox regression models were used for analysis of prognostic factors and estimating odd ratios with 95% confidence intervals.

Results

The estimated 2-year and 5-year overall survival (OS) was 88.9% (95% CI: 0.7693 – 0.9485) and 77.7% (95% CI: 0.6400 – 0.8664) respectively. Based on the local treatment received, the 5-year OS was 73.3% for surgery only, 83.3% for radiotherapy only and 76.0% for patients that had both surgery and radiotherapy

In total, 18 (33.3%) of the 54 patients experienced recurrence; isolated distant recurrence occurred in 15 patients, the commonest site being the lungs (55.6%), followed by the

spine (14.8%) and the pelvis (14.8%). Combined local and distant recurrence occurred in 3 patients. The site of local recurrence in these three (3) was the pelvis. The estimated 5-year event free survival (EFS) for all patients was 65.8% (95% CI: 0.5130 – 0.7691). The estimated 5-year EFS was 63.6% (95% CI: 0.2042 – 0.8045) for surgery only, while for radiotherapy only, 66.7% (95% CI: 0.3771 – 0.8234) and for both surgery and radiotherapy 66.2% (95% CI: 0.4347 – 0.8149). No patient in this cohort of ES/pPNET had isolated local recurrence or failure following local treatment.

Patients with tumour size ≥ 8cm are 2.54 times likely to have local or distant tumour recurrence compared to patients with tumours < 8cm (OR: 2.54, 95% CI: 1.05-6.13, p=0.019). Patients with high pretreatment lactose dehydrogenase (LDH) level (at diagnosis), had approximately 5 times increased odds of recurrence compared with those with normal LDH (OR: 4.59, 95% CI: 1.34-15.74, p=0.010). The factors associated with recurrence were tumour size (OR: 2.64, 95% CI 1.08-6.45) and LDH (OR: 4.01, 95% CI 1.15-13.99). For mortality, LDH level (p<0.001) and disease recurrence (p<0.001).

Conclusion

Overall disease control in ES/pPNET is comparable for patients treated with surgery or definitive radiotherapy with chemotherapy. The risk of local failure is commoner in patients treated with definitive radiotherapy than surgery. Although radiation therapy is frequently applied in unfavorable disease group, local control outcomes are good and in many cases similar to surgical treatment outcomes. Distant failures account for the majority of relapses in this disease; therefore there is need for better and improved systemic therapies for both local and distant disease control.

Keywords: ES/pPNET, surgery, radiotherapy (RT), disease recurrence, overall survival (OS), event free survival (EFS), lactate dehydrogenase (LDH)

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I have experienced your guidance day by day. You are the one who enabled me to complete my degree. I will keep on trusting you for my future.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/NOMENCLATURE

AJCC: American Joint Cancer

Committee CD: Cluster of differentiation

CEO: Chief Executive Officer

CESS: Cooperative Ewing sarcoma studies

CHBAH: Chris Hani Baragwanath Academic Hospital

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

COG: Children's Oncology Group

CT: Computed tomography

DNA: Deoxyribonucleic acid

ECOG: Eastern Cooperative Oncology Group

EICESS: European Intergroup cooperative Ewing sarcoma studies

ESFT: Ewing Sarcoma Family of Tumours

ES: Ewing Sarcoma

ERG: ETS- related gene

ETS: Erythroblastosis virus transforming sequence

ETV1: ETS translocation variant 1

EURO-EWING-99: EUROpean Ewing tumour Working Initiative of National Groups 1999

EFS: Event free survival

EWS: Ewing Sarcoma gene

F-18 FDG: 18- Fluorine Fluorodeoxyglucose FEV: Fifth Ewing variant

FFPE: Formalin fixed paraffin embedded

FLI: Friend leukemia virus integration site

FISH: Fluorescence in situ hybridization

Gy: Gray (unit dose of radiation)

HDCT: High dose chemotherapy

HREC: Human Research Ethics Committee

IHC: Immunohistochemistry

IQR: Interquartile range

LDH: Lactate dehydrogenase

MRI: Magnetic resonance imaging

MV: Megavoltage

NCCN: National Comprehensive Cancer Network

NCR-SA: National Cancer Registry, South Africa

NGS: Next generation sequence

OS: Overall survival

PAS: Periodic acid Schiff

PET Positron emission tomography

PFS: Progression free survival

pPNET: peripheral Primitive Neuro ectodermal Tumour

PORT: Post-operative radiotherapy

RNA: Ribonucleic acid

RT: Radiotherapy

RT-PCR: Reverse transcriptase polymerase chain reaction

SRCS: Small round cell sarcoma

TBI: Total body irradiation

VACA: Vincristine, Actinomycin D, Cyclophosphamide, Adriamycin (Doxorubicin)

VACD: Vincristine, Actinomycin, Cyclophosphamide, Doxorubicin

VDC/IE: Vincristine, Doxorubicin, Cyclophosphamide, Ifosfamide, Etoposide

WLI: Whole lung irradiation

Appendices

1. Introduction

1.1. Background

Ewing Sarcoma family of tumours (ESFT) comprising of peripheral Primitive Neuroectodermal Tumour (pPNET) and Ewing sarcoma (ES) were first described by Stout in 1918 for a tumour originating from the ulnar nerve and James Ewing in 1921 for a diffuse endothelioma, involving the diaphysis of the long bone [1-3]. These small round cell tumours, (ES and pPNET) are the second most common primary malignant bone tumours in children and adolescents. They represent almost 3% of primary paediatric malignancies [4]. They are aggressive bone and soft tissue malignancies which usually metastasize to the lungs, bone marrow and bone. The use of multimodality approach in the management of localized disease improves treatment outcomes, with a survival rate of about 70% over the years [5]. However, 30% of patients develop local recurrence and or distant metastases, typically 2-10 years after the primary diagnosis [4,6]. After the introduction of chemotherapy (Doxorubicin, Vincristine, actinomycin D and cyclophosphamide (VACD regimen), the 5 year overall survival rate of local disease increased from 28% to 65% in the 1970s [7,8]. Chemotherapy was initially used as systematic treatment to control metastasis, and later in a neoadjuvant setting to enhance local control with confirmed efficacy [8]. The current local control strategies include: radiotherapy, surgery or combined surgery and radiotherapy [9]. Local control is an important therapy to improve the overall survival rate and local control rate of ES/pPNET as a result of advances in chemotherapy schedules, surgical and radiotherapy techniques. Local treatment is recommended after chemotherapy for all patients. ES/pPNET are radiation- sensitive, the proportion of patients whose primary tumours are treated with radiation alone has steadily declined because of improved surgical approaches

[10]. Zogopoulos, et al., suggested that surgery is the most effective method for local treatment, while radiotherapy should be used sparingly [11]. The debate over whether surgery and radiotherapy are comparable in terms of local control continues, and optimal local control strategies as well as the timing for these local therapies post chemotherapy for ES/pPNET remains unclear [12]. Surgery is preferred in children due to the negative effect of radiotherapy on growth and development, and is usually applied in small, peripheral and resectable tumors while radiotherapy is applied in larger, central tumors [13]. The increasing use of surgery as local control modality has led to re-evaluation of the indications for radiotherapy. It is important to remember that surgery and radiotherapy are complimentary modalities in the management of ES/pPNET, and not competitive [14].

The influence of systemic chemotherapy on local control has been investigated and studies have shown reduced local recurrences, with improvement in 5 years local control of disease [15, 16]. In an ideal situation, local treatment should be individualized taking into consideration various factors based on patient characteristics, the likely benefit and harm of the treatment modality and preference of the patient. The selection strategy should be planned with the goal of optimizing local tumor control while minimizing therapy related adverse effects [17].

1.2. Epidemiology of Ewing Sarcoma/Primitive Neuroectodermal Tumours

The Ewing sarcoma family of tumours (ESFT) is the second most common malignant bone tumor found in children, adolescents and young adults. Approximately, 250 new cases are diagnosed per year in the United States, with an annual incidence of 3 cases per 1 million children less than 15 years of age [5].

Limited data on ES/pPNET is available in Africa, and none is available from the local National Cancer Registry, South Africa. However, few case series and reports have been reported in South Africa [18, 19, 20]. Around 80% of cases occur in patients younger than 18 years, less than 1% of cases are found in adults more than 40 years of age [21]. The most common age for occurrence of ESFT is in adolescents and young adults (10-19 years) and approximately 15% of cases occur in children less than 10 years of age [22]. The mean age for diagnosis is 14-15 years of age while increasing age at diagnosis leads to worse outcomes. The incidence is significantly higher in Caucasians as compared to Asians and Africans, and there is slight male preponderance [23,24]. The tumour most often begins in the extremities followed by the pelvis, but can occur in any bone. Less often, it can occur in extraosseous tissues i.e soft tissues of the chest, abdomen, and other sites [25-27]. Most cases present with primary localized disease, whereas approximately 20–25% present with metastatic disease at initial diagnosis [28].

1.3. Literature review

Ewing sarcoma/peripheral primitive neuroectodermal tumour (ES/pPNET) is rare, with approximately 250 to 300 new cases per year for both osseous and extraosseous tumours in the United States. It is the 3rd most common primary malignant neoplasm of bone and soft tissues in children, after osteosarcoma and rhabdomyosarcoma, respectively [29, 30]. Extraosseous tumours constitute 20%–40% of all ES/pPNET and most often are found in the soft tissues of the trunk and extremities, followed in frequency by head and neck, retroperitoneum, and other sites, especially those associated with skeletal muscle. Rarer still are organ-based sites, including the female genital tract, adrenal gland, and kidney [31,32]. Familial cases have been reported, and extraosseous ES/pPNET may rarely occur as a 2nd malignant neoplasm [33].

