Transplantation and Cellular Therapy 28 (2022) 196–202

Transplantation and Cellular Therapy

journal homepage: www.tctjournal.org

ASTCT American Society for Transplantation and Cellular Therapy

Full Length Article
Pediatric

Association Between the Magnitude of Intravenous Busulfan Exposure and Development of Hepatic Veno-Occlusive Disease in Children and Young Adults Undergoing Myeloablative Allogeneic Hematopoietic Cell Transplantation

Tim Bognàr[1,*], Imke H. Bartelink[2], Toine C.G. Egberts[1], Carin M.A. Rademaker[1], A. Birgitta Versluys[3], Mary A. Slatter[4], Morris Kletzel[5], Christa E. Nath[6], Geoffrey D.E Cuvelier[7], Rada M. Savic[8], Christopher Dvorak[9], Janel R. Long-Boyle[10], Morton J. Cowan[10], Henrique Bittencourt[11], Robbert G.M. Bredius[12], Tayfun Güngör[13], Peter J. Shaw[14], Marc Ansari[15,16], Moustapha Hassan[17], Maja Krajinovic[18], Georg Hempel[19], Sarah Marktel[20], Robert Chiesa[21], Yves Théoret[22], Troy Lund[23], Paul J. Orchard[23], Robert F. Wynn[24], Jaap Jan Boelens[25], Arief Lalmohamed[1]

[1] *Department of Clinical Pharmacy, University Medical Center Utrecht/Wilhelmina Children's Hospital, Utrecht, the Netherlands*
[2] *Amsterdam University Medical Center, Location VUmc, Amsterdam, the Netherlands*
[3] *Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands*
[4] *Paediatric Haematopoietic Stem Cell Transplant Unit, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom & Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom*
[5] *Stem Cell Transplant Program, Ann & Robert Lurie Children's Hospital/Northwestern University, Chicago, Illinois*
[6] *Children's Hospital at Westmead, Sydney, New South Wales, Australia*
[7] *Pediatric Blood and Marrow Transplantation, CancerCare Manitoba, University of Manitoba, Winnipeg, Manitoba, Canada*
[8] *Departments of Allergy/Immunology/Bone Marrow Transplantation, Clinical Pharmacy, or Bioengineering & Therapeutic Sciences of the University of California San Francisco (UCSF), San Francisco, California*
[9] *Division of Pediatric Allergy/Immunology/Bone Marrow Transplantation, University of California San Francisco (UCSF), San Francisco, California*
[10] *Department Clinical Pharmacy and Division of Pediatric Allergy/Immunology/Bone Marrow Transplantation, University of California San Francisco (UCSF), San Francisco, California*
[11] *Transplant and Cell Therapy Program. Hematology-Oncology Division, CHU Sainte-Justine, University of Montreal, Montreal, Quebec, Canada*
[12] *Department of Pediatrics, Willem Alexander Children's Hospital, Leiden University Medical Center, Leiden, The Netherlands*
[13] *Division of Stem Cell Transplantation and Children`s Research Center (CRC), University Children`s hospital Zürich, Switzerland*
[14] *Blood Transplant and Cell Therapies Program, The Children's Hospital at Westmead, Sydney, NSW Australia, Clinical Professor, Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia*
[15] *Cansearch Research Platform in Pediatric Oncology and Hematology, Faculty of Medicine, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland*
[16] *Division of Pediatric Oncology and Hematology, Department of Women, Child and Adolescent, University Geneva Hospitals, Geneva, Switzerland*
[17] *ECM, KFC, Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden*
[18] *Centre de Cancérologie Charles-Bruneau Centre de recherche - Hôpital Sainte-Justine Montréal, Montréal, Québec, Canada*
[19] *University of Münster, Münster, Germany*
[20] *Stem Cell Program, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[21] *Great Ormond Street, Hospital for Children London, United Kingdom & Stem Cell Program, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[22] *Clinical Pharmacology Laboratory, OPTILAB and Research Center, CHU Sainte-Justine Montréal, Montreal, Québec, Canada*
[23] *Division of Pediatric Blood and Marrow Transplantation, Masonic Children's Hospital, University of Minnesota, Minneapolis, Minnesota*
[24] *The Royal Manchester Children's Hospital, Manchester, United Kingdom*
[25] *Stem Cell Transplantation and Cellular Therapies, Memorial Sloan-Kettering Cancer Center, New York, New York*

Article history:
Received 13 October 2021
Accepted 13 January 2022

A B S T R A C T

Intravenous busulfan is widely used as part of myeloablative conditioning regimens in children and young adults undergoing allogeneic hematopoietic cell transplantation (HCT). Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a serious clinical problem observed with busulfan-based conditioning

Financial disclosure: See Acknowledgments on page 201.
*Correspondence and reprint requests: Tim Bognàr, Department of Clinical Pharmacy, Wilhelmina Children's Hospital/University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.
E-mail address: t.bognar-2@umcutrecht.nl (T. Bognàr).

https://doi.org/10.1016/j.jtct.2022.01.013

Key Words:
Hematopoietic cell transplantation
Busulfan exposure
Veno-occlusive disease
Sinusoidal obstruction syndrome

HCT. The development of VOD/SOS may be associated with busulfan exposure. Getting more insight into the association between busulfan exposure and the development of VOD/SOS enables further optimization of dosing and treatment strategies. The objective of this study was to assess the association between the magnitude of busulfan exposure and the occurrence of VOD/SOS in children and young adults undergoing myeloablative conditioning with a busulfan-containing regimen before allogeneic HCT. In this observational study we included all patients who underwent allogeneic HCT with intravenous busulfan as part of the conditioning regimen at 15 pediatric transplantation centers between 2000 and 2015. The endpoint was the development of VOD/SOS. The magnitude of busulfan exposure was estimated using nonlinear mixed effect modeling and expressed as the maximal concentration (Cmax; day 1 and day 1 to 4 Cmax), cumulative area under the curve (AUC; day 1, highest 1-day AUC in 4 days, and 4-day cumulative AUC), cumulative time above a concentration of 300 μg/L, and clearance on day 1. A total of 88 out of 697 patients (12.6%) developed VOD/SOS. The number of alkylators in the conditioning regimen was a strong effect modifier; therefore we stratified the regression analysis for the number of alkylators. For patients receiving only busulfan as one alkylator (36.3%, n = 253), cumulative busulfan exposure (>78 mg × h/L) was associated with increased VOD/SOS risk (12.6% versus 4.7%; odds ratio [OR] = 2.95, 95% confidence interval [CI] 1.13 to 7.66). For individuals receiving busulfan with one or two additional alkylators (63.7%, n = 444), cumulative busulfan exposure (≤78 and >78 mg × h/L) did not further increase the risk of VOD/SOS (15.4% versus 15.2%; OR = 1.03, 95% CI 0.61 to 1.75). The effect of the magnitude of busulfan exposure on VOD/SOS risk in children and young adults undergoing HCT is dependent on the number of alkylators. In patients receiving busulfan as the only alkylator, higher cumulative busulfan exposure increased the risk of VOD/SOS, whereas in those receiving multiple alkylators, the magnitude of busulfan exposure did not further increase this risk.

Intravenous busulfan is widely used as part of myeloablative conditioning regimens in children and young adults undergoing allogeneic hematopoietic cell transplantation (HCT). Busulfan has high interpatient and intrapatient pharmacokinetic variability that can be only partly explained by pharmacokinetic or patient-specific determinants. Finding the optimal busulfan exposure remains a challenge, with underexposure being associated with graft failure and disease recurrence and overexposure being associated with toxicity. The optimal therapeutic window is narrow: therapeutic drug monitoring (TDM) targeting a cumulative busulfan area under the curve (AUC_{cum}) of 78 to 101 mg × h/L after intravenous administration, which leads to optimal treatment outcomes in mixed malignant and nonmalignant diseases [1,2].

Despite the routine practice of TDM and its proven clinical benefits, hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) remains a relevant clinical issue with busulfan-based conditioning HCT, with a reported overall incidence of 13.7% [3]. The pathogenesis of VOD/SOS is complex: owing to an imbalance in procoagulant, inflammatory, and fibrinolytic processes, sinusoidal blood flow is obstructed, leading to hepatic endothelial damage and ultimately to necrosis. VOD/SOS is clinically characterized by jaundice, hyperbilirubinemia, hepatomegaly, ascites, weight gain, and pain in the right upper quadrant of the abdomen [4].

Several studies suggest an association between the magnitude of busulfan exposure and the development of VOD/SOS, with mixed results. High busulfan peak concentration (Cmax) and 4-day cumulative AUC have been linked to a higher incidence of VOD/SOS in children [2,5], but various other studies did not find an association [6–8]. Therefore it remains unclear what strategies should be used to minimize the risk of VOD/SOS in individuals requiring intravenous busulfan. The objective of the present study was to assess the association between the magnitude of intravenous busulfan exposure and VOD/SOS development in children and young adults undergoing myeloablative conditioning with a busulfan-containing regimen before allogeneic HCT.

METHODS

Setting, design, and study population

In this observational multicenter study, we included all patients who received an allogeneic HCT with intravenous busulfan as part of the conditioning regimen at 15 pediatric transplantation centers in the Netherlands, Germany, Austria, United States, Australia, Canada, Switzerland, Italy, and the United Kingdom between 2000 and 2015 and who underwent busulfan pharmacokinetic blood sampling. Ethical approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht. The study design has been described previously [1]. Data collection started after patients had provided written informed consent in accordance with the Helsinki Declaration or after approval was given by the research ethics board of the participating center.

Patient-specific, demographic, medication-related, and HCT-related variables were registered by the transplantation centers, during a minimum follow-up period of 6 months after conditioning. Blood sampling, busulfan dose adjustments, and HCT-related procedures were performed according to local protocol. The initial busulfan dose was calculated based on actual body weight. Busulfan was mostly administered over 4 consecutive days as a 3-hour or 4-hour infusion administered once a day or a 2-hour or 4-hour infusion (depending on center) administered 2 or 4 times a day. Plasma samples were analyzed with validated high-performance liquid chromatography, gas chromatography, or liquid chromatography–mass spectrometry assays, all according to Good Laboratory Practices. A small but unknown number of patients receiving busulfan, cyclophosphamide, and melphalan participated in a VOD/SOS prophylaxis trial and received defibrotide as part of VOD/SOS prophylaxis.

Veno-occlusive disease/sinusoidal obstruction syndrome and secondary outcomes

The outcome of interest was the occurrence of VOD/SOS as defined by the modified Seattle or Baltimore criteria [9,10]. According to the modified Seattle criteria, VOD/SOS is defined as the occurrence of 2 or more of the following criteria within 30 days of HCT: hepatomegaly or pain in the upper right quadrant, hyperbilirubinemia ≥34 μmol/L (≥2 mg/dL), and ≥2% weight gain [10]. According to the Baltimore criteria, VOD/SOS is defined as the occurrence of hyperbilirubinemia ≥34 μmol/L (≥2 mg/dL) and 2 or more of the following symptoms within 21 days of HCT: hepatomegaly, ascites and ≥5% weight gain [9]. VOD/SOS diagnosis was performed by a clinician of the participating center according to local protocol using the Baltimore or Seattle criteria.

Event free survival (EFS), graft-failure/relapse of disease, and transplant-related mortality were also evaluated as secondary endpoints. Transplant-related mortality was defined as death from causes unrelated to the disease. Graft failure was defined as nonengraftment or rejection. Events for EFS included graft failure, relapse of disease, or death and was defined as survival from HCT to last contact. Patients who did not experience an event were censored at the time of last contact.

Magnitude of busulfan exposure

The magnitude of busulfan exposure was characterized in several ways:

- Cumulative 4-day AUC (AUC_{cum}), defined as the cumulative exposure over 4 days of therapy
- Maximal AUC on any given treatment day (AUC_{max}), defined as the maximal AUC during therapy
- AUC on day 1 (AUC_{day1}), defined as the cumulative exposure on day 1
- Maximal concentration ($Cmax_{day1}$) on either day 1 or on any given treatment day (Cmax).
- Cumulative time (%) above a concentration of 300 μg/L ($C_{>300\ \mu g/L}$)
- Busulfan clearance on day 1 (Cl_{d1}); poor, medium, and fast metabolizers were identified by calculating the difference between individual and mean population clearance (Cl_{delta})

These measures of busulfan exposure were calculated from the raw concentration–time data using nonlinear mixed effect modelling (NONMEM). A previously published, externally validated busulfan pharmacokinetic model was used to fit the raw concentration–time data [11,12].

Potential confounders/effect modifiers

Biological plausibility and available literature suggest that the following determinants may influence the development of VOD/SOS and were therefore considered potential confounders or effect modifiers: gender, body weight, age, disease status (malignant/non-malignant), donor/recipient matching status, graft-versus-host disease prophylaxis regimen, and number of alkylators.

Data analysis

To investigate the association between the magnitude of busulfan exposure and the occurrence of VOD/SOS, the above-mentioned characteristics of the magnitude of busulfan exposure were compared in patients with and without VOD/SOS using descriptive statistics and multivariate logistic regression analysis (IBM SPSS Statistics, version 25.0.02). Continuous variables were first grouped into predefined tertiles or quartiles, and when a correlation was found, they were included as continuous data in spline logistic regression analyses. Confounders or effect modifiers were identified using stepwise logistic regression analysis with a change-in-estimate procedure ($P < .1$). To investigate the effect of the use of 2 diagnostic criteria for VOD/SOS, a sensitivity analysis was performed, in which we excluded the centers that diagnosed VOD/SOS according to the Baltimore criteria. In case continuous variables were included in the final multivariate model, the cutoff value of these variables were calculated using receiver operating characteristic curve analysis and decision tree analysis (chi-square automatic interaction detection). EFS, graft-failure/relapse, and transplant-related mortality were estimated by the Kaplan-Meier method.

RESULTS

A total of 697 patients received an HCT with intravenous busulfan as part of their conditioning regimen, with a median age of 4.7 years (range 0.04 to 30.4 years) and a median body weight of 18.0 kg (range 2.7 to 117.8 kg; the patient characteristics are displayed in Table 1). Estimated EFS at 1 and 2 years after HCT was 70.7% and 65.6%, respectively. Estimated probability of graft failure, transplant-related mortality, and graft failure/relapse at 2 years was 7.6%, 14.2%, and 20.1%, respectively.

In total, 36.3% (n = 253) received only busulfan as an alkylator, 53.2% (n = 371) received cyclophosphamide or melphalan in combination with busulfan (2 alkylators), and 10.5% (n = 73) received cyclophosphamide and melphalan in combination with busulfan (3 alkylators). The most frequently used conditioning regimens were busulfan/cyclophosphamide (39.7%, n = 277), busulfan/fludarabine (28.8%, n = 201) and busulfan/cyclophosphamide/melphalan (10.5%, n = 73). The model and results of the population pharmacokinetic refit of all individual raw concentration–time profiles and method of calculation of each pharmacokinetic parameter are shown in Supplementary Table S4 and Supplementary Figure S2. Individual clearance and volume of distribution of the first compartment was well estimated, but in the full dataset, we observed shrinkage to the mean value in the inter-occasion variability. Therefore, in

Table 1
Patient Characteristics of the Total Patient Population

	N = 697	%
Patient demographics		
Gender		
Male	431	61.8%
Female	266	38.2%
Age (years)		
0-2	226	32.4%
2-5	134	19.2%
5-12	189	27.1%
>12	148	21.2%
Weight (kg)		
<10	151	21.7%
10-20	240	34.4%
20-30	110	15.8%
>30	196	28.1%
Donor-related characteristics		
Diagnosis		
Malignant	298	42.8%
Nonmalignant	369	52.9%
Missing	30	4.3%
Donor		
Unrelated	429	61.5%
Family	254	36.4%
Missing	14	2.0%
Matching status		
Matched	352	50.5%
Mismatch	293	42.0%
Missing	52	7.5%
Hematopoietic cell transplantation-related characteristics		
Busulfan in combination with		
Fludarabine	296	42.5%
Cyclophosphamide	418	60.0%
Melphalan	99	14.2%
Etoposide	8	1.1%
Number of alkylators in the conditioning regimen		
1: Busulfan	253	36.3%
2: Busulfan + Cyclophosphamide or Melphalan	371	53.2%
3: Busulfan + Cyclophosphamide + Melphalan	73	10.5%
Chemotherapy regimen		
Busulfan + Cyclophosphamide	277	39.7%
Busulfan + Cyclophosphamide + Melphalan	73	10.5%
Busulfan + Cyclophosphamide + Fludarabine	42	6.0%
Busulfan + Fludarabine	201	28.8%
Busulfan + Cyclophosphamide + Etoposide	5	0.7%
Busulfan + Fludarabine + Clofarabine	12	1.7%
Busulfan + Fludarabine + Melphalan	11	1.6%
Missing	76	10.9%
T-cell depletion		
Yes	39	5.6%
No	234	33.6%
Missing	424	60.8%
Serotherapy		
Yes	495	71.0%
No	144	20.7%
Missing	58	8.3%
Graft-versus-host disease prophylaxis regimen		
None	17	2.4%
Cyclosporine	56	8.0%

(*continued*)

Table 1 (*Continued*)

	N = 697	%
Cyclosporine + Methotrexate	212	30.4%
Cyclosporine + Prednisolone	60	8.6%
Cyclosporine + Mycophenolic Acid	81	11.6%
Cyclosporine + Methotrexate + Prednisolone	32	4.6%
Missing/other	239	34.3%

the present analysis, change in clearance over time could not be included in our NONMEM analyses.

Incidence of veno-occlusive disease/sinusoidal obstruction syndrome and association with busulfan exposure

In total, 12.6% (n = 88) of patients were diagnosed with VOD/SOS according to the modified Seattle or Baltimore criteria. Univariate regression analysis showed that the magnitude of busulfan exposure was not associated with the development of VOD/SOS in the total study population (Table 2). The number of alkylators in the conditioning regimen was a strong effect modifier (Table 2, $P < .001$): in patients receiving busulfan in combination with cyclophosphamide or melphalan (2 alkylators, in combination with other nonalkylator chemotherapy drugs), there was a trend toward an increased risk of developing VOD/SOS as compared to patients receiving only busulfan as alkylator (13.2% versus 7.9%, odds ratio [OR] = 1.59, 95% confidence interval [CI] 0.91 to 2.77), whereas in patients who received busulfan in combination with cyclophosphamide and melphalan (3 alkylators, in combination with other nonalkylator chemotherapy drugs), the risk of developing VOD/SOS increased fourfold (26.0% versus 7.9%, OR = 4.09; 95% CI 2.02 to 8.30) (Table 3 and Supplementary Table S1). Therefore, we stratified the univariate and multivariate regression analysis for the number of alkylators (Tables 2 and 3). The number of alkylators in the conditioning regimen was equally distributed between patients with a malignant and a nonmalignant disease status (Supplementary Table S2). The disease status, graft-versus-host disease prophylaxis regimen, and donor/

Table 2
The Association Between Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome and Busulfan Exposure

	Patients Receiving Busulfan as the Only Alkylator (n = 253)*				Patients Receiving Busulfan + Cyclophosphamide and/or Melphalan (n = 444)*			
	Total (n)	VOD/SOS, n = 20, % (n)	OR	95% CI	Total (n)	VOD/SOS, n = 68, % (n)	OR	95% CI
AUC_{cum} (mg × h/L)*								
≤78	150	4.7% (7)	Reference group		223	15.2% (34)	Reference group	
>78	103	12.6% (13)	2.95	1.13-7.66	221	15.4% (34)	1.03	0.61-1.75
AUC_{max} (mg × h/L)								
<12.5	115	9.6% (11)	Reference group		226	13.5% (36)	Reference group	
12.5–17.5	67	4.5% (3)	0.44	0.12-1.65	49	10.2% (5)	0.74	0.27-2.02
>17.5	71	8.5% (6)	0.86	0.30-2.44	129	20.9% (27)	1.55	0.88-2.73
AUC_{day1} (mg × h/L)								
<12.5	34	0.0% (0)	Reference group		107	17.8% (19)	Reference group	
12.5–17.5	123	8.9% (11)	—		171	12.3% (21)	0.64	0.32-1.28
>17.5	96	9.4% (9)	—		166	16.9% (28)	0.90	0.47-1.74
Cl_{d1} (L/h/kg)								
<0.20	103	9.7% (10)	Reference group		130	13.8% (18)	Reference group	
0.20–0.24	72	9.7% (7)	0.78	0.26-2.34	159	15.7% (25)	0.80	0.40-1.61
>0.24	78	3.8% (3)	0.26	0.06-1.11	155	16.1% (25)	0.78	0.38-1.61
$C_{>300\ \mu g/L}$ (%)								
<33.3	52	0.0% (0)	Reference group		34	26.5% (9)	Reference group	
33.3–66.6	91	8.8% (8)	—		152	19.7% (30)	0.65	0.27-1.56
>66.6	110	10.9% (12)	—		258	11.2% (29)	0.36	0.15-0.87
Cmax (μg/L)								
<1450	62	9.7% (6)	Reference group		138	16.7% (23)	Reference group	
1450–2250	75	10.7% (8)	1.12	0.36-3.48	159	10.7% (17)	0.60	0.30-1.19
>2250	116	5.2% (6)	0.51	0.16-1.67	147	19.0% (28)	1.09	0.58-2.03
$Cmax_{day1}$ (μg/L)								
<1250	77	7.8% (6)	Reference group		143	16.1% (23)	Reference group	
1250–2600	69	11.6% (8)	1.57	0.51-4.78	170	10.0% (17)	0.62	0.31-1.23
>2600	107	5.6% (6)	0.70	0.22-2.28	131	21.4% (28)	1.32	0.70-2.46
Metabolizer type								
Slow metabolizer	116	8.6% (10)	Reference group		189	16.9% (32)	Reference group	
Medium metabolizer	45	11.1% (5)	1.30	0.41-4.08	100	16.0% (16)	1.01	0.52-1.99
Fast metabolizer	92	5.4% (5)	0.60	0.20-1.82	155	12.9% (20)	0.79	0.43-1.46

The results of the logistic regression analysis have been corrected for age and sex. OR indicates adjusted odds ratio for sex and age; AUC_{cum} = cumulative AUC, AUC_{max} = maximal AUC on any given treatment day, AUC_{day1} = AUC on day 1, Cl_{delta} = clearance of each individual versus the mean population clearance, Cl_{d1} = clearance day 1, $C_{>300\ \mu g/L}$ = cumulative time above a concentration of 300 μg/L, $Cmax_{day1}$ = maximal concentration on day 1, Cmax = maximal concentration, VOD/SOS = veno-occlusive disease/sinusoidal obstruction syndrome.

* The number of alkylators in the conditioning regimen was a strong effect modifier (busulfan versus busulfan and cyclophosphamide or melphalan), $P < .001$ (AUC_{cum} as continuous variable), $P = .058$ (AUC_{cum} >78 mg × h/L).

Table 3
Final Multivariate Logistic Regression Models of the Association Between Busulfan Exposure and Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome

	Total Study Population (N = 697)		Patients Receiving Only Busulfan As An Alkylator (N = 253)		Patients Receiving Busulfan in Combination With Cyclophosphamide, Melphalan, or Both (N = 444)	
	OR	95% CI	OR	95% CI	OR	95% CI
Busulfan	Reference		–		–	
Busulfan + Cyclophosphamide or Melphalan	1.59	0.91-2.77	–		Reference	
Busulfan + Cyclophosphamide + Melphalan	4.09	2.02-8.30	–		2.63	1.40-4.95
Sex (female)	1.76	1.11-2.78	0.97	0.37-2.56	2.17	1.27-3.71
Age (y)	0.94	0.90-0.98	0.99	0.92-1.06	0.91	0.85-0.96
AUC_{cum} ≤78 mg × h/L	Reference		Reference		Reference	
AUC_{cum} >78 mg × h/L	1.39	0.88-2.21	2.95	1.13-7.66	1.10	0.64-1.88

Patients were stratified by the number of alkylators (busulfan as the only alkylator and busulfan + cyclophosphamide and/or melphalan). AUC_{cum} = cumulative 4-day area under the curve.

recipient matching status did not predict VOD/SOS development. Multiple, non-busulfan related, determinants (female sex, younger age) appeared to be strong predictors of VOD/SOS development (Table 3). A cutoff value of 2.89 years for age was identified using receiver operating characteristic curve analysis and decision tree analysis. In the total patient population, VOD/SOS risk increased approximately threefold in patients aged ≤2.89 years (19.4% versus 8.1%, OR = 2.81, 95% CI 1.76 to 4.51).

In patients receiving only busulfan (in combination with non-alkylator chemotherapy) as an alkylator (36.3%, n = 253), cumulative busulfan exposure (>78 mg × h/L) was associated with increased VOD/SOS risk (12.6% versus 4.7%, OR = 2.95; 95% CI 1.13 to 7.66), whereas in patients who received busulfan in combination with other alkylators (cyclophosphamide, melphalan, or both; total of 2 or 3 alkylators; 63.7%, n = 444), busulfan exposure was not associated with a further increase in VOD/SOS risk (15.2% versus 15.4%). When using a continuous variable instead of categorical variables for the AUC_{cum}, these associations remained (data not shown). Consistent with this finding, the spline regression analysis showed that a high AUC_{cum} (>78 mg × h/L) increased the probability of developing VOD/SOS in patients receiving busulfan as the only alkylator, whereas in patients receiving busulfan and cyclophosphamide or melphalan (multiple alkylators), the risk was not dependent on the AUC_{cum} (Figure 1). To perform a

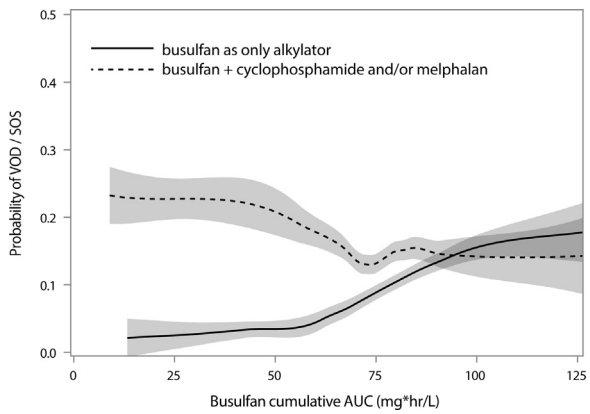

Figure 1. Spline regression analysis of the association between VOD/SOS and busulfan AUC_{cum} stratified by the number of alkylators and adjusted for age, sex, and number of alkylators (only in the multiple alkylator group). In patients who received only busulfan as an alkylator (*solid line*), cumulative busulfan exposure was associated with increased VOD/SOS risk, whereas in patients receiving multiple alkylators (*dashed line*) the risk remained similar and was not dependent on AUC_{cum}.

sensitivity analysis, we excluded the centers that diagnosed VOD/SOS according to the Baltimore criteria. This analysis showed that the overall results were not affected by the use of 2 different sets of diagnostic criteria for VOD/SOS.

DISCUSSION

This study shows that in patients receiving busulfan as the only alkylator, high busulfan AUC_{cum} (>78 mg × h/L) was associated with an approximately threefold higher risk of developing VOD/SOS. In patients receiving cyclophosphamide and/or melphalan in combination with busulfan in the conditioning regimen, the magnitude of busulfan exposure was not associated with a further increase in VOD/SOS risk. Multiple, non-busulfan-related, determinants (female sex, younger age) appeared to be strong predictors of VOD/SOS development.

Our results show that patients receiving three alkylators had a higher incidence of VOD/SOS than patients receiving a single alkylator (26.0% versus 7.9%, Supplementary Table S1). The observed increase in busulfan toxicity in patients receiving multiple alkylators may be explained by a number of different factors. First, cyclophosphamide and melphalan may potentiate busulfan toxicity or vice versa because all these agents are metabolized by conjugation to glutathione (GSH) through glutathione S-transferase (GST) [13–16]. Therefore simultaneous metabolism of these agents may lead to a rapid depletion of GSH and to the accumulation of hepatotoxic metabolites. Second, busulfan may be a better substrate for GST than other alkylating agents [15,17], potentially inhibiting the detoxification of cyclophosphamide or melphalan by displacing these substrates from GST, leading to additional toxicity. Third, busulfan may inhibit and even deplete GST [15], leading to decreased clearance of melphalan [18–20] and cyclophosphamide [15,21], which may also potentiate toxicity. Although the increase in busulfan toxicity may be explained by various biological mechanisms, it is important to note that some patients were diagnosed with diseases that have an increased risk of VOD/SOS (e.g., osteopetrosis, hemophagocytic lymphohistiocytosis, thalassemia, or neuroblastoma) and/or had more likelihood of previous exposure to chemotherapy that may also predispose patients to VOD/SOS. Unfortunately, the study design does not allow for previous exposure to chemotherapy or hepatotoxic drugs (e.g., ozogamicin-antibody conjugates) to be analyzed as a potential determinant. In addition, pre-existing liver disease or damage is an important risk factor for VOD/SOS, but unfortunately these data were not available.

Various studies have investigated the effect of GST genetic polymorphisms on VOD/SOS development, with mixed results [22–28]. These polymorphisms have been linked to busulfan pharmacokinetics and clearance in particular, with patients

having high GST-metabolizing capacity being at risk of developing VOD/SOS trough rapid depletion of intracellular GSH [28]. In 2020, a model for GSH depletion over time was proposed, which describes a decrease in busulfan clearance and increase in busulfan exposure in older patients [29]. This decrease in clearance in adults cannot be explained by a difference in GSH levels, as Gibbs et al. [30] demonstrated that GSH levels in children are similar to those in adults, but may be attributed to differences in GST expression. This is substantiated by evidence suggesting that children express higher levels of GST [30,31], leading to a more rapid depletion of intracellular GSH and the formation of a potentially toxic busulfan–GSH conjugate as compared to adults. Consistent with this hypothesis, we found that young age is an important determinant for VOD/SOS.

Finally, a number of important limitations need to be considered. First, dosing of busulfan guided by TDM was routine care in the majority (N = 14/15) of the participating transplantation centers, limiting variability in exposure and the possibility of studying the extremes in this population. Second, 2 different sets of criteria (Seattle and Baltimore criteria) were used to diagnose VOD/SOS in the participating treatment centers, which may have introduced bias. However, as shown in the sensitivity analysis, the overall results were not affected by the use of 2 different sets of diagnostic criteria for VOD/SOS. The Seattle and Baltimore criteria are outdated and not designated for the pediatric population. Moreover, both the Seattle and Baltimore criteria are considered to be stricter than the new pediatric European Society for Blood and Marrow Transplantation criteria for VOD/SOS [32]. Owing to the use of these strict criteria in our study, only the more severe cases of VOD/SOS may have been identified. Third, the incidence of VOD/SOS may have been underestimated, because a small number of patients received defibrotide and/or ursodeoxycholic acid as VOD/SOS prophylaxis. Fourth, because VOD/SOS severity was not the primary exposure of interest upon original data collection, VOD/SOS severity was not adequately assessed in the majority of the participating centers and was therefore not incorporated. Finally, patient inclusion took place over a long period, during which outcomes may have been improved and HCT treatment protocols may have been altered according to new scientific insights, for example, the introduction of reduced-intensity conditioning regimens and reversal of the order of application of chemotherapy. The strength of this study is its large study population, with patients recruited at 15 different international HCT centers.

In conclusion, in this large, multicenter study, high cumulative exposure to busulfan was associated with VOD/SOS in children and young adults receiving busulfan as the only alkylator as part of the HCT conditioning regimen. However, in patients receiving multiple alkylators, risk increased markedly but was not associated with busulfan exposure.

ACKNOWLEDGMENTS

Financial disclosure: There are no disclosures to report.

Conflict of interest statement: M. Ansari received traveling grants from Jazz pharmaceuticals. H. Bittencourt is on the advisory board from Jazz Pharmaceutics. T. Güngör is on the advisory board from Jazz Pharmaceutics. C.C. Dvorak is a consultant for Jazz Pharmaceuticals. J.J. Boelens is a consultant for Avrobio, BlueRock, Race Oncology, Advanced Clinical, Omeros, and Sanofi and on the advisory board for Medexus, Equillium, Sobi.

Authorship statement: T.B., T.C.G.E., I.H.B., C.M.A.R., A.B.V., J.J. B. and A.L. contributed to the conception, study management and design of the study. T.B., I.H.B., T.C.G.E. and A.L. contributed to data analysis and manuscript writing. All authors critically reviewed the manuscript and approved the final version.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.01.013.

REFERENCES

1. Bartelink IH, Lalmohamed A, van Reij EML, et al. Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. *Lancet Haematol*. 2016;3(11):526–536.
2. Feng X, Wu Y, Zhang J, et al. Busulfan systemic exposure and its relationship with efficacy and safety in hematopoietic stem cell transplantation in children: A meta-analysis. *BMC Pediatr*. 2020;20(1):1–25.
3. Corbacioglu S, Jabbour EJ, Mohty M. Risk factors for development of and progression of hepatic veno-occlusive disease/sinusoidal obstruction syndrome. *Biol Blood Marrow Transplant*. 2019;25(7):1271–1280.
4. Mohty M, Malard F, Abecassis M, et al. Sinusoidal obstruction syndrome/veno-occlusive disease: current situation and perspectives—a position statement from the European Society for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 2015;50(6):781–789.
5. Philippe M, Neely M, Rushing T, et al. Maximal concentration of intravenous busulfan as a determinant of veno-occlusive disease: a pharmacokinetic-pharmacodynamic analysis in 293 hematopoietic stem cell transplanted children. *Bone Marrow Transplant*. 2019;54(3):448–457.
6. Schechter T, Perez-Albuerne E, Lin TF, et al. Veno-occlusive disease after high-dose busulfan–melphalan in neuroblastoma. *Bone Marrow Transplant*. 2020;55(3):531–537.
7. Michel G, Valteau-Couanet D, Nguyen L, et al. Weight-based strategy of dose administration in children using intravenous busulfan: clinical and pharmacokinetic results. *Pediatr Blood Cancer*. 2012;58(58):90–99.
8. Bartelink IH, Bredius RGM, Belitser SV, et al. Association between busulfan exposure and outcome in children receiving intravenous busulfan before hematologic stem cell transplantation. *Biol Blood Marrow Transplant*. 2009;15(2):231–241.
9. Jones R J, Kamthorn S, Lee SK, et al. Veno-occlusive disease of the liver following bone marrow transplantation. *Transplantation*. 1987;44(6):778–783.
10. McDonald GB, Hinds MS, Fisher LD, et al. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med*. 1993;118(4):255–267.
11. Bartelink IH, Van Kesteren C, Boelens JJ, et al. Predictive performance of a busulfan pharmacokinetic model in children and young adults. *Ther Drug Monit*. 2012;34(5):574–583.
12. Bartelink IH, Boelens JJ, Bredius RGM, et al. Body weight-dependent pharmacokinetics of busulfan in paediatric haematopoietic stem cell transplantation patients: towards individualized dosing. *Clin Pharmacokinet*. 2012;51(5):331–345.
13. de Jonge ME, Huitema ADR, Rodenhuis S, Beijnen JH. Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet*. 2005;44(11):1135–1164.
14. Myers AL, Kawedia JD, Champlin RE, et al. Clarifying busulfan metabolism and drug interactions to support new therapeutic drug monitoring strategies: a comprehensive review. *Expert Opin Drug Metab Toxicol*. 2017;13(9):901–923.
15. Hassan M, Ljungman P, Ringdén O, et al. The effect of busulphan on the pharmacokinetics of cyclophosphamide and its 4-hydroxy metabolite: time interval influence on therapeutic efficacy and therapy-related toxicity. *Bone Marrow Transplant*. 2000;25(9):915–924.
16. Bouligand J, Boland I, Valteau-Couanet D, et al. In children and adolescents, the pharmacodynamics of high-dose busulfan is dependent on the second alkylating agent used in the combined regimen (melphalan or thiotepa). *Bone Marrow Transplant*. 2003;32(10):979–986.
17. Gibbs JP, Yang JS, Slattery JT. Comparison of human liver and small intestinal glutathione S- transferase-catalyzed busulfan conjugation in vitro. *Drug Metab Dispos*. 1998;26(1):52–55.
18. Lee JL, Gooley T, Bensinger W, et al. Veno-occlusive disease of the liver after busulfan, melphalan, and thiotepa conditioning therapy: incidence, risk factors, and outcome. *Biol Blood Marrow Transplant*. 1999;5(5):306–315.
19. Bailey HH, Mulcahy RT, Tutsch KD, et al. Phase I clinical trial of intravenous L-buthionine sulfoximine and melphalan: an attempt at modulation of glutathione. *J Clin Oncol*. 1994;12(1):194–205.
20. Willcox A, Wong E, Nath C, et al. The pharmacokinetics and pharmacodynamics of busulfan when combined with melphalan as conditioning in adult autologous stem cell transplant recipients. *Ann Hematol*. 2018;97(12):2509–2518.
21. Rezvani AR. Cyclophosphamide followed by intravenous targeted busulfan for allogeneic hematopoietic cell transplantation: pharmacokinetics and clinical outcomes. *Bone*. 2008;23(1):1–7.
22. Ansari M, Krajