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ARTICLE

Sinusoidal obstruction syndrome/veno-occlusive disease after high-dose intravenous busulfan/melphalan conditioning therapy in high-risk Ewing Sarcoma

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Abstract

This mono-institutional observational study was conducted to determine incidence, severity, risk factors, and outcome of sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) in high-risk Ewing sarcoma (ES) patients treated with intravenous busulfan and melphalan (BU-MEL) followed by autologous stem cell transplantation (ASCT). During the past 10 years, 75 consecutive ES patients resulted evaluable for the analysis. After diagnosis of SOS/VOD, defibrotide therapy was started as soon as the medication was available. The variables analyzed as potential risk factors were: gender, patient's age at diagnosis, primary tumor site, disease stage, and prior radiation therapy (RT) given, focusing on RT liver exposure. The median age at diagnosis was 18.8 years. Five patients developed moderate to severe SOS/VOD (cumulative incidence, 6.67%). None of 32 pediatric patients (≤17 years) developed SOS/VOD ($p = 0.0674$). In univariate analysis, prior RT liver exposure resulted statistically significant ($p = 0.0496$). There was one death due to severe SOS/VOD. This study reports the largest series of high-risk ES patients treated with intravenous BU-MEL before ASCT. The incidence of SOS/VOD was lower when compared with other studies that used oral busulfan. Any prior RT liver exposure should be avoided. Earlier defibrotide treatment confirms to be effective.

Introduction

Ewing sarcoma (ES) family tumors are the second most common malignant bone tumor in children, adolescents, and young adults [1]. Patients have historically been stratified as high-risk according to presence of metastases, large tumor volume, and poor response to induction chemotherapy [2–5]. The consolidation therapy combining busulfan and melphalan (BU-MEL) followed by autologous stem cell transplantation (ASCT) have been evaluated in several clinical trials for high-risk ES patients improving the prognosis [6–11], although its role remains under investigation.

Sinusoidal obstruction syndrome (SOS), previously called veno-occlusive disease (VOD), is a potential fatal complication that can occur after ASCT [12]. Defibrotide has demonstrated safety and efficacy in treating severe SOS/VOD [13, 14], with more favorable outcomes when used early after clinical diagnosis [15, 16]. Recently, the European Society for Blood and Marrow Transplantation (EBMT) proposed new definitions for diagnosis and severity criteria for SOS/VOD in children and adults, in order to allow an earlier identification of patients requiring immediate therapeutic intervention [17, 18].

The incidence and severity of SOS/VOD in patients who received ASCT varies across different prospective studies,

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depending upon the study design, the patient populations, and the clinical criteria used for the diagnosis [19]. Most of the studies reporting the incidence of SOS/VOD in ES patients used oral busulfan in the conditioning regimen before ASCT [6–11]. Studies based on busulfan pharmacokinetics have shown that the absorption and metabolism of oral busulfan have wide inter-patient variability, resulting in more severe adverse effects associated with elevated busulfan levels [20–22]. The development of intravenous busulfan has led to reduced pharmacokinetic inter-patient variability for both children and adults [23, 24], thus reducing the risk of related toxicity [25, 26].

To date, the use of intravenous busulfan in high-risk ES was reported for limited cohorts of patients [27, 28]. Since 2007, the Rizzoli Orthopaedic Institute, Bologna, has started the transplant activity for high-risk ES patients by using intravenous busulfan in the BU-MEL conditioning regimen before ASCT. The purpose of this mono-institutional observational study was to analyze incidence, severity, risk factors, and outcome of SOS/VOD in this patient population.

Patients and methods

Patient eligibility

Between January 1, 2007 and December 31, 2016, high-risk ES patients aged 0–39 years were included in this study if they were treated in our institution with intravenous BU-MEL and ASCT according to protocols reviewed and approved by the institution review-board and ethical committee [6, 7, 29–32]. Follow-up of patients was through June 30, 2017. High-risk ES patients were considered if they had (1) localized disease histologically or radiologically poorly responsive after initial chemotherapy, (2) lung or pleural metastases or single bone metastasis, (3) disseminated disease or bone marrow metastasis, and (4) relapsed disease with metastases. Patients with histological poor chemotherapy-induced necrosis or with radiological incomplete disappearance of the soft tissue component were considered as poor responders [7, 33]. Presence of metastases was investigated using computed tomography (CT) of the chest, bone scintigraphy or positron emission tomography (PET), and bone marrow (BM) biopsies. Informed consent was obtained from all patients or legal guardians. Only data from those patients who completed the treatment and were followed at Rizzoli Institute over the period were used for this analysis.

Local therapy

Surgery aimed to obtain wide margins was the preferred local treatment. Radiation therapy (RT) was given whenever surgery was not feasible or was marginal or intralesional [34]. RT of the primary site was delivered with hyperfractionated-accelerated modalities, with a total planned dose from 42 Gy to 54 Gy [6, 33]. RT was planned and performed using 3D conformational RT or intensity modulated radiation therapy in order to obtain the optimal planning target volume dose coverage and the least possible exposure of the surrounding organ-at-risk. The doses and volume of the involved organ were quantified by the dose–volume histogram analysis. The minimum rest period from the end of RT and the beginning of BU-MEL was 6 weeks.

Stem cell collection

In case of BM involvement, bilateral BM aspirates were performed before stem cell collection to confirm clearance of the bone marrow. Autologous peripheral blood stem cells collected after mobilizing chemotherapy course and granulocyte colony-stimulating factor (GCSF) stimulation were used in all cases. The recommended cell dose per procedure was $\geq 3 \times 10^6$ CD34 cells/kg of body weight (BW).

Pretreatment evaluations

Before ASCT, all patients underwent full re-staging, including chest CT, bone scintigraphy or PET, and standard radiographs or magnetic resonance imaging or CT of the primary site. All patients underwent baseline liver ultrasonography and were assessed for adequate renal, cardiac, pulmonary, neurological, and liver function, including serum liver examinations.

Conditioning regimen

The BU-MEL conditioning regimen consisted of intravenous busulfan (Busilvex; Pierre Fabre Medicament, Boulogne, France), four daily doses calculated on kgBW over 4 days (days −6 to −3) combined with melphalan (on day −2), followed by stem cell rescue on day 0. Intravenous busulfan doses were modified according to kgBW (1.0 mg/kg for BW <9 kg; 1.2 mg/kg for BW ≥9 kg to <16 kg; 1.1 mg/kg for BW 16–23 kg; 0.95 mg/kg for BW >23 kg to ≤34 kg; and 0.8 mg/kg for BW >34 kg). Intravenous melphalan (140 mg/m^2) was given 24 h after the final busulfan dose. ASCT was performed 48 h after melphalan.

Supportive care

All patients received anti-seizure prophylaxis, standard antimicrobial prophylaxis, and oral ursodeoxycholic acid. GCSF (5 µg/kg per day) was administered from day 3 after ASCT to the end of neutropenia.

Definition of SOS/VOD

Diagnosis of SOS/VOD was defined by the Baltimore criteria [35], without the classical limit of 21 days after ASCT: presence of bilirubin ≥2 mg/dl and at least two of the following clinical findings: (1) ascites, (2) unexplained weight gain ≥5% above baseline, (3) hepatomegaly increased above baseline. Severity was graded according to the Bearman toxicity scale [36]. Liver ultrasonography was performed at the time of the clinical diagnosis of SOS/VOD to confirm hepatomegaly and/or ascites and to exclude differential diagnoses [37]. Liver ultrasonography with doppler evaluation was performed to confirm complete remission (CR). CR was defined as the resolution of all signs and symptoms of the SOS/VOD diagnostic criteria. The diagnostic and severity criteria used were retrospectively re-defined by using the new diagnostic criteria and severity-grading system proposed by the EBMT [17, 18].

Treatment of SOS/VOD

Defibrotide therapy was started as soon as the medication was available after diagnosis of SOS/VOD at the dose of 6.25 mg/kg BW every 6 h until CR and for a minimum of intended duration of 21 days.

Risk factors for SOS/VOD

The variables analyzed as potential risk factors were: gender, patient's age at diagnosis (≤17 years and 18–39 years), primary tumor site (extremity and axial skeleton), disease stage (localized and metastatic), and prior RT given, focusing on the analysis of prior RT liver exposure. All patients with metastatic disease at diagnosis or at relapse were considered together when compared with patients with localized disease.

For the evaluation of RT liver exposure, mean absorbed dose (Gy) to the liver and liver volume percentage that received more than 30 Gy were used as reference values, which values are reported as significant for the occurrence of radiation-induced liver disease [38]. For the purpose of the study, liver volume percentage that received more than 12 Gy was also calculated. The patients who received RT with calculated liver exposure were considered separately from all other patients who received RT.

Statistical analysis

Patient characteristics, follow-ups, and outcomes data were collected in our institutional database.

The cumulative incidence of SOS/VOD was estimated by cumulative incidence functions, presented with 95% confidence intervals. Survival status of SOS/VOD patients was calculated at day +100 post-transplant. Patient characteristics and clinical data were compared using χ^2 tests (or Fisher exact test), as appropriate. The variables considered for univariate analysis were: gender, patient's age at diagnosis, primary tumor site, disease stage, and prior RT grouped as no RT, RT with liver exposure, or other RT. Statistical significance was defined as a two-tailed P-value <0.05.

Survival data were calculated for patients with follow-up greater than 5 years and excluding patients enrolled in ongoing treatment protocols [30, 31]. The Kaplan–Meier method was used to estimate 5 years overall survival (OS) and event-free survival (EFS). OS was the time from the start of chemotherapy to death or the last follow-up. EFS was the time from the start of chemotherapy to disease progression, recurrence, second malignancies, death from treatment-related complications, or the last follow-up. Statistical analyses were performed using SAS software 9.3 (SAS Institute Inc., Cary, NC).

Results

Patient characteristics

During the 10-year period, 77 consecutive ES patients treated with ASCT after intravenous BU-MEL were eligible for this study. Two patients were excluded from the analysis: one patient had central venous catheter-related sepsis during busulfan treatment and did not complete the conditioning regimen; another patient resulted lost to follow-up once returned to the country of origin shortly after completing the treatment. Therefore, 75 patients who completed the treatment BU-MEL with ASCT and were followed at our institution were evaluable. Characteristics of the 75 evaluable patients are shown in Table 1. The median age at diagnosis was 18.8 years (range 3.9–39.4 years). Forty-eight (64%) patients were male and 32 (43%) were children (≤17 years old). Twenty-six (35%) patients had axial skeleton location and 33 (44%) had metastatic disease at diagnosis or at relapse. Thirty-two patients (43%) underwent RT, nine children and 23 adults. Twenty patients (four children and 16 adults) received RT on the axial skeleton. Among these, six patients received spinal RT with a total dose between 42 and 54 Gy.

Incidence and severity of SOS/VOD

Five out of 75 evaluable patients met the SOS/VOD criteria described above, with cumulative incidence of 6.67% (95% CI: 2.83–15.28%). The clinical diagnosis of SOS/VOD was based on the Baltimore criteria, considering late SOS/VOD

Table 1 Clinical characteristics of the evaluable patients ($n = 75$)

Characteristic	No. of patients	%
Gender		
Male	48	64
Female	27	36
Age at diagnosis		
Median, years	18.8 (3.9–39.4)	
<17	32	43
>18	43	57
Primary tumor site		
Extremity	49	65
Axial skeleton		
Pelvis	16	21
Spine	5	7
Ribs	2	3
Other	3	4
Disease stage		
Localized	42	56
Metastatic		
at diagnosis	26	35
at relapse	7	9
Metastatic sites		
Lung only	21	
Bone	6	
Lung + bones	5	
Lung + bone + lymphnodes	1	
Local treatment		
Surgery	43	57
Radiation Therapy	21	28
Surgery + radiation therapy	11	15

onset too, and corresponded in all cases with the diagnostic criteria proposed by the EBMT [17, 18]. Clinical characteristics of patients at the time of SOS/VOD diagnosis are shown in Table 2. All five patients were ≥18 years old, with a median age of 20 years (range 18–37 years). The median time within SOS/VOD occurred after ASCT was day 27 (range day 8–40). All patients required supportive care in the hospital setting. There was one death due to multi-system organ failure for SOS/VOD. This patient started as Bearman grade II-EBMT grade severe SOS/VOD and was the only case in which a delay in starting defibrotide therapy occurred due to temporary unavailability of the drug in the country along the year 2010. Progressive SOS/VOD after defibrotide therapy occurred. An attempt with intravenous tissue plasminogen activator (rTPA) was done but the patient subsequently died due to multi-system organ failure, thus retrospectively defined as Bearman grade IV. Four patients started defibrotide treatment within 3 days from clinical diagnosis. These patients achieved CR at day 3, 3, 5, and 13, respectively, after starting defibrotide treatment. At clinical presentation, all patients were SOS/VOD grade II according to Bearman toxicity scale [36], whereas the severity re-definition according to the EBMT severity-grading system [18] resulted moderate in two cases and severe in three cases.

Survival outcome

The overall mortality to day +100 post-transplant was one out of five patients with SOS/VOD (20%). Survival data were calculated for 31 ES high-risk patients with follow-up >5 years: 13 patients with localized poorly responsive disease, eight patients with lung-only metastases or single bone metastasis, three patients with disseminated disease, and seven patients with relapsed disease. Eleven were pediatric patients. At analysis, 20 (64.5%) of 31 patients were still alive. With a median follow-up of 81 months after diagnosis (range: 10–132 months), 17 (54.8%) patients were event free. There was one toxic death due to severe SOS/VOD, as described above. Ten patients died because of progressive disease. The 5-year OS rate and EFS rate in the 31 patients were 67.74% (95% CI: 48.35–81.16) and 57.89% (95% CI: 38.72–72.98), respectively (Fig. 1).

Analysis of risk factors for SOS/VOD

The univariate analysis of risk factors analyzed is shown in Table 3. Prior RT liver exposure resulted statistically significant ($p = 0.0496$). Two adult patients received RT with calculated liver exposure, as detailed in Table 4. The patient who developed severe SOS/VOD and died due to multi-system organ failure received RT to liver with a mean dose of 9 Gy and the percentages of liver volume exposed to 12 Gy and 30 Gy were 28% and 2%, respectively. The patient who received lower liver mean absorbed dose and lower liver volume exposition developed transient hyperbilirubinemia and transaminitis grade 2, according to CTCAE vers. 4.0 [39], and did not meet the criteria for diagnosing SOS/VOD. Compared with adults (≥18 years), none of the 32 children developed SOS/VOD ($p = 0.0674$), according to diagnostic criteria used in this study [17, 35].

Discussion

The cumulative incidence of SOS/VOD observed in our series was 6.67%, which is less than the incidence (ranging from 16 to 25%) reported from other studies on ES patients treated with BU-MEL and ASCT [6, 8–10]. However, all these studies used oral busulfan in combination with melphalan. In the EuroEwing 99 protocol, busulfan was used orally calculated by body surface area or by kgBW [40, 41].

Table 2 Disease characteristics, clinical presentation, and outcome of five patients with SOS/VOD

Age (years)	Indication for ASCT	Primary site	Sites of metastases	Local treatment	SOS/VOD diagnosis					
					Days since ASCT	Clinical presentation	Severity-grading *Bearman #EBMT	Interval from dx and start of DF (days)	CR after start of DF (days)	Outcome
27	Metastatic	Femur	T12 vertebra Lung	Sx (femur) XRT (T12)	8	First clinical symptoms 2 days; bilirubin = 3.05 mg/dl; weight gain >5%; ascites; painful hepatomegaly; ultrasound reversal of portal flow	*Grade II #Severe	46		Died for MOF
37	Localized Poor responder	Ilium		XRT	24	First clinical symptoms 7 days; bilirubin = 2.5 mg/dl; transaminases >2 ULN; weight gain >5%; ascites; painful hepatomegaly; ultrasound flat and slow portal flow	*Grade II #Moderate	1	13	Alive
18	Metastatic	Ischium	Lungs	XRT (ischium)	27	First clinical symptoms 4 days; bilirubin = 2.18 mg/dl; transaminases >6 ULN; weight gain >5%; ascites; painful hepatomegaly; ultrasound slow portal flow	*Grade II #Severe	3	5	Died for progression disease
18	Localized Poor responder	Fibula		Sx	40	First clinical symptoms 7 days; painful hepatomegaly; weight gain >5%; ascites with need of paracentesis	*Grade II #Moderate	3	3	Alive
20	Localized Poor responder	Femur		Sx XRT	9	First clinical symptoms 3 days; bilirubin = 2.99 mg/dl; weight gain >5%, ascites; painful hepatomegaly	*Grade II #Severe	1	3	Alive

SOS/VOD sinusoidal obstruction syndrome/veno-occlusive disease, *ASCT* autologous stem cell transplantation, *dx* diagnosis, *Sx* surgery, *XRT* radiation therapy, *DF* defibrotide, *CR* complete remission, *MOF* multi-organ failure, *ULN* upper limit of normal

* Severity-grading according to Bearman toxicity scale [36]

Severity-grading according to EBMT criteria [18]

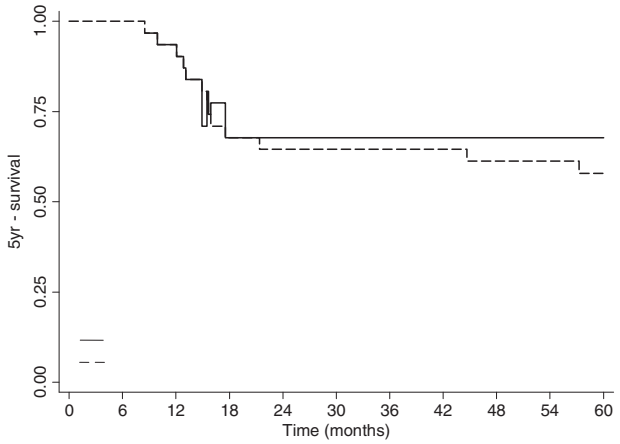

Fig. 1 Five years overall survival and five years event-free survival of 31 high-risk Ewing sarcoma patients treated with intravenous busulfan–melphalan. Five years OS = 67.74% (95% CI: 48.35–81.16), 5 years EFS = 57.89% (95% CI: 38.72–72.98)

Table 3 Univariate analysis of risk factors for SOS/VOD among 75 patients who received intravenous busulfan/melphalan conditioning therapy

Risk factors	*n*	SOS/VOD	%	*p*-Value
Gender				
Male	48	4	8.3	
Female	27	1	3.7	0.6483
Age at diagnosis				
<17	32	0	0	
18–39	43	5	11.6	0.0674
Primary tumor site				
Extremity	49	3	6.1	
Axial skeleton	26	2	7.7	1.0000
Disease stage				
Localized	42	3	7.1	
Metastatic	33	2	6.1	1.0000
Radiation therapy before transplant				
No	43	1	2.3	
RT with liver exposure	2	1	50	
Other RT	30	3	10	0.0496

SOS/VOD sinusoidal obstruction syndrome/veno-occlusive disease

Intravenous busulfan results less toxic than oral busulfan by maintaining the drug distribution within its narrow therapeutic window [42]. Patients receiving busulfan dosed to target AUC values showed lower incidence of SOS/VOD for intravenous busulfan when compared with oral busulfan [42–44]. In addition, intravenous busulfan is dosed according to different kgBW strata in order to achieve the target drug distribution [42], whereas oral dosing based on either kgBW or body surface area may result in considerable inter-patient variability and different busulfan systemic exposure [45]. Taking all these observations together, we

Table 4 Clinical details and outcome of patients with radiation therapy liver exposure prior to BU-MEL

Age (years)	Indication for ASCT	Primary site	Sites of metastases	Irradiated site	RT dose (Gy)	RT to liver mean dose (Gy)	Liver volume exposed to 12 Gy (%)	Liver volume exposed to 30 Gy (%)	Hepatic toxicity	Days from ASCT	Outcome
27	Metastatic	Femur	T12 vertebra Lungs	T12 vertebra	42	9	28	2	*Grade IV SOS/VOD	8	Died for SOS/VOD
23	Localized Poor responder	T6 vertebra		From T3 to T9 vertebra	43.5	5	18	0	#Grade 2 Bilirubin <2 mg/dl Transaminases >2 ULN	7	Alive

ASCT autologous stem cell transplantation, *RT* radiation therapy, *Gy* grays, *ULN* upper limit of normal, *SOS/VOD* sinusoidal obstruction syndrome/veno-occlusive disease

* Severity-grading according to Bearman toxicity scale [36]

CTCAE (Common Terminology Criteria for Adverse Events v 4.03) [39]

speculate that the use of intravenous busulfan in our series may in part explain the lower incidence of SOS/VOD we report when compared with other studies that used oral busulfan.

In this study, the rate of SOS/VOD observed in 32 children was 0% compared to 11.6% in 43 adults. This finding is in contrast with the higher incidence of SOS/VOD in children than that reported in adults [17]. Differences in pharmacokinetics and pharmacodynamics between children and adults are well known. Studies have shown a higher rate of busulfan clearance in children than in adults [20, 46]. The busulfan bioavailability in children appears to be more variable than in adults and is weight dependent, leading to different dosing strata, ranging from doses of 0.80 to 1.20 mg/kgBW [42]. However, despite these observations it seems difficult to explain the different incidences of SOS/VOD among children and adults by assuming a different toxic effect exerted by intravenous busulfan. Thus, we speculated that children of our series had been subject of some selection.

We focused on the possible correlation between SOS/VOD incidence and any prior liver exposure to RT. In two adult patients, spinal RT partly involved the liver. Both patients received liver mean RT dose and liver volume exposition >30 Gy significantly lower than values reported as associated with a risk of radiation-induced liver disease >5% [38]. Nazemi et al. reported a high incidence of SOS/VOD in patients with medulloblastoma treated with 36 Gy craniospinal RT prior to the busulfan containing conditioning regimen and supposed that the RT dose to the left lobe of the liver was presumably closer to 12 Gy [47]. If we correlate this observation with our results, it is conceivable that even limited liver exposures to RT prior to BU-MEL can play a role in the incidence and severity of SOS/VOD, due to the combined toxic effect of RT and the sensitizing effect of busulfan.

By analyzing our pediatric population who received RT, only four were treated with RT on the axial skeleton and, among these, none received spinal RT or received any calculated RT liver exposure. Therefore, the absence of SOS/VOD cases among children may also be a result of excluding several patients at risk, such as those with prior liver radiation exposure. Given the small number of SOS/VOD cases we observed, these results must be interpreted cautiously and need to be further explored in larger and multicentric studies.

An increased risk for SOS/VOD or other severe complications with the combination of RT delivered to the axial sites and BU-MEL was reported by other authors [6, 48, 49]. The Euro-Ewing 99 trial amended the protocol avoiding busulfan in those patients who had received RT to axial sites for reasons of anticipated toxicity [40, 49]. Strategies to prevent severe toxicity in those patients who received RT on the axial skeleton also included the use of treosulfan instead of busulfan [50]. Since 2011, we started using treosulfan–melphalan (TREO-MEL) before ASCT in high-risk ES patients who had previously received RT to central axial sites. The TREO-MEL study is currently running in our institution and will be subject for future communication.

Finally, four out of five patients with SOS/VOD achieved CR after early start of defibrotide treatment. This finding confirms that earlier defibrotide treatment is associated with more favorable outcomes [15, 16].

The limitations of this study are related to its retrospective nature. However, as a mono-institutional study, it has the advantage of using a single database where data are entered for prospective evaluations. Patients were also treated with the same conditioning regimen and same supportive and prophylactic therapies, within treatment protocols they did not undergo substantial changes over the period.

In summary, with this study, we emphasize the importance of recognizing patients at increased risk of developing severe SOS/VOD in order to implement strategies to reduce its incidence. Defibrotide is an effective therapy but severe SOS/VOD still represents a challenge, requiring timely recognition and treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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