1.3.1. Molecular biology of ES/pPNET: Genetic and molecular characterization

It is generally recognized that ES/pPNET represents one and the same neoplastic process, as demonstrated by the overlapping morphologic features, including ultrastructure, immunophenotype, and a set of molecular genetic abnormalities [3,34,]. These tumours are characterized pathologically as small round cell blue tumours and cytogenetically by a family of translocation genes. In ES/pPNET-associated translocation, the erythroblastosis virus transforming sequence domain (ETS) can be contributed from any of the family members of genes [34]. In about 85%, ES/pPNET harbour the translocation t(11;22)(q24;q12). At the molecular genetic level, the chromosome 22q12 breakpoints are clustered within a single gene designated EWS (for Ewing sarcoma) and the chromosome 11q24 breakpoints are within a gene called FLI-1 (a gene homologous to the Friend leukemia virus integration site 1) [35]. The FLI-1 gene is a member of a large family of DNA-binding transcription factors that are implicated in the control of cellular proliferation, development and tumourigenesis of ES/pPNET [36,37]. As a result of the t(11;22)(q24;q12) translocation, there is fusion of the EWS gene (which encodes for an RNA-binding protein of unknown functions, and is located at 22q12) with the human homologue of the murine FLI-1 gene, a member of the ETS family of transcription factors [38]. Approximately, 10% of ES/pPNET exhibit a 2nd translocation, t(21;22)(q22;q12) which fuses EWS to ETS-related gene (ERG) [39,40]. The remaining 5% of ES/pPNET exhibit rare variant translocations. In these rare instances, the ES is fused to the ETS domains of ETV1 (ETS translocation variant 1; t(7;22)(q22,12)), E1AF (E1A factor; t(17;22)(q12;q12)) or FEV (fifth Ewing variant; t(2;22)(q33;q12)) [41-44]. Both EWS-FLI-1 and EWS-ERG gene fusions are heterogeneous because of variability in genomic breakpoint locations leading to EWS-FLI-1 transcripts with a variety of exons from the EWS and FLI-1 or ERG genes [39]. Two most frequently observed in-frame fusions (EWS/FLI-1) or rearrangements have been documented, type 1 and type 2. Type 1 occurs between EWS exon 7 and FLI-1 exon 6,(in 65% of cases) while type 2 occurs between EWS exon 7 and FLI-1 exon 5 (in 25% of cases) [35,45]. Several studies have shown that the survival of patients whose Ewing tumor contains the type 1 EWS-FLI-1 rearrangement (*EWS* exon 7 fused

to *FLI-1* exon 6) is significantly better than those with other rearrangement types [45-47]. Evidence has also emerged, implicating the poor initial response to chemotherapy and the presence of non–type 1 EWS/FLI1 rearrangement with adverse outcome. Studies have shown that EWS-FLI-1 induces a cancer stem cell population in paediatric, but not adult human, mesenchymal stem cells by direct repression of microRNA-145 [48].

Several studies have also shown that EWS-FLI-1 exerts both activating and repressive effects on gene expression and that both effects are important for ES/pPNET oncogenesis [49,50]. In addition to the EWS-ETS rearrangements, other genetic changes have been described in ES/pPNET, including numerical and structural chromosomal aberrations in approximately 80% of cases [51,52]. Among the most common aberrations are gains of chromosomes 8 and 12 seen in 50% and 30% of cases, respectively, gains of chromosome 2 and 1q, an unbalanced translocation t(1:16), and deletions at the short arm of chromosome 1 [53,54]. Deletion of chromosome 1 is reported to be associated with unfavourable outcome in patients with localized disease [53]. Up to 15 different chromosomes, other than those relating to EWS–ETS gene rearrangements, have been implicated in ESFT, although much of this has been in the form of individual patient data.

It is important to evaluate the frequency and clinical importance of these additional chromosomal aberrations in large clinical outcome studies, because a more complex karyotype with multiple chromosomal aberrations appears to be associated with poor outcome and may be prognostically powerful in ESFT [55-57].

The degree of neuronal differentiation is used for histopathologic sub-classification in ES/pPNET, with classical Ewing sarcoma, characterized by minimal evidence of neural differentiation. pPNET, displays marked evidence of neural differentiation by standard microscopy (presence of pseudorosettes), electron microscopy, or immunohistochemistry (two or more neuronal markers, including neuron-specific enolase, Leu-7, or synaptophysin) [31].

1.3.2. Pathologic diagnosis of Ewing sarcoma/ Primitive Neuroectodermal Tumour

The current pathologic diagnosis of ES/pPNET depends on tumour morphology, immunohistochemistry findings, and a demonstration of a Ewing sarcoma- specific translocation by standard cytogenetics, fluorescence in situ hybridization (FISH), or reverse transcriptase-polymerase chain reaction (RT-PCR) assays[58,59].

Morphologically, the typical histology of ES/PNET shows a lobular growth pattern of tumour cells that are monomorphic, small round blue staining cells with scanty cytoplasm. The cells demonstrate positive periodic acid-Schiff staining because of cytoplasmic glycogen and electron microscopy may reveal these glycogen stores [58,60,61]. Atypical or large cell variant of ES/PNET may show increased nuclear size or more pronounced atypia [58]. PNETs typical display primitive Homer-Wright pseudorosette formation, and electron microscopy may reveal additional features suggestive of neural differentiation, such as neurosecretory granules or rarely Flexner-Wintersteiner rosette or primitive neuroepithelium [58, 62].

Immunohistochemistry [IHC] plays a crucial role in differentiating ES and pPNET from other small round cell tumours of children and young adults. The most useful antigen in the diagnosis of ES appears to be CD99 (protein product of MIC-2 gene), both ES and pPNET display prominent CD99 immunostaining in a membranous pattern [63, 64]. Multiple series have found strong CD99 immunostaining in more than 90% of cases of ES and pPNET [63-65]. Lymphoblastic lymphomas, leukemias, rhabdomyosarcomas, desmoplastic small round cell tumours, synovial sarcomas, solitary fibrous tumours, extrarenal malignant rhabdoid tumours, neuroendocrine tumours, and mesenchymal chondrosarcomas may demonstrate immunoreactivity to MIC-2 /CD 99 [66]. In order to exclude these other tumours, a panel of IHC stains such as muscle markers (desmin, muscle- specific actin, myoD1, myogenin), S-100, epithelial markers (epithelial membrane antigen, cytokeratin), INI-1, and lymphoid markers (CD45, CD30, TdT, T-cell and/or B-cell markers) are employed [60,63,65].

Many molecular techniques have proven useful for the detection of the EWS translocations that is associated with ES and pPNET, including (FISH), Southern

blotting, DNA-based polymerase chain reaction (PCR), and RNA-based PCR (also referred to as [RT]-PCR) [67]. The translocations associated with ES and pPNET can be readily identified using RT-PCR by the presence of fusion transcripts in tumour cells, and this technique has become a mainstay in molecular diagnosis. RT-PCR is extremely specific and sensitive and allows detection of very low levels of tumour cells even when they are present among large numbers of normal cells [68, 69]. A comparison of RT-PCR and FISH analyses of ES/pPNET indicates that these approaches are complementary [70].

An advantage of using RT-PCR is its ability to distinguish between the t(11;22) and t(21;22) translocations, this technique can distinguish specific subtypes of translocations that have been shown to be of a potential prognostic value [68]. Although RT-PCR has been shown to be a specific method for the detection of EWS-FLI-1 and EWS-ERG chimeric transcripts present in ES from (formalin fixed paraffin embedded) tissues, final interpretation of the results must be viewed along with the other features, including clinical history, histologic, and IHC data [68,70-72]. Currently, a diagnosis of ES/PNET can be confirmed only by molecular pathology, which is mandatory when cases have unusual clinical and pathological features. FISH- based detection of *EWSR1* rearrangements and or PCR (RT- PCR), detection of *FET–ETS* gene fusions specific for EWS have been used for the past 25 years as a diagnostic tool [69,73]. The use of next-generation sequencing is advisable for Small Round Cell Sarcomas (SRCS) in which FISH and/or RT-PCR cannot confirm the diagnosis of ES/pPNET [74].

The definitive diagnosis of ES should be made (or reviewed) at a sarcoma reference center by biopsy, providing sufficient material for conventional histology, IHC, molecular pathology and bio-banking [75].

1.3.3. Imaging work up for ES/PNET

Diagnostic imaging work up for ES/PNET should begin at clinical presentation, and this must include appropriate staging of the disease for metastases, which are detected in about 25% of patients [76]. Approximately 30-40% of patients will develop local or distant recurrence after treatment which will modify the outcome as poor

prognosis. The most common sites of metastases are the lungs, pleural spaces, the skeletal system, and the bone marrow or a combination of any of these [76]. Locoregional lymph node involvement is rare in this disease entity.

Traditional imaging modalities used to assess ES/PNET include plain x-ray film, computed tomography (CT), magnetic resonance imaging (MRI), bone scintigraphy and fluorine-18 fluorodeoxyglucose (18F-FDG) positron emission tomography (PET) or PET/CT [77-80]. Bone marrow biopsy and aspiration are also recommended as part of diagnostic/staging work up [81].

For primary bone tumours, plain radiograph in two planes is the initial radiological investigation [75], while the chest CT, with or without contrast is the examination of choice for evaluating the lungs, the most common site for metastases from bone sarcomas [82]. CT remains useful for local staging to characterize some lesions of the spine, pelvis, and scapula when plain radiographs have proven inadequate to evaluate the bony architecture [82]. MRI with and without contrast remains the mainstay of diagnostic imaging for soft-tissue sarcomas. Its ability to help define the greatest dimension of the tumor allows MRI to play a role in staging. When MRI is contraindicated or not available, CT with contrast can be useful for local imaging. For osseous primary, MRI also helps define the extent of intramedullary involvement, which aids surgical planning. Chest CT has been shown to be more sensitive than fluoro-deoxyglucose positron emission tomography (PET)/CT in detecting pulmonary metastases from both osseous and soft-tissue sarcomas [83]. Bone scintigraphy helps to determine or exclude the presence of multiple bone metastases [82]. PET scan is useful for staging the tumour, to rule out distant bony and soft-tissue (lung or lymph nodes) metastasis and to assess skip lesions in equivocal conditions [84]. The 2021 National Comprehensive Cancer Network guidelines suggest PET/CT and or bone scan; and possibly bone marrow biopsy and/ or screening MRI of the spine and pelvis, CT of the chest with or without contrast as clinically indicated; MRI with or without CT (both with contrast) of the primary site; for initial staging of ES [85]. Bilateral bone marrow biopsy and aspirate are also part of routine work-up, but many experts question the additional benefit of bone marrow biopsy and aspirate when the PET scan is negative [81].

Historically, the Musculoskeletal Tumor Society system for bone sarcomas and the Enneking system for soft-tissue sarcomas are the original staging systems developed by orthopaedic surgeons [86]. Currently, the American Joint Committee on Cancer (AJCC) staging system is what is widely accepted for staging of cancers [87]. In addition to being more consistent with the tumour (T), node (N) and metastases (M) convention used for staging tumours, a number of tumour factors which help to predict disease prognosis have been incorporated into the staging system [87]. Bone and soft tissue sarcomas are staged based on histologic type, tumour grade, size, location, as well as the location and presence of metastases [87].

With the advent of different diagnostic modalities used in the work up of bone and soft tissue sarcomas, including Ewing sarcoma and PNETs, patients are broadly categorized as having localized or metastatic disease. This broad classification has helped greatly in disease prognostication as multimodal approach to treatment is now being used for most cancers.

1.3.4. Treatment approach

The current standard treatment for ES/PNET involves the integration of appropriate systemic therapies (neoadjuvant and or adjuvant chemotherapy) with local treatment such as surgery, radiotherapy (RT), or a combination of both modalities [7, 88, 89].

1.3.4.1. Chemotherapy

The general approach to the treatment of ES in both North America and Europe involves neoadjuvant chemotherapy followed by local treatment with either surgery and or radiation therapy, followed by adjuvant chemotherapy.
Multiagent chemotherapy is a critical component of the definitive management of ES. The current standard of care in North America (Children's Oncology Group) includes vincristine, doxorubicin (adriamycin), and cyclophosphamide alternating with ifosfamide and etoposide (VDC/IE), given every two weeks [88-90]. This regimen has

been shown to be effective in patients with localized ES in single as well as multi-institution collaborative trials in the United States and Europe. The standard treatment algorithm recommended using this neoadjuvant multi-agent chemotherapy regimen is at least 9 weeks prior to surgery to decrease tumour and increases the probability of achieving a complete resection with microscopically negative margins [85]. Adjuvant chemotherapy following surgical resection improves relapse free survival (RFS) and overall survival (OS) in a majority of patients [88,89, 90-93]. For patients with metastatic disease, vincristine, actinomycin D, cyclophosphamide, and doxorubicin (adriamycin) (VACA/VACD) alternating with IE did not improve outcomes for patients over VACA alone [91]. Similarly, there was no improvement in survival with dose-intensified VDC/IE, or with high-dose melphalan, etoposide, total body irradiation, and autologous stem-cell transplantation and local control [91, 94]. A report from the Children's Oncology Group (COG) by Yock TI et.al, showed that there was a local control benefit from VACA-IE chemotherapy regimen and that this benefit appeared to persist irrespective of the local control modality used for localized pelvic ES patients [16]. In the Pediatric Oncology Group-Children's Cancer Group (POG-CCG) study (INT-0091), 398 patients with localized ES were randomized to receive chemotherapy with VACD alone or VACD alternating with ifosfamide and etoposide (VACD-IE) for a total of 17 cycles [89]. The 5-year EFS rate was significantly higher in the VACD-IE group than in the VACD alone group (69% and 54%, respectively; P=0.005). The 5-year OS rate was also significantly better among patients in the VACD-IE group (72% and 61% respectively; P=0.01). VACD-IE was also associated with lower incidences of local failure (11%) compared with VACD (30%) irrespective of the type of local control therapy, 5 year cumulative incidences of local failure were 30% in the VACD arm compared with 11% in the VACD-IE arm (16).

The European (International Society of Pediatric Oncology) strategy has historically implemented initial chemotherapy with VIDE (vincristine, ifosfamide, doxorubicin and etoposide) at maximum tolerated dosage within the shortest possible intervals, which was considered as the best neoadjuvant chemotherapy in the EUROpean Ewing tumour Working Initiative of National Groups 1999 (EURO-E.W.I.N.G 99) clinical trial [95]. Differences in the European and North America approaches to the management

of ES include a unique neoadjuvant chemotherapy regimen, increased use of combined modality treatment for primary tumour control, and a variation in adjuvant chemotherapy according to prognostic factors, with increased use of high-dose chemotherapy and stem-cell rescue in ongoing European protocols. Following induction therapy, the role of high-dose chemotherapy (HDCT) followed by stem-cell rescue has been extensively studied in high-risk ES, including localized disease, based on dose intensity relationships [96-99]. The result from these studies showed that high-dose therapy followed by hematopoietic cell transplant (HDT/HCT) is associated with potential survival benefit in patients with localized disease. The EURO-EWING 99 and Ewing-2008 randomized trial particularly showed BuMel (busulfan and melphalan) HDT- treated patients who received VIDE regimen as induction, with improved EFS and OS, when compared to VAI-treated patients with localized ES with predefined high-risk factors [97].

The Euro Ewing 2012 (EE2012) was a randomized analysis conducted to compare the induction and consolidation regimens for newly diagnosed ES in both the United States and Europe. Patients were randomized into two treatment arms, treatment arm A received the European regimen: VIDE induction therapy followed by VAI (vincristine, dactinomycin and ifosfamide) or VAC consolidation. Treatment arm B received the U.S regimen: compressed VDC/IE neoadjuvant followed by IE/VC consolidation. The randomization was closed in May 2019 after a preliminary analysis demonstrated improved EFS and OS in the VDC/IE arm. The VDC/IE regimen proved superior to the European regimen in terms of the PFS or EFS and OS with similar toxicity profiles [90,100].

In ES, chemotherapy was initially used as systematic treatment to control metastasis, and later in a neoadjuvant setting to enhance local control with confirmed efficacy [9]. This strategy of upfront induction chemotherapy results in higher rates of complete resection with negative margins and a reduced need for adjuvant radiation therapy [93].

The NCCN expert panel recommends adjuvant chemotherapy following surgery (wide excision or amputation) for all patients regardless of surgical margins [85]. The panel

strongly recommends that the duration of chemotherapy following surgery should be between 28 and 49 weeks depending on the type of chemotherapy regimen and the dosing schedule [88,89,92]. For patients with metastases limited to the lungs or pleura, the R2 randomization of the Euro-Ewing99 study tested megatherapy using Busulfan-Melphalan (Bu-Mel) conditioning followed by stem-cell infusion versus VAI consolidation chemotherapy and whole lung irradiation (WLI) based on encouraging European data [101,102]. No benefit to Bu-Mel HDCT was demonstrated and significantly more patients in the Bu-Mel arm experienced severe acute toxicities [103]. In contrast, the Italian Sarcoma Group/Scandinavian Sarcoma Group (ISG/SSG) IV trial reported encouraging results (five-year OS near 50% for patients with lung, pleural, or isolated bone metastasis) using induction chemotherapy followed by both Bu-Mel HDCT and whole lung radiotherapy in case of lung metastases [102].

In the non-lung metastatic population, intensive induction chemotherapy using different drugs (VIDE, VDC/IE ± temozolomide and Irinotecan) also remains the standard of care in Europe before local treatment of the primary tumour and metastases [104].
In this retrospective review of patients with localized Ewing sarcoma/ PNET, the EURO-EWING '99 protocol was used as neoadjuvant and adjuvant systemic therapy at the two academic hospitals.

1.3.4.2. Local treatment for local control of disease

Ewing sarcoma is a radiosensitive tumour, both surgery and radiation therapy (RT) represents effective local treatment modalities. The long term outcome (5- year survival) was < 10% with high incidence of local and systemic recurrence following RT alone [10]. Recent trends for local management in ES have favoured the surgical approach [10, 14, 105,]. The goal of local therapy for the primary tumour is to ensure that the entire volume of tissue involved at diagnosis is treated [10,14]. Complete surgical excision, where feasible, is regarded as the best modality of local control, because of the higher risk of local recurrence (LR) when RT is used as the sole treatment. Surgery must involve excision of all tissues originally involved before induction chemotherapy (not just the tumour tissue remaining following dimensional

shrinkage on chemotherapy) [106]. The benefits of surgical resection include providing prognostic information regarding histologic response after chemotherapy, preventing radiotherapy-associated late effects such as growth inhibition and secondary malignancies [107]. Surgery also helps with improved local control for some tumour locations [108]. The impact of different local therapy approaches was investigated in 1058 patients with localized ES through the Cooperative Ewing's Sarcoma Studies (CESS) 81, CESS 86, and the European Intergroup Ewing's Sarcoma (EICESS) 92 trials [14]. Surgery was used when possible, followed by RT in patients with poor histologic response or with incomplete resection. Preoperative RT was performed for patients with expected close resection margins. The local failure rates were similar after surgery with or without postoperative RT (7.5%) and after preoperative RT (5.3%). In contrast, the local failure rate was significantly higher after definitive RT (26.3%, P=0.001) [14]. Choice of local control modality (surgery, surgery combined with radiotherapy, or radiotherapy alone) and its impact on clinical outcomes such as survival and relapse in patients with localized ES is the subject addressed by Zhu, *et al* [109].

RT with definitive intent alone is usually used instead of surgery if complete surgical excision is not possible and in cases with challenging local sites such as axial or spinal tumours where surgery will be unacceptably morbid [14,110]. Adjuvant RT (45-60Gy) significantly reduces LR in patients with large volume tumours (>200mls), poor histological response or inadequate surgical margins and is recommended in these circumstances [14,111]. Adjuvant RT is also considered in patients with non-sacral pelvic ES regardless of surgical margins, tumour volume or histological response, and it has been shown to have superior local control and survival outcomes compared with surgery alone [112]. The use of modern RT techniques with the ability to deliver high doses and minimize dose to normal tissue, including heavy particles has also been considered whenever it is technically more appropriate, especially in paediatrics patients and young adults [113]. Tumour size and RT dose have been shown to be predictive of local control rates in patients with localized ES treated with chemotherapy and definitive RT [114,115]. In the postoperative setting, RT treatment volumes are defined according to histologic response to chemotherapy, given adequate margins, (45 Gy) while for gross residual disease, a total cumulative dose (55.8 Gy) is

recommended [85].

1.4. Aims and objectives

1.4.1. Aim

To determine the effectiveness of treatment of ES/pPNET in children treated in the paediatric oncology units at Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital.

1.4.2. Study objectives

The objectives of this study are:

1. To compare effectiveness between surgery and RT in the management of ES/pPNET in local control and event-free survival.
2. To assess prognostic factors in relation to modalities for local control.
3. To compare recurrence rate between local control modalities i.e, surgery versus RT.

2. Research methodology

2.1. Study design and setting

This is a retrospective review of medical records of paediatric patients diagnosed with Ewing sarcoma/Primitive Neuroectodermal Tumor (ES/PNET) at Chris Hani Baragwanath Academic Hospital (CHBAH) and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), and treated at the Radiation Oncology department through the paediatrics unit between 01 January 2000 and 31 December 2015.

2.2. Study population and study sample

A total number of 54 pediatrics patients, age less than 18 years and with the diagnosis of ES/PNET were included for data analysis for the study.

1. Inclusion criteria

- All children diagnosed with ES/PNET, (bone or soft tissue) less than 18 years at diagnosis, without metastasis, i.e localized disease

- Children treated for ES/PNET at Paediatric Oncology Units of Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Paediatric Oncology Units from 01 January, 2000 to 31 December, 2015

2. Exclusion criteria

- Patients with metastatic disease
- Patients with refractory or recurrent Ewing sarcoma/PNET
- Patients on palliative treatment
- Patients treated primarily by oncologists elsewhere or those with incomplete medical records

2.3. Sequence of treatment and follow up

The patients received six (6) cycles of VIDE (vincristine, ifosfamide, dactinomycin, and etoposide) as neoadjuvant chemotherapy after which, they were then considered for local treatment within few weeks post neoadjuvant chemotherapy. Post local therapy, seven (7) cycles of adjuvant chemotherapy using VAC regimen was then given. Tumor response assessment was then done with CT/MRI scan. Decision on the appropriate choice of local treatment in the form of surgery, radiation therapy or both is determined by the MDT(multidisciplinary team) and patients for radiation therapy are initiated on treatment two (2) weeks post neoadjuvant chemotherapy while those for surgery (partial or total resection of tumor or limb amputation) were also done within few weeks post neoadjuvant chemotherapy. Patients with inadequate surgical margins (positive margins or partial tumour resection or intra-lesional margins) were considered for post-operative radiation therapy (PORT) using megavoltage technology, from 6-18 MV photons and this was done two (2) weeks after surgery or as soon as the surgical wound was healed. Follow up was done 3 monthly within the first 2 years post treatment with routine blood tests and imaging modalities such as chest x-ray, CT, MRI and PET/CT in selected patients especially for treatment assessment or in identifying residual or recurrent disease.

2.4. Data collection and management

Relevant data was extracted from the patients' files onto a data collecting sheet, and thereafter imported to an excel spreadsheet for ease of analysis. Demographics (date of birth/age at diagnosis, gender and race). Clinical data including tumour characteristics such as: histological type (ES or pPNET), with EWSR1 translocation status/IHC parameters, site, size and tumour grade. Others include pathological diagnostic tests, imaging modalities (plain radiographs, Chest x–ray, CT/ MRI scan or PET/CT scan), lactose dehydrogenase (LDH) level, performance status using the European Cooperative Oncology Group (ECOG) assessment, specific treatment patient received (chemotherapy regimen, type of surgery, dose of radiation), pattern/site of disease recurrence and follow up status (alive or dead) (see Appendix 1 for data collection sheeting sheet).

2.5. Data processing and statistical analysis

Data was entered into secure Microsoft Excel software. Categorical variables were expressed as frequencies and percentages while continuous variables were expressed as median [(Interquartile range (IQR)]. The associations between patient's socio-demographic and disease variables with local treatment received were presented using Pearson's chi squared test for categorical variables and analysis of variance for the continuous variable follow-up period.

Kaplan–Meier survival curves were constructed and stratified by local treatment received for the analyzed cohort for overall survival (OS) and event free survival (EFS). Event-free survival (EFS) was calculated from the time of diagnosis to the date of an event (any relapse or recurrence or mortality from any cause) or date of last follow-up or last patient contact, at which time the patient was censored. Overall survival (OS) was defined from time of diagnosis to death or date of last follow up. Survival comparisons were performed using the log-rank test. Factors associated with recurrence and mortality, were analysed using univariate and multivariate logistic regression analyses, reporting odds ratio and 95% confidence interval. Variables significant at p-value <0.1 on univariate analysis were considered in the multivariate model. On multivariate analysis, two-sided p-values of <0.05 was considered

significant throughout. Analysis was performed using Stata version 16 (Stata Corp Ltd, College Station, TX).

2.6. Ethics

Permission to access patients' medical records was obtained from the Chief Executive Officers (CEO) of the two academic hospitals (Appendix 2 and 3).
Ethics approval was sought from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand. The Ethics clearance certificate number M181087 (Appendix 4).

3. Results

3.1. Patients' characteristics

A total of 54 patients met the minimum inclusion and exclusion criteria for this study. The patients' age, ranged from 3 months to 18 years [median age of 8 years]. The majority of the patients (n=21, 38.9%) were within the age group "0-4" years, 10 (47.6%) of whom were females and 11 (52.4%) were males,(Figure 3.1) while 59.3% (n=32) were 10 years old and younger, with 22 (40.7%) older than 10 years as shown in Table 3.1.The majority of the patients (n=35, 64.8%) were blacks. All patients had core biopsy and molecular genetic profiling of tumor was done and EWSFL1/EWSR1 rearrangement or fusion was present in 31 (57.4%) patients, the rest of the patients (n=22, 42.6%) were not tested however had immunohistochemistry (S-100, synaptophysin, NSE and CD99). The predominant histopathological type was Ewing sarcoma (n=31, 57.4%) and the commonest site of tumor was the extremities (n=24, 44.4%), followed by pelvic tumour (22.2%), then truncal tumors (11.1%). Other tumour site of the primary tumour; head and neck, spine and the kidney. Over 60% (n=35) of the tumors were less than 8 cm in size, grade 4 (n=47, 87%), and most of the patients (n=34, 63%) had high levels of lactate dehydrogenase (LDH) at time of diagnosis. The various diagnostic imaging work up tests done during initial tumor assessment and follow up period post treatment, is shown in Figure 3.2. All the patients were assessed

with Chest x- ray, almost all the patients (n=53, 98.5%) had CT scan while 68.5% (n=37) had MRI and only 8 patients (15.1%) had PET/CT scan.

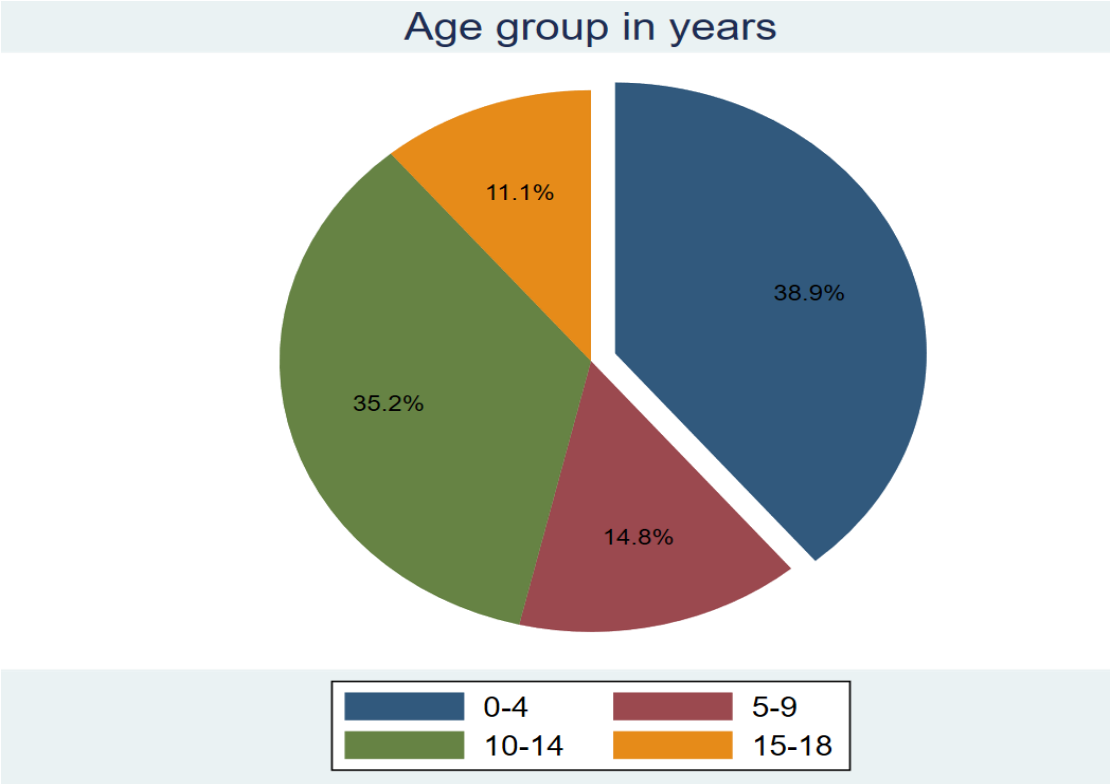

Figure 3.1 Distribution of age in years at diagnosis

Table 3.1 Socio-demographic and clinical characteristics of the patients

Characteristics	N=54	%
Age groups (years)		
0-4	21	38.9
5-9	8	14.8
10-14	19	35.2
15-18	6	11.1
Age		
≤10 years	32	59.3
>10 years	22	40.7
Age (Mean ± S.D)	8.1 ± 5.0	
Age [Median (IQR)]	8.0 (3.2-12.0)	
Gender		
Female	29	53.7
Male	25	46.3
Race		
Black	35	64.8
Non-Black	19	35.2
Histology*		
EWS	31	57.4
PNET	23	42.6
Tumour site		
Extremities	24	44.4
Pelvis	12	22.2
Trunk	6	11.1
Organ (Kidney)	4	7.4
Head and neck	4	7.4
Spine	4	7.4
Tumour size		
< 8cm	35	64.8
≥8cm	19	35.2
Tumour grade		
Grade 3	7	13.0
Grade 4	47	87.0
LDH		
High	34	63.0
Normal	20	37.0
ECOG		
Good	24	44.4
Poor	30	55.6

*EWS (Ewing Sarcoma), PNET (Primitive Neuroectodermal Tumor)

3.2. Patients' treatment

Most of the patients (n=53, 98.1%) had neoadjuvant chemotherapy while all of them (n=54, 100%) had at least one form of local treatment (surgery only; n=11, 20.4%),

(radiation therapy only; n=18, 33.3%) and (surgery with radiation therapy; n=25, 46.3%). Overall, 66.7% (n=36) had surgery post chemotherapy; complete resection of tumor was achieved in 57.4% (n=31), while 33.3% (n=18) were not offered any form of surgery. The vast majority of the patients (n=44, 81.5%) had adjuvant radiation therapy and in most of them, (n=37, 84.1%) the median radiation dose of RT given was 50.4- 55.8 Gy as shown in Table 3.2.

Table 3.2: Treatment received by EWS/PNET patients, follow-up and disease outcome

Characteristics	N=54	%
Chemotherapy treatment	**Frequency**	**Percentage (%)**
Yes	53	98.1
No	1	1.9
Surgery		
No	18	33.3
Yes	36	66.7
Surgical resection		
No resection	18	33.3
Complete resection	31	57.4
Partial resection	5	9.3
Radiotherapy		
No	11	18.5
Yes	43	81.5
Radiotherapy dose, n=43		
36.0Gy	4	9.3
45.0Gy	2	4.7
50.4Gy	25	58.1
55.8Gy	12	27.9
Radiotherapy dose [mean ± SD]	50.7 ± 5.0	
Radiotherapy dose [median (IQR)]	50.4 (50.4-55.8)	
Local treatment modality, n=54		
Surgery only	11	20.4
Radiotherapy only	18	33.3
Surgery and radiotherapy	25	46.3
Disease recurrence		
Yes	18	33.3
No	36	66.7
Follow-up status		
Alive	36	66.7
Dead	18	33.3

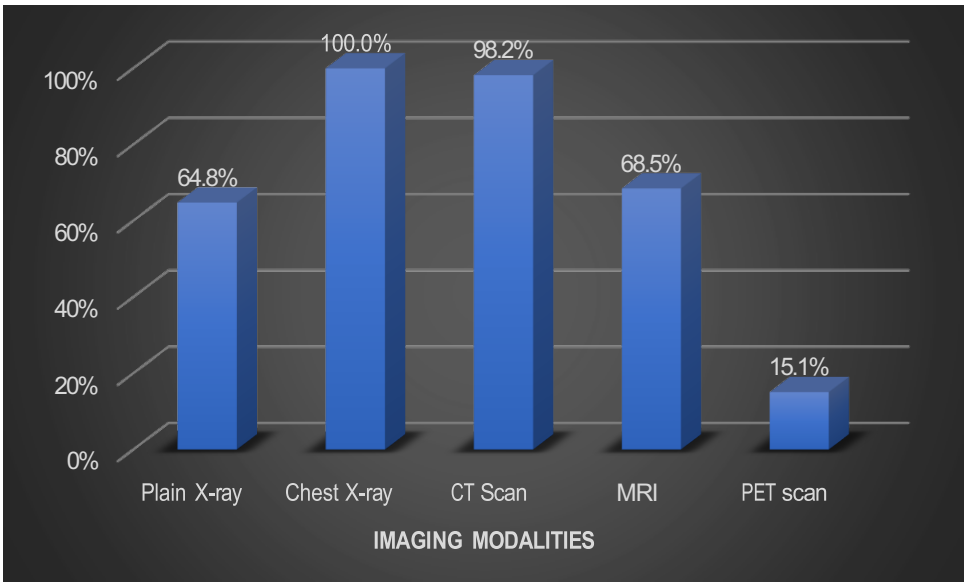

Figure 3.2: Diagnostic imaging workup during initial tumor assessment and follow up

3.3. Patients' outcome

The median follow-up of patients alive at last contact in this cohort was 84 (IQR 60-144) months. Those patients that had surgery and radiotherapy as local treatment had the longest median follow-up period 84 (IQR 60-144) months. Eighteen (33.3%) patients developed disease recurrence and 16 (26.9%) died, two patients with recurrence were still alive by the last date of follow up, while another two patients without evidence of disease recurrence also demised. The remaining 36 (67.7%) patients were alive. The highest proportion of those that died was in the 15-18 age group with 66.4% (n=4/6) of death in this age group category, and much more in the age category of 10 years and above (10-18 years) (44%) while for those less than 10 years (0-9 years) it was 23% [Table 3.3]. There is a trend with age and mortality, the older the patient, the increased likelihood of dying. The estimated 2-year and 5-year overall survival (OS) for all patients was 88.9% (95% C.I 0.7693 - 0.9485) and 77.7% (95% CI 0.6400 – 0.8664) respectively [Figure 3.3]. Based on the local treatment received, the 5-year OS was 73.3% for surgery only, 83.3% for radiotherapy only and 76.0% for patients that had both surgery and radiotherapy [Figure 3.4]. The estimated 5-year event free survival (EFS) for all patients was 65.8% (95% CI 0.5130 -0.7691). The estimated 5-year EFS for surgery was 63.6% (95% CI 0.2042 - 8045) for surgery only, while for radiotherapy only, 66.7% (95% CI 0.3771 - 0.8231) and for both surgery

and radiotherapy 66.2% (95% CI 0.4347 - 0.8149) [Figure 3.6]. Overall, for the entire cohort, isolated distant recurrence occurred in 15 patients, the commonest site being the lungs (55.6%), followed by the spine (14.8%) and the pelvis (14.8%). Combined local and distant recurrence occurred in 3 patients. The site of local recurrence in these three (3) was the pelvis (Table 3.3). Two of these three patients with local recurrence had RT only while the third patient had both surgery and RT. No patient in this cohort of ES/PNET had isolated local recurrence or failure following local treatment (Table 3.5).

Table 3.3 Disease recurrence type versus site of primary tumour

Site of primary tumour	Local recurrence only N=0	(%)	Distant recurrence only N=15	(%)	Local + Distant recurrence N=3	(%)	P value
Extremities	0	0	9	60.0	0	0	0.099
Pelvis	0	0	3	20.0	3	100	
Trunk	0	0	0	0.0	0	0	
Organ (Kidney)	0	0	1	6.7	0	0	
Head and Neck	0	0	2	13.3	0	0	
Spine	0	0	0	0.0	0	0	

Table 3.4 Gender and follow-up status distribution by age group

	Age group in years					
	0-4	5-9	10-14	15-18	Total	P value
	N=21 (%)	N=8 (%)	N=19 (%)	N=6 (%)	N=54 (%)	
Gender						0.187
Female	10 (47.6)	4 (50.0)	9 (47.4)	6(100.0)	29 (53.7)	
Male	11 (52.4)	4 (50.0)	10 (52.6)	0 (0.0)	25 (46.3)	
*Total	21 (38.9)	8 (14.8)	19 (35.2)	6 (11.1)	54 (100.0)	
Follow-up status						0.305
Alive	16 (76.2)	6 (75.0)	12 (63.2)	2 (33.3)	36 (66.7)	
Dead	5 (23.8)	2 (25.0)	7 (36.8)	4 (66.4)	18 (33.3)	
*Total	21 (38.9)	8 (14.8)	19 (35.2)	6 (11.1)	54 (100.0)	

*Total (Row percentage shown), p-value (Fisher's Exact test)

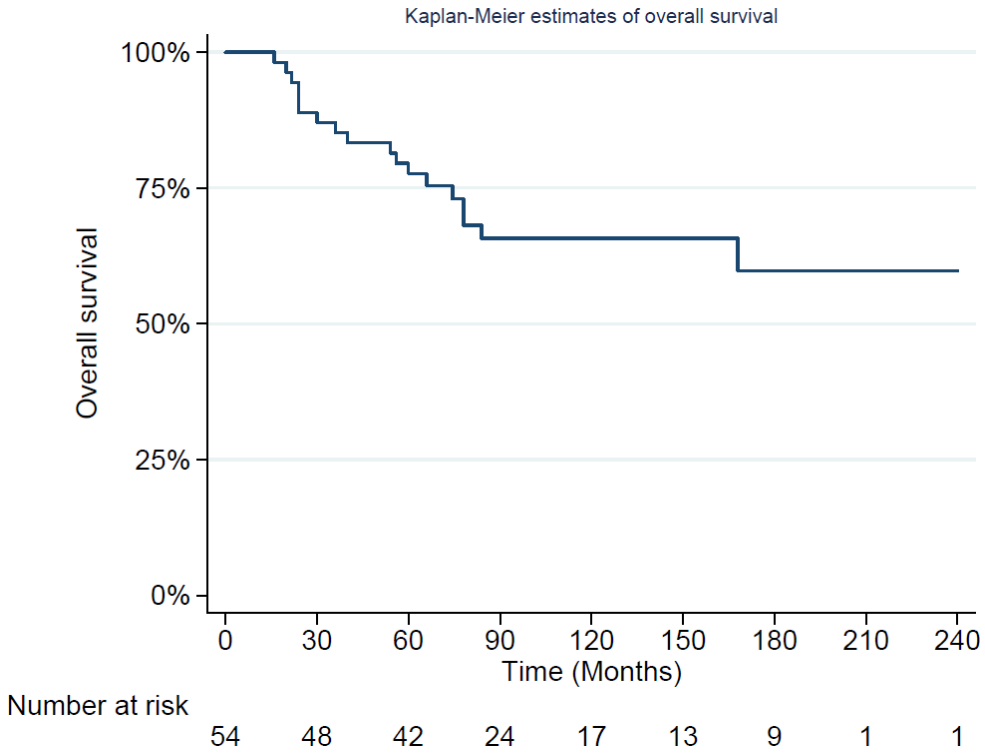

Figure 3.3 Kaplan-Meier estimate of overall survival in children diagnosed with EWS and PNET

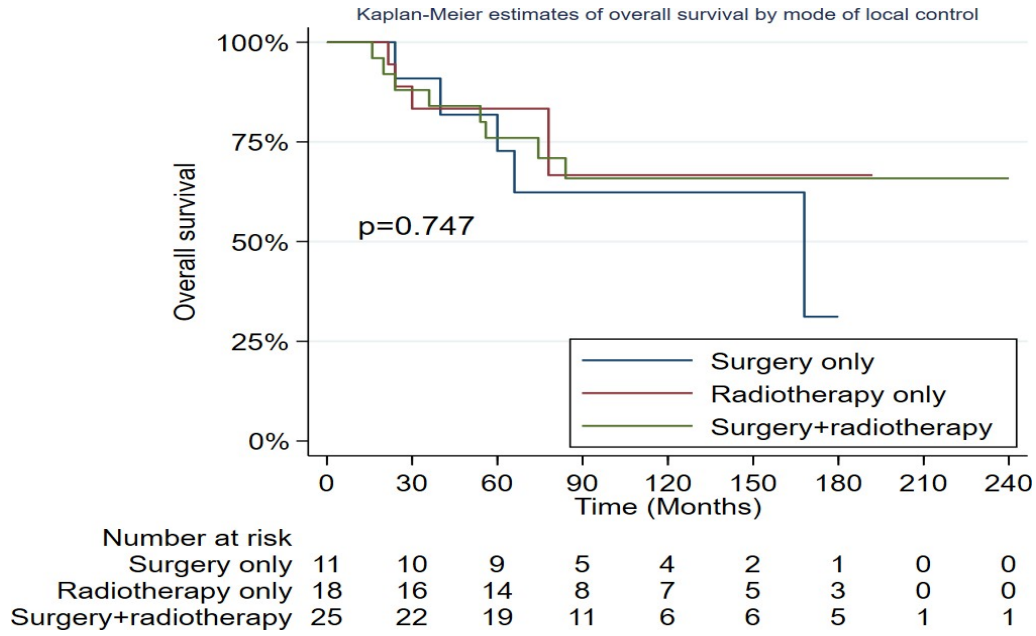

Figure 3.4 Kaplan-Meier estimate of overall survival by local treatment modality in children diagnosed with EWS and PNET

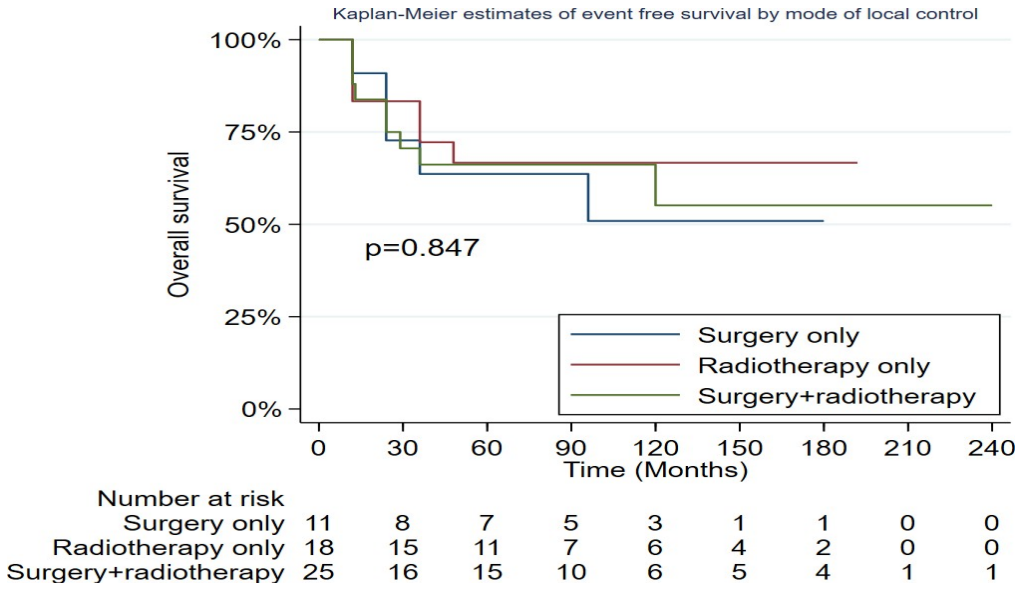

Figure 3.5 Kaplan-Meier estimate of events free survival by local treatment modality in children diagnosed with EWS and PNET

Table 3.5 Association of patient and disease characteristics with local treatment modality

	Local treatment modality								
Characteristic	**Surgery only**		**Radiotherapy only**		**Surgery + Radiotherapy**		**Total**		**P-value***
Row (%)	**N=11**	**20.4%**	**N=18**	**33.3%**	**N=25**	**46.3%**	**N=54**	**100.0**	
Age									0.753
≤ 10 years	7	63.6	12	66.7	13	52.0	32	59.3	
>10 years	4	36.4	6	33.3	12	48.0	22	40.7	
Gender									0.451
Female	4	36.4	10	55.6	15	60.0	29	59.3	
Male	7	63.6	8	44.4	10	40.0	25	40.7	
Race									0.630
Black	9	81.8	11	61.1	16	64.0	36	66.7	
Non-Black	2	18.2	7	38.9	9	36.0	18	33.3	
Tumour site									**0.026**
Axial (Central)	3	27.3	15	83.3	12	48.0	30	55.6	
Appendicular (Extremities)	8	72.7	3	16.7	13	52.0	24	44.4	
Extremities	*8*	*72.7*	*3*	*16.7*	*13*	*52.0*	*24*	*44.4*	**0.000**
Pelvis	*0*	*0.0*	*8*	*44.4*	*4*	*16.0*	*12*	*22.2*	
unk	*0*	*0.0*	*1*	*5.6*	*5*	*20.0*	*6*	*11.1*	
Viscera/Organ (Kidney)	*1*	*9.1*	*0*	*0.0*	*3*	*12.0*	*4*	*7.4*	
Head and Neck	*2*	*18.2*	*2*	*11.1*	*0*	*0.0*	*4*	*7.4*	
Spine	*0*	*0.0*	*4*	*22.2*	*0*	*0.0*	*4*	*7.4*	
Tumour size									0.064
< 8cm	5	45.5	11	61.1	19	76.0	35	64.8	
≥ 8cm	6	54.5	7	38.9	6	24.0	19	35.2	

Tumour grade									**0.020**
Grade 3	0	0.0	0	0.0	7	28.0	7	13.0	
Grade 4	11	100.0	18	100.0	18	72.0	47	87.0	
Histology									0.605
EWS	8	72.7	8	44.4	15	60.0	31	57.4	
PNET	3	37.3	10	55.6	10	40.0	23	42.6	
LDH									0.999
High	7	63.6	12	66.7	16	64.0	35	64.8	
Normal	4	36.4	6	33.3	9	36.0	19	35.2	
ECOG status									**0.028**
Good	8	72.7	4	22.2	12	48.0	24	44.4	
Poor	3	27.3	14	77.8	13	52.0	30	55.6	
Chemotherapy									0.223
No	0	0	1	5.6	0	0	1	1.9	
Yes	11	100	17	94.4	25	100	53	98.1	
Recurrence									0.455
No	6	54.5	13	72.2	17	68.0	36	66.7	
Local recurrence only	0	0	0	0	0	0.0	0	0.0	
Distant recurrence only	5	45.5	3	16.7	7	28.0	15	27.8	
Local and distant recurrence	0	0	2	11.1	1	4.0	3	5.5	
Sites of metastases at recurrence (n=27)									0.426
Lungs	*5*	*71.4*	*4*	*50.0*	*6*	*50.0*	*15*	55.6	
Spine	*1*	*14.3*	*2*	*25.0*	*1*	*8.3*	*4*	14.8	
Pelvis	*0*	*0.0*	*2*	*25.0*	*2*	*16.7*	*4*	14.8	
Head and Neck	*1*	*14.3*	*0*	*0.0*	*0*	*0.0*	*1*	3.7	
Intra-abdominal	*0*	*0.0*	*0*	*0.0*	*3*	*25.0*	*3*	11.1	
Follow-up time in months, median (IQR)	66 (60-132)		78 (60-144)		84 (60-108)		84 (60-144)		0.832

P-value* (Fisher's exact), variables significant at P <0.05 shown in bold face**, IQR** (Interquartile range), **EWS** (Ewing Sarcoma), **PNET** (Primitive Neuroectodermal Tumor)

Socio-demographic and disease characteristics of the patients are listed in Table 3.4 according to the local treatment modality. All the patients (n=54) received at least one form of local treatment, 11(20.4%) patients had surgery, 18 (33.3%) had radiotherapy, while 25 (46.3.0%) patients had both surgery and radiotherapy (Table 3.5). There was a significant difference between the local treatment received for tumour site, tumour grade and European Cooperative Oncology Group (ECOG) score/performance status.

Those that received radiotherapy only were more likely to have had axial tumour (p=0.026) and more likely with poor ECOG score (p=0.028).

The median follow-up of patients alive at last contact in this cohort was 84 (IQR 60-144) months, with those that had both surgery and radiotherapy having the longest median follow-up period of 84 (60-108) months (Table 3.5).

Table 3.6 Univariate analysis showing risk factors associated with EWS/PNET recurrence

	Disease recurrence					
	No		Yes		Univariate analysis	P-value
Row (%)	N=36	66.7%	N=18	33.3%	*Hazard Ratio (95% CI)*	
Age						0.117
≤10 years	24	66.7	8	44.4	1.00 (Reference)	
>10 years	12	33.3	10	55.6	1.64 (0.68-3.94)	
Gender						0.700
Female	20	55.6	9	50.0	1.00 (Reference)	
Male	16	44.4	9	50.0	1.10 (0.46-2.65)	
Race						0.314
Non-Black	11	30.6	8	44.4	1.00 (Reference)	
Black	25	69.4	10	55.6	0.87 (0.35-2.14)	
Tumour site						0.561
Axial (Central)	21	58.3	9	50.0	1.00 (Reference)	
Appendicular (Extremities)	15	41.7	9	50.0	1.16 (0.48-2.78)	
Tumour size						**0.001**
< 8cm	29	80.6	6	33.3	1.00 (Reference)	
≥ 8cm	7	19.4	12	66.7	2.54 (1.05-6.13)	
Tumour grade						0.775
Grade 3	5	13.8	2	11.1	1.12 (0.26-4.90)	
Grade 4	31	86.2	16	88.9	1.00 (Reference)	
Histology						0.120
ES	18	50.0	13	72.2	2.34 (0.85-6.43)	
PNET	18	50.0	5	27.8	1.00 (Reference)	
ECOG						0.245
Good	18	50.0	6	33.3	1.00 (Reference)	
Poor	18	50.0	12	66.7	1.32 (0.54-3.22)	
LDH						**0.005**
Normal	18	50.0	2	11.1	1.00 (Reference)	
High	18	50.0	16	88.9	4.59 (1.34-15.74)	
Chemotherapy						0.667
No	1	2.8	0	0.0	1.00 (Reference)	
Yes	35	97.2	18	100.0	1.82 (0.24-13.64)	

Local treatment modality						0.829
Surgery only	7	19.5	4	22.2	1.16 (0.36-3.78)	
Radiotherapy only	13	36.1	5	27.8	0.89 (0.32-2.50)	
Surgery and Radiotherapy	16	44.4	9	50.0	1.00 (Reference)	

LDH (Lactate dehydrogenase), **EWS** (Ewing Sarcoma), **PNET** (Primitive Neuro-ectodermal Tumor), **CI** (Confidence interval)

Patients with tumour size ≥ 8cm are 2.54 times likely to have tumour recurrence compared to patients with tumours < 8cm (OR: 2.54, 95% CI 1.05-6.13, p=0.019). Patients diagnosed with appendicular tumours (extremities) (p=0.529) and ES (p=0.080) have increased odds of disease recurrence, though not significant. Patients with high pretreatment LDH level (at diagnosis), have approximately 5 times increased odds of recurrence compared with those with normal LDH (OR: 4.59, 95% CI: 1.34-15.74, p=0.010) (Table 3.6).

Table 3.7 Multivariate analysis showing risk factors associated with EWS/PNET recurrence

Multivariate analysis				
	Hazard Ratio (95% CI)	**P-value**	***Hazard Ratio (95% CI)**	**P-value**
Tumour size		**0.042**		**0.033**
< 8cm	1.00 (Reference)		1.00 (Reference)	
≥ 8cm	2.50 (1.03-6.04)		2.64 (1.08-6.45)	
LDH		**0.016**		**0.029**
Normal	1.00 (Reference)		1.00 (Reference)	
High	4.55 (1.32-15.63)		4.01 (1.15-13.99)	

*Model adjusted for age and tumour histology

On multivariate analysis, adjusting for age and tumour histology, the factors associated with recurrence in children diagnosed with ES/PNET are tumour size (OR: 2.64, 95% CI 1.08-6.45) and LDH (OR: 4.01, 95% CI 1.15-13.99) (Table 3. 7)

Table 3.8 Univariate and Multivariate analyses showing risk factors associated with mortality

	Alive		**Died**		**Univariate analysis**	**P-value**	**Multivariate analysis**	**P-value**
Row (%)	**N=36**	***66.7%***	**N=18**	***33.3 %***	**Hazard ratio (95 CI)**		**Hazard ratio (95 CI)**	
Age						0.176		
≤ 10 years	24	66.7	8	44.4	1.00 (Reference)			
>10 years	12	33.3	10	55.6	1.89 (0.74-4.80)			

Gender						0.543		
Female	21	58.3	8	44.4	1.00 (Reference)			
Male	15	41.7	10	55.6	1.34 (0.52-3.40)			
Race						0.645		
Non-Black	13	36.1	6	33.3	1.00 (Reference)			
Black	23	63.9	12	66.7	1.26 (0.47-3.43)			
Tumour site						0.912		
Axial (Central)	21	58.3	9	50.0	1.00 (Reference)			
Appendicular (Extremities)	15	41.7	9	50.0	1.05 (0.42-2.66)			
Tumour size						0.114		
< 8cm	26	72.2	9	50.0	1.00 (Reference)			
≥ 8cm	10	27.8	9	50.0	2.11 (0.84-5.32)			
Tumour grade						0.281		
Grade 3	4	11.1	3	16.7	1.99 (0.57-6.92)			
Grade 4	32	88.9	15	83.3	1.00 (Reference)			
Histology						0.331	N S	
ES	17	47.2	6	33.3	1.34 (0.50-3.59)			
PNET	19	52.8	12	66.7	1.00 (Reference)			
LDH						**<0.001**		
Normal	20	55.6	0	0.0	Undefined		Omitted	
High	16	44.4	18	100.0	1.00 (Reference)			
ECOG						0.881		
Good	16	44.4	8	44.4	1.00 (Reference)			
Poor	20	55.6	10	55.6	1.07 (0.42-2.72)			
Recurrence						**<0.001**	1.00 (Reference)	**<0.001**
No	34	94.4	2	11.1	1.00 (Reference)		19.43 (4.36-86.56)	
Yes	2	5.6	16	88.9	18.10 (4.14-79.05)			
Chemotherapy						0.999		
No	1	2.8	0	0.0	1.00 (Reference)			
Yes	35	97.2	18	100.0	1.82 (0.24-13.64)			
Local treatment Modality						0.763		
Surgery only	6	16.7	5	27.8	1.42 (0.46-4.33)			
Radiotherapy only	13	36.1	5	27.8	0.90 (0.30-2.76)			
Surgery + Radiotherapy	17	47.2	8	44.4	1.00 (Reference)			

Eighteen (33.3%) patients died while 36 (66.7%) were alive. The factors associated with mortality in patients diagnosed with ES and PNET on univariate analysis are lactate dehydrogenase (LDH) level ($p<0.001$) and disease recurrence ($p<0.001$). On multivariate analysis, disease recurrence was the only factor associated with mortality with those that had recurrence having 19 times increased odds of mortality compared to those without recurrence (OR:19.43, 95% CI 4.36-86.56), $p<0.001$) Table 3.8

4. Discussion

With the median follow up duration of 84 months, in this cohort of patients, we retrospectively reviewed the outcome of ES/PNET local control using multimodality approach to treatment. Our cohort comprising a heterogeneous set of anatomic location of diverse primary tumour sites in the Ewing Sarcoma family of tumours. Only paediatric patients with localized disease at initial presentation were included in our study which to the best of our knowledge is the first audit of ES/PNET in our local setting. Local therapy is a critical component of the multimodal treatment strategy employed in ESFT.

Our study showed that patient treated with RT only and Surgery with RT, especially for extremities and pelvic tumours comprise the highest risk group for experiencing treatment failure. Overall local recurrence rate of 5.6%. Conversely, an overall distant recurrence rate was 33.3%, despite multimodality approach in patients' management. Most of our patients had tumour size that was less than 8 cm and the vast majority was in the extremities (both osseous and soft tissue primary) and they benefitted from primary surgery. Recent trends for local management of ES/PNET have favored the surgical approach [10, 14,105]. Cumulative incidence of local and distant recurrence was lowest in this group of patient who had surgery only (11.1%). The group of patients who had RT only, even though with the majority of tumours less than 8cm, had a high cumulative incidence of recurrence (27.8%). The group that had surgery with post- operative RT also had most tumours, less

than 8 cm, with the highest cumulative incidence of recurrence of 44.4%. Overall disease control and estimated overall survival was 88.9% and 77.7% at 2 years and 5 years OS respectively, and did not differ according to mode of local control. Du Bois et al., demonstrated that although there is no significant difference in overall survival between local treatment modalities for Ewing sarcoma patients, RT is associated with high risk of local failure. In our study, the somewhat paradoxical similar OS for all patients treated regardless of the local modality, with similar EFS reflects relatively low contribution of local failure to overall disease survival in ESFTs. Our result showed that most patients with axial (central) tumours had definitive RT as the primary treatment for local control of disease (p=0.026) and this group of patients also had poor ECOG (p=0.028). Furthermore, our result also showed that older patients and patients treated with RT, especially for extremity and pelvic tumours, comprise the highest risk group that experienced treatment failure. This finding is consistent with the literature [108,115,116]. Extremity location was a site associated with more incidence of distant failure. In this study, only a few patients with extremity and pelvic tumours were considered for RT only, two of the three patients who had RT only for pelvic tumour, developed recurrence and eventually succumbed to their disease. Patients (n=5/13, 38.5%) who had surgery as well as RT for extremity tumours eventually developed distant recurrence and also succumbed to their disease. Only two patients who had surgery only (n=2/13, 15.4%) developed distant recurrence and subsequently demised.

Historically, comparing outcomes of surgery and RT is not possible since surgically treated tumours are mainly located at the extremities, and are amenable to surgical resection. Those tumours treated with RT, are usually centrally located at the pelvis, spine, head and neck areas, where surgery is not possible without serious injury due to close proximity to many critical structures [13,16,117-120]. In this current study, similar results were obtained for tumours treated with RT only. The incidence of local and distant recurrence in the tumours of the pelvis (n=3/8, 37.5%), the only record of local disease recurrence in our study. For surgery combined with RT for pelvic tumours, there was a higher incidence of distance failure (n=3/4, 75%) but lower incidence of local failure (n=1/4, 25%). Conversely,

various studies assert that there is no significant difference in local control and event-free survival outcomes of patients with Ewing sarcoma treated surgically or with RT [121, 122]. Sixty patients were evaluated in the Memorial Sloan-Kettering study; definitive RT results were reported in 72% of patients with non-extremity tumors and 77% 3-year local control. A significant difference was not detected between definitive RT and preoperative and postoperative RT groups with regard to local control (p= 0.91) [123]. In a study from the University of Florida evaluating 35 patients with Ewing sarcoma who had tumors in the pelvic and sacral regions, 5-year local control was 100% in the postoperative RT arm and 64% in the definitive RT arm. When patient and tumor characteristics were evaluated, it was seen that tumor volume was larger in the definitive RT arm compared to that in the surgical arm [118]. In our study, other non- extremity tumours (truncal, head/neck, spine and kidney), had lower risk of recurrence, with definitive or post-operative RT as compared to surgery only.

Our study further showed that the majority of our patients with recurrence developed pulmonary metastasis (n=15, 83%), and the lungs being the only identifiable site of disease in 50%, while 27.8% for bone or bone marrow metastasis. Stachelek et al, reported similar finding in a cohort of 94 patients with localized Ewing sarcoma, pulmonary metastasis was recorded in 82% of the patients with the lungs being the only site of disease in 53% [124]. In our study, local disease recurrence was recorded in two patients with pelvic tumours, those treated with RT only and then in another treated with combined surgery and RT. Ahmed et.al, reported similar finding which showed that the incidence of local treatment failure was highest in pelvic tumours treated with RT only as compared with surgery ($p< 0.01$) [108]. Moreover, Stachelek et al, also showed similar patient outcome compared to this present study, in terms of disease recurrence and estimated overall survival at 5-years. Twenty-one versus eighteen patients with recurrent disease, fourteen versus fifteen patients having isolated distant recurrence, and three patients each in the respective studies had combined local and distant recurrence. The 5-year disease overall survival in their study was 79 % (versus 77.7% in this present one).

In our series, isolated local disease recurrence was not recorded, however, the importance of achieving good local control cannot be over-emphasized, outcome after local recurrence is often dismal [6]. The type of local control influences outcome. Our study showed that tumour size as well as LDH level at diagnosis were predictors of disease recurrence. Due to selection bias, some patients with large tumour size (≥ 8 cm) in the extremities and pelvis were considered for combined Surgery and RT. Overall, this cohort of patients had the highest cumulative incidence of recurrence as negative surgical margins were not achieved, as they were inadvertently offered intra-lesional excision of tumour. Literature has shown that patients treated with surgery plus RT had similar outcomes to patients treated with surgery alone [10]. Current North American standard practice discourages “debulking” surgeries in which gross residual tumour is anticipated [10]. Instead, combined surgery plus RT is reserved for cases which surgical margins are unexpectedly positive. Our results suggest that disease control is acceptable with this approach. Several studies have reported about the tumour size as an important prognostic factor in predicting disease outcome, with most showing that larger tumour size (≥ 8 cm) or volume in excess of 100-200mls confer poorer EFS or OS [5, 6,116,125-128]. A few studies have also indicated the significance of high base line or pretreatment LDH (two times the normal level) which leads to poorer EFS [27,129,130]. On univariate analysis, lactate dehydrogenase (LDH) level ($p<0.001$) and disease recurrence ($p<0.001$) were the only predictors of mortality in our cohort. Our study did not implicate any other documented prognostic factors such as the age, gender, tumour site, or tumour type as a predictor of any adverse event.

Poor response to neoadjuvant chemotherapy is regarded to be the most important predictive factor affecting local disease control in ES/PNET [9,131,132]. Although local control outcomes are good in localized disease, both local control and overall survival results are poor in disease refractory to chemotherapy [133].

4.1. Limitations of the study

ES/PNET being rare tumours, hence retrospective design of our study was a major limitation as well as the small sample size over a long period, hence the wide confidence intervals in the analysis. Other relevant data such as histological analysis of surgical tissue for pathological response assessment following neoadjuvant chemotherapy, and objective assessment of treatment response using standardized criteria on imaging were not available. Due to the small sample size, subgroup analysis of tumour site versus local treatment modality was not done. Selection bias remains a major confounding factor in the determination of the choice of modality for achieving local control of disease in ES/PNET. Randomized clinical trials in ES/PNET still appear to be practically a difficult study that cannot be carried out to date.

4.2. Recommendations

Multi institution based studies should be conducted locally in-order to further validate the results from this current study.

4.3. Conclusions

Overall disease control in Ewing sarcoma/PNET is comparable for patients treated with surgery or definitive radiation. The risk of local failure is commoner in patients treated with definitive radiation than surgery. The target of local therapy is to choose the most appropriate treatment for any patient, by providing minimum morbidity and best tumour control. Although radiation therapy is frequently applied in unfavourable disease group, local control outcomes are good and in many cases similar to surgical treatment outcomes. Resection of tumour should be avoidable and curative radiation therapy should be planned in tumours that cannot be resected with satisfactory margins. Combined surgery plus RT should be reserved for patients whose surgical margins are unexpectedly positive. Distant failures account for the majority of

relapses in this disease; therefore there is need for better and improved systemic therapies for both local and distant disease control.

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Appendix 1 – Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Ncumisa T. Ndamase (Student number: 1595963) am a student

registered for the degree of Masters in Medicine (MMed) in the academic year 2022.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 01 September 2022

Appendix one [1]

Appendix 2 - CMJAH CEO's Letter

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Email:Nolwazi.Mzila@gauteng.gov.za
Tell: (011): 488-4812
17 September 2018

Dear Dr. N. T. Ndamase

STUDY TITTLE: Local Control of Ewing's Sarcoma/Primitive Neuroectodermal Sarcoma Using Multimodality Approach at Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Hospital Peadiatric Oncology: A Retrospective Review.

Permission to conduct the above mentioned study is provisional approved. Your study can only commence once Ethics approval is obtained. Please forward a copy of your Ethics Clearance Certificate as soon as the study is approved by the Ethics Committee for the CEO's office to give you the final approval to conduct the study.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Clinical Director
DATE: 18/9/2019

Approved / not approved

Ms. G. Bogoshi
Chief Executive Officer
DATE: 18-09-2018

Appendix 3 Chris Hani Baragwanath CEO'S let

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 27th September 2022

TITLE OF PROJECT:

Local Control of Ewing's Sarcoma/Primitive Neuroectodermal Sarcoma using Multimodality Approach at Charlotte Maxeke Academic Hospital and Chris Hani Baragwanath Hospital Paediatric Oncology: A retrospective Review.

UNIVERSITY: Witwatersrand

Principal Investigator: Dr N Ndamase

Department: Radiation Oncology

Supervisor : Prof Poole

Permission Head Department (where research conducted): Yes

NHRD No. GP_202209_031

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

Recommended
(On behalf of the MAC)
Date: 27/09/2022

pp .....
Approved/Not Approved
Hospital Management
Date: 29.09.2022

Appendix 4: Ethics Letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr NT Ndamase
School of Clinical Medicne
Department of Radiation Sciences
Division of Radiation Oncology
Medical School

E-mail: Vinay.Sharma@wits.ac.za

CC: Supervisor: Professor J Poole and Dr J Kotzen <Janet.Poole@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 20/11/2018

REF: *R14/49*

PROTOCOL NO: **M181087** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Local control of ewing sarcoma/primitive neuroecto-dermal sarcoma multimodality approach at Charlotte Maxeke Johanesbug and Chris Hani Baragwanath Academic Hospitals Paediatric Oncology Units*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix four [4]

Appendix 5: Ethics Letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr NT Ndamase

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M181087

NAME: (Principal Investigator)	Dr NT Ndamase
DEPARTMENT:	School of Clinical Medicne Department of Radiation Sciences Division of Radiation Oncology Medical School University
PROJECT TITLE:	Local control of ewing sarcoma/primitive neuroecto-dermal sarcoma multimodality approach at Charlotte Maxeke Johanesbug and Chris Hani Baragwanath Academic Hospitals Paediatric Oncology Units
DATE CONSIDERED:	26/10/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor J Poole and Dr J Kotzen
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/11/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore reports and re-certification will be due early in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

RESEARCH DATA COLLECTION SHEET (Clearance certificate number: M181087)

Name: Y☒ No☐

GT NO: Y☒ No☐ DXT NO: Y☒ No☐ DOB: Y☒ No☐

Age in years: Y☒ No☐

Gender:

Female Y☒

Male Y☒

Race: Black☒ Non-black☒

Weight: Y☒ Height: Y☒

First visit date: Y☒

Tumour site:

Central Y☒ No☐

Extremities Y☒ No☐

Tumour size:

<8cm Y☒ No☐

>8cm Y☒ No☐

Appendix five [5]

Tumour grade:

Grade 3 Y☒ No☐

Grade 4 Y☒ No☐

Histology:

Core biopsy Y☒ N☐

Trephine biopsy Y☒ N☐

EWS Y☒ No☐

PNET Y☒ No☐

Immunohistochemistry:

Synaptophysin Y☒ N☐

S-100 Y☒ N☐

EWS-FL1 Y☒ N☐

NSE Y☒ N☐

CD99 Y☒ N☐

EWSR1 Y☒ N☐

LDH:

Normal Y☒ No☐

High Y☒ No☐

ECOG status:

Good Y☒ No☐

Poor Y☒ No☐

Imaging:

Chest X-ray Y☒ N☐

Plain X-ray Y☒ No☐

CT Scan Y☒ No☐

MRI Scan Y☒ No☐

PET Scan Y☒ No☐

Recurrence Y☒ No☐

Chemotherapy regimen:

1. Euro-Ewings99 Y☒ N☐ Dates: Y☒ N☐

Local treatment modality:

1. Surgery alone Y☒ N☐
2. Radiotherapy alone Y☒ N☐
3. Surgery and radiotherapy Y☒ N☐

Surgery:

Date Y☒ N☐

Radiotherapy:

Start date Y☒ N☐
Completion date Y☒ N☐

Radiation dose:

1. 36.0 GY Y☒ N☐
2. 45.0 GY Y☒ N☐
3. 50.4 Gy Y☒ N☐
3. 55.8 Gy Y☒

Local effects:

Erythema Y☒ N☐
Dry desquamation Y☒ N☐
Skin hyperpigmentation Y☒ N☐
Soft tissue fibrosis Y☒

Follow up status:

Alive Y☒ N☐

Dead Y☒ N☐