Title page

Original Article

Title: Adolescent-Adult Non-metastatic Ewing sarcoma- Experience from a large developing country

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Word Count for:

Abstract: 239

Main Text (excludes title page, abstract, Conflicts of Interest, Acknowledgments, References, Tables, Figures, and Legends):3106

Total number of Tables: 3

Total number of Figures: 3

Total number of supporting information files: 1

Short running Title: Adolescent-Adult Non-metastatic Ewing sarcoma

Keywords: Non-metastatic; Ewing sarcoma; Adolescent-adult; EFT-2001; Low-middle income countries (LMICs)

Funding: No research support received for this study

Institutional Ethics Committee Permission: Taken

Abbreviations

ES	Ewing Sarcoma
LMIC	Low and middle income country
EFT-2001	Ewing's family of tumors-2001
COG	Children's oncology group
ICT	Induction chemotherapy
MCT	Maintenance chemotherapy
PS	Performance status
LDH	Lactose dehydrogenase
SAP	Serum alkaline phosphatase
OS	Overall survival
EFS	Event free survival

Abstract

Background

Outcomes of Ewing sarcoma (ES) in low and middle income countries lags behind the rest of the world owing to multiple tumoral, logistical and socio-economic factors. The data of outcomes and toxicity in these countries is sparse, especially in the adolescent and adult (AA) population and merits exploration

Procedure

This was a retrospective analysis of prospectively collected data of non-metastatic AA-ES patients, who received standard institutional combination chemotherapy regimen (EFT-2001) along with surgery or definitive radiotherapy. Various cohorts were analyzed for treatment-related toxicities, event- free survival (EFS) and overall survival (OS).

Results

There were 235 patients (primary safety cohort, PSC) with median age of 23 years. One hundred and ninety six were treatment naïve (primary efficacy cohort, PEC) and of these 119 had surgery. In PEC, at a median follow up of 36.4 months, estimated 5 year EFS and OS were 60.9% (95% CI 53.1% - 69.9%) and 84.5% (95% CI 77.7% - 91.9%), respectively. Of these, 158 complying with intended treatment, had an estimated 5 year EFS of 63.1% (95% CI 54.8%-72.6%). In multivariate analysis, good prognostic factors included longer symptom duration, ≥ 99% necrosis and treatment completion. Among PSC, grade 3-4 toxicities were febrile-neutropenia (50.6%), anemia (55.3%), peripheral neuropathy (15.7%), with 3 (1.3%) chemo-toxic deaths.

Conclusions

The outcomes of AA non-metastatic ES patients treated with EFT-2001 regimen were comparable to those reported by others, with acceptable toxicity and can be considered as standard-of-care, especially in LMICs .

Keywords: Non-metastatic; Ewing sarcoma; Adolescent-adult; EFT-2001; Low-middle income countries (LMICs)

Introduction

Ewing sarcoma (ES) is the second most common bone and soft tissue tumor in children and young adults, comprising 15% of all primary bone tumors. Ewing sarcoma family of tumors includes skeletal ES, extra-osseous ES, Askin's tumors, and peripheral neuro-ectodermal tumors[1]. It arises from primitive neuroepithelial cells and has a propensity for early micro-metastasis to distant locations. Management of these tumors includes use of multiple modalities like aggressive chemotherapy, limb salvage or amputation surgery and radiation The multidisciplinary management of non-metastatic ES has resulted in 5-year overall survival (OS) of around 70-80%. However, there is a considerable (~20%) gap in survival statistics between the high and low-middle income countries (LMICs)[2,3]. Hurdles such as finances, malnourishment, poor patient literacy, treatment abandonment, delayed and advanced presentation lower the outcomes in LMICs[4]. Inadequate compliance to treatment protocols has a direct implication on the survival in these tumors[5]. Additionally, these risk factors have led to poor tolerance of the standard western dose dense regimens like the Children's oncology group protocol, and have led to adoption of more practical and less toxic regimens in LMICs, especially among the vulnerable population[6,7]. Adolescents and adults (AA) make up the major group of patients with ES and currently, there is sparse data available regarding outcomes and prognostic markers especially from LMICs, including India and merits exploration. The current

study analyzed the toxicity, survival outcomes and prognostic markers of these patients, treated at our institution with an in-house protocol and compared them with published literature.

Methods

This was a retrospective analysis of prospectively collected data of histologically confirmed non-metastatic ES patients in AAs ,over 15 years of age, who were worked up and received at least partial treatment at Tata Memorial Centre, a tertiary oncological centre, between January 2013 to December 2018 with the standard in-house Ewing family of tumors (EFT)-2001 regimen[8,9]. Before starting treatment, all patients had radiographs and magnetic resonance imaging of the affected area. Staging workup included a whole-body Positron emission tomography - computed tomography (PET-CT) scan, complete haemogram, renal and liver function tests, serum lactate dehydrogenase (LDH) echocardiography, and diethylenetriaminepentaacetic acid scan (in some cases) to assess baseline organ functions and fitness for receiving chemotherapy. Anemia was defined as per WHO criteria i.e. hemoglobin (Hb) <12 gm/dl in adult females, <13 gm/dl in adult males, and hypoalbuminemia as albumin <3gm/dl. Patients were also referred to a nutritionist, as per clinicians' discretion and deficiencies were corrected by using intravenous (IV) or oral supplements, as appropriate. Patients were counseled for fertility preservation options like sperm banking before initiation of treatment. The in-house standard EFT -2001 chemotherapy protocol (49 weeks duration),

as shown in figure 1, was used with primary granulocyte colony-stimulating factor (G-CSF) prophylaxis. It consisted of sequential cycles of VIME (V=Vincristine 1.5mg/m2 on day 1, I= Ifosfamide 2000 mg/m2 day1 to day5, M=Mesna 600 mg/m2 at 0,3,6 and 9 hrs of ifosfamide, E= Etoposide 100mg/m2 day1 to day5) and VAC (V=Vincristine 1.5mg/m2 on day 1, A= Adriamycin 60mg/m2 on day 1, C= Cyclophosphamide 600mg/m2 on day 1) in the induction phase followed by VCD (V=Vincristine 1.5mg/m2 on day 1, C= Cyclophosphamide 600mg/m2 on day 1, D= Actinomycin-D 1mg/m2 on day 1), VIME and VAC sequences in the chemo radiation and maintenance phase. Some non-extremity ES patients were also treated with standard Children's Oncology Group (COG) protocol[6]. Planned dose reductions in subsequent cycles were based on the occurrence of clinically significant hematologic and/or non-hematologic toxicities and as per clinician's discretion. Toxicities were documented using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 till November 2017 and thereafter version 5.0[10,11]. After completion of the induction chemotherapy (ICT) at 10 weeks, patients were reassessed with PET scans and underwent either surgery (limb salvage or amputation for extremity lesions, or wide local resection for non- extremity lesions) or definitive radiation therapy (RT), based on the extent and clinico-radiologic response. The histological response was assessed using Huvos' necrosis grading[12]. However, grade of histological necrosis did not lead to alteration in the chemotherapy protocol. Patients with positive margins, significant

tumor burden at the time of presentation, contamination of tumor by prior biopsy or other means, and poor necrosis were considered for adjuvant RT after discussion in our multidisciplinary tumor board. In patients, receiving definitive RT, another response assessment PET-CT scan was done at 12 weeks of completion of RT. Follow up data was retrieved from EMR as well as updated via telephonic follow-up. The study was conducted according to the good clinical practice guidelines, and the guidelines laid by the Indian Council for Medical Research. Institute review board permission was obtained.

Statistical Analysis:

The data was entered into an excel worksheet and analyzed using the Statistical Package for Social Sciences (SPSS), software version 24 (SPSS, Chicago, IL). Standard statistical techniques were applied for descriptive statistics like mean (standard deviation), median [range or interquartile range, or 95% confidence intervals (95%CI)] for quantitative variables, and the chi-square test was used for comparison of qualitative variables. Descriptive statistics were represented as median or percentage, and various comparisons were made using the $\chi 2$ test or Mann-Whitney U test, as appropriate. Survival was estimated using the Kaplan- Meier method and compared using the log-rank test. Primary efficacy cohort (PEC) included all the treatment naïve enrolled patients and patients who were lost to follow-up were appropriately censored. Additionally, a per-protocol (PP) analysis was also conducted in patients who complied with

the intended protocol with resultant completion of treatment or non-completion of treatment due to progressive disease or tolerance issues. The factors found to be significant on univariate analysis were subsequently tested in multivariate analysis to identify independent prognostic predictors. Toxicity was reported for the entire cohort (primary safety cohort, PSC) who received EFT-2001. Event-free survival (EFS) was defined as the duration between the date of diagnosis and the date of first event, inclusive of progression without complete remission, relapses following complete remission, second malignancy, or death, whichever occurred first. Overall survival (OS) was defined as the duration between the date of diagnosis and the date of death from any cause or date of last follow-up in patients who were still alive. Follow up details were collected from electronic medical records and by telephonic follow-ups and censoring was done appropriately. The data cut-off date was considered as 30th September 2020. Post-relapse survival (PRS) was defined as the time from the date of relapse until the last documented follow-up or death. Various potentially prognostic factors were correlated with survival outcomes as below:

i) Patient-related factors: baseline age, gender, comorbidities, Eastern Cooperative Oncology Group (ECOG) performance status, and nutritional parameters (Hb, albumin), duration of symptoms.

ii) Tumor related factors: tumor burden indicators (size, serum LDH, and alkaline phosphatase (SAP)), primary site, presence of pathological

fracture or skip metastasis, regional lymph node involvement, post-ICT necrosis.

iii) Treatment related factors: local therapy (surgery vs RT vs both), and grade ≥ 3 treatment-induced toxicities.

Results

There were 1169 ES patients registered at our center during the 5 year period from 2013 to 2018, of which nearly half were either metastatic (37.4%), recurrent (8.2%), or with doubtful metastases (5.2%). The remaining 575 (49.2%) were non-metastatic. Of the non-metastatic patients, 308 constituted the AA cohort, and 235 were offered EFT-2001 protocol and analyzed. These patients formed the primary safety cohort (PSC). Of the remaining AA cohort, 37 patients received the COG protocol, 7 received other multidrug chemotherapy protocol, 1 patient with aplastic anemia was considered for palliative intent therapy, while the rest did not take any treatment at our center (Figure 2). Of the 235 (100%) patients treated with EFT-2001 protocol, 196 (83.4%) were treatment naïve and comprised the primary efficacy cohort (PEC), while 39 (16.6%) were prior treated. The median age among the whole cohort (PSC) was 23 (range 15-61) years with 23(9.8%) ≥40 years of age. A majority were male patients (n=159, 67.7%). Skeletal primary was noted in 155 (66%), extremity was the site of primary in 114 (48.5%) and the commonest sub-site was femur (n=30, 12.8%). The baseline median tumor size was 8.8 cms (IQR 5.4 –

11.5 cm) serum LDH and SAP were elevated in 66.7% and 42.9%, respectively and 52% were anemic (Table 1).

Among the primary efficacy cohort (n=196), 140 (59.6%) completed the intended treatment inclusive of the ICT, local therapy and MCT. Treatment was stopped by treating oncologists due to progression in 13 (5.6%) and toxicity in 5 (2.1%) cases. These 158 patients (140+13+5) (68.1%), constituted the per-protocol (PP) cohort (Figure 2). The remaining 17 (7.2%) defaulted prior to local therapy and 21 (8.9%) after local therapy and were not included in the PP population (Figure 2). Among the PEC, local modality was surgery in 119 (60.7%) patients and 2 (1.7%) had positive margins. Necrosis of ≥90% was observed in 55 (46.2%) and ≥99% in 35 (29.4%) patients. Definitive RT was delivered in 69 (29.4%) patients. Post 12 weeks of definitive RT, complete metabolic response on PET was documented in 45 (65.2%) of these patients.

Survival Outcomes

I) Primary efficacy cohort (n=196)(Figure 3A, 3B)

The median follow-up in surviving patients was 36.4 (Interquartile range (IQR) 20 – 55) months.

a) Event-free survival (EFS) and patterns of failure:

At the time of analysis, the median EFS was 82.2 (95% CI 82.2- NA) months. Estimated 5-year EFS was 60.9% (95% CI 53.1% - 69.9%). At the time of analysis, there were 60 (30.6%) events- 44 (22.5%) relapses and 13 (6.6%) on treatment progressions and 3 chemotoxic deaths (1.5%) deaths. Among the relapse/progressions, 25 (12.8%) had distant failures,

17 (8.7%) had loco-regional and 15 (7.7%) had both distant and loco-regional failures.

b) Overall survival:

There were 21 deaths of which 18 (9.1%) were disease related while 3 (1.5%) were due to chemotherapy toxicity. At the time of analysis, the median OS was not reached. The OS estimates at 5 years was 84.5% (95% CI 77.7% - 91.9%). The median PRS was 32.6 (95% CI 27.8- NA) months.

II) Per-protocol analysis (n=158):

The median follow-up in surviving patients was 39 (IQR 26- 57) months in PP population.

a) Event-free survival:

At the time of analysis, the median EFS was not achieved. Estimated 5-year EFS was 63.1% (95% CI 54.8%-72.6%). There were 48 (30.3%) events including 34 (21.5%) relapses, 13 (8.2%) on treatment progressions, and 3 (0.6%) chemo-toxic deaths. Of these relapses/progressions, -21 (13.3%) were distant, 12 (7.6%) were loco-regional and 14 (8.9%) had both failures. The median PRS was 27.9 (95% CI 4.1 – 51) months.

b) Overall survival:

Median OS was not achieved. 5 year estimates of OS were 91.6% (95% CI 86.1% - 97.5%). Of the 10 deaths, 9 (5.7%) were disease related and one (0.63%) was attributed to chemotoxicity.

III)Prior treated cohort (n=39):

The median follow up was 31 (IQR 11 – 36.6) months in patients who received any form of treatment prior to reaching our institute.

a) Event-free survival:

Median EFS was 33 (95% CI 22.1 - NA) months. Estimated 5-year EFS was 48% (95% CI 32.6% - 70.7%).

b) *Overall survival*:

Median OS was not achieved. The OS estimate at 5-year was 55.8% (95% CI 34.3% - 90.8%). Median PRS was not achieved.

IV) Primary safety cohort :(*n=235)*

The median follow up of surviving patients was 35 months (IQR 20 – 52 months).

a) Event-free survival (EFS) and patterns of failure:

At the time of analysis, the median EFS was 82.2 (95% CI 53 - NA) months. Estimated 5-year EFS was 57.6% (95% CI 50.2% - 66.1%). At the time of analysis, there were 77 (32.8%) events; 51 (21.7%) relapses, 16 (6.8%) progression on treatment, 10 deaths of which 7(3%) were disease related and 3 (1.3%) were due to chemotoxicity. Among the relapses/progressions, 32 (13.6%) had distant failures, 17 (7.2%) had loco-regional and 18 (7.7%) had both distant and loco-regional failures.

b) Overall survival:

There were 30 deaths, 27 (11.4%) disease related, and3 (1.5%) due to chemotherapy toxicity. At the time of the analysis, the median OS was not reached. The OS estimate at 5 years was 80.8% (95% CI 74% - 88.3%). The median PRS was 32.6 (95% CI 23- 42) months.

Toxicity (n=235)(Table 2A)

Toxicity analysis was carried out in the entire cohort which comprised the primary safety population (n=235). Significant grade 3 or more hematological toxicities were febrile neutropenia in 119 (50.6%), thrombocytopenia in 76 (32.3%), and anemia in 130 (55.3%). Among the non-hematological grade 3/4 toxicities, peripheral-neuropathy was seen in 37 (15.7%), hepatotoxicity in 17 (7.2%), diarrhea in five (2.1%), constipation in three (1.3%), stomatitis in 3 (1.3%) and cardiac toxicity in 5 (2.1%).There were additional 46 (20%) patients who had grade 2 peripheral neuropathy. The permanent dose modifications for the entire regimen were carried out predominantly for hematological toxicities in 34 (14.5%) cases. There were transient or permanent alterations in vincristine doses and /or frequency of administration in additional 83 (35.3%) cases due to peripheral neuropathy (≥ grade2), predominantly during the maintenance phase.

Factors Correlating with Outcomes:

Factors found significant in univariate analysis are shown in supplemental Table S1.

Multivariate analysis (table 3): In primary safety cohort (N=235), non-visceral primary (HR=0.33,p= 0.028),tumor size ≤8cm (HR=0.47,p= 0.004), surgery as local therapy (HR=0.58, p=0.040), completion of treatment (HR= 0.31, p<0.001) and necrosis of ≥99% (HR= 0.22 ,p= 0.002) in surgically treated patients, were independent predictors for superior EFS.

In the primary efficacy cohort (n=196), longer symptom duration (HR- 0.93, p=0.033), completion of treatment (HR= 0.32; p<0.001) and necrosis of ≥99% (HR=0.30,p= 0.012) in surgically treated patients were independent predictors for superior EFS .

Multivariate analysis couldn't be performed in case of OS for the less number of events. However univariate analysis showed extremity primary (HR=0.43, p=0.037) was a good prognostic factor for OS.

Discussion:

There were 1169 ES patients registered during the study period. A higher proportion of metastatic patients (37.4%) reflect a possible referral bias to our center, which is among the leading tertiary cancer centers of South-East Asia. A significant proportion of patients present with significant delays in diagnosis, with or without prior treatment, and resultant upstaging, which is common in LMICs[13]. Among the treatment-naive cases, 28% were metastatic and is comparable to other studies[14,15].

Among the entire cohort (N=235), the median age was 23 years which appears slightly higher. However, this study population included was over 15 years of age and is comparable with other studies[16,17]. Male predominance was noticed in our cohort, similar to others literature[13,16].Our findings of the majority of ES being skeletal and the femur as the commonest primary site are in sync with the SEER database and other studies[17–19]. In the pre-chemotherapy era, less than 20% of patients with ES survived. In the current era, with the use of multimodality therapy, EFS

rates have increased to 60-70% for localized disease[6,20–23] .Our 5-year EFS is 60.9% (95% CI 53.1% - 69.9%) and 63.1% (95% CI 54.8%-72.6%), in the PEC and in the per-protocol population, respectively(Table 2B). Notably, these internationally comparable outcomes with the in-house EFT-2001 protocol were achieved, despite the majority of the patients being nutritionally dispossessed with large tumor loads (reflected by higher LDH, SAP, and large tumor size)[6,24]. Outcomes of prior untreated population were significantly superior to prior treated patients; among prior untreated patients, those who completed intended treatment (PP population) had a superior outcome (Figure 3 C and D). This emphasizes the need to educate the community including practitioners, for timely referral to tertiary cancer centers with adequate expertise[13,25]. Not surprisingly, completing the intended treatment (compliance) had a positive impact on the outcome and is supported by literature[5] Treatment abandonment is quite prevalent in most LMICs due to socio-economic factors, illiteracy, poor logistics and social stigma[26–28].

We observed no statistically significant correlation of age and gender with survival as noted in other studies[16,29].Patients with longer symptom duration, indicative of relatively indolent disease, had better prognosis as reported in another study as well[30]. Larger tumor size in our study predicted worse EFS as concluded by other studies as well[31,32].Extremity primary fared better in univariate analysis, in concurrence with literature ; however, in multivariate analysis, only non-visceral primary was found as

an independent prognosticator[33]. Surgery as definitive therapy was associated with superior survival as shown by other studies as well[34,35]. Based on current evidence, surgery is the preferred local modality of treatment at our center (64%) which is in accord with the widely prevalent practice worldwide[36]. Majority had limb-salvage surgery with negative margins (96.5%), indicating the high-quality of oncological treatment at our institute. Tumor necrosis ≥99% was independently associated with better EFS which is in sync with published studies[12,37]. Most common grade 3/4 toxicities such as febrile neutropenia, thrombocytopenia, and anemia are comparable to other intensive protocols[6,38,39]. Notably, nutritional deficiencies and higher tumor burden along with delayed presentation, collectively lead to anemia of chronic disease which is also a contributing factor, especially in LMICs[29]. The incidence of grade 2 and above peripheral neuropathy was higher than other studies, requiring dose or frequency modifications in 35.3% (predominantly in maintenance phase), perhaps due to higher cumulative vincristine dose (58.5 mg/m2) in our protocol[38,39]. Another study further confirms that peripheral neuropathy is predominantly seen in the adult rather than in the pediatric age group[40]. Notably, there is differential tolerance in young children and adults and demands precision therapy with possibly less VCR dose and frequency in adults especially during maintenance phase. There was 3.4% cardiac toxicity requiring intervention, which is comparable to published literature[12].

The study has limitations including its single institute, non-randomized nature with inherent biases. However, conducting randomized studies in rare tumors like ES are extremely challenging. Meaningful inferences can be drawn from good-quality studies wherein relatively large numbers of patients are treated uniformly, like the current study[29]. Though, not a population-based study, our cohort represents a large majority of patients treated in the real-world setting, which can be generalized and applied in similar populations. Notably, in LMICS, with resource constraints, post relapse follow-up is a challenge especially when the patients are not on active treatment. Patients who defaulted were appropriately censored at their last recorded follow up visit. This may lead to pseudo-inflation of the OS. However, EFS remains a robust outcome measure as variations in treatment, compliance and post relapse follow-up can affect the OS.

Conclusion

The outcomes and toxicity of AA patients treated with in-house EFT-2001 protocol in this relatively large cohort of uniformly treated patients from India are comparable to the international studies and are widely applicable especially in LMICs. EFT-2001 chemotherapy regimen can be considered standard of care for adolescent and adult non-metastatic ES patients. Treatment naïve patients who comply with the intended treatment fared better re-emphasizing the need to educate the community and caregivers regarding optimal referral and compliance to treatment in non- metastatic Ewing sarcomas.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Figure and table legends

Table 1: Baseline characteristics

Table 2: Outcome comparison with published literature

Table 2A (Safety comparison): Grade 3 or higher toxicity comparison with published literature:

Table 2B (Efficacy comparison): Survival comparison with published literature

Table 3: Significant prognostic factors in multivariate analysis of event free survival (EFS)

Figure 1: EFT-2001 chemotherapy protocol consisted of sequential cycles of VIME and VAC in the induction phase followed by VCD, VIME and VAC sequences in the chemo radiation and maintenance phase.

V=Vincristine 1.5mg/m2 iv push on D1(Maximum dose of vincrsitine was 2mg)

I= Ifosfamide 2000 mg/m2 iv infusion over 2 hrs D1-D5,

M=Mesna 600 mg/m2 at 0,3,6 and 9 hrs of ifosfamide,

E= Etoposide 100mg/m2 iv over 1−2 hours D1-D5

A= Adriamycin 60mg/m2 m2 iv over 6 hours on D1,

C= Cyclophosphamide 600mg/m2 iv over 30 minutes on D1,

D= Actinomycin-D 1mg/m2 iv push on D1.

Figure 2:Consort diagram of this study. ES= Ewing sarcoma, ICT=Induction chemotherapy; RT=Radiotherapy; ECRT=Extra-corporeal radiotherapy; EBRT=External beam radiotherapy.

*these two groups constituted the per-protocol (PP) cohort.

Figure 3:

Figure 3A. Event free survival (EFS) of primary efficacy cohort

Figure 3B. Overall survival (OS) of primary efficacy cohort

Figure 3C.Comparison of event free survival (EFS) of prior untreated (primary safety cohort) and prior treated cohort.

Figure 3D. Comparison of overall survival (OS) of prior untreated (primary safety cohort) and prior treated cohort.

Supplemental Table S1: Significant factors in univariate analysis for event free survival (EFS) and overall survival (OS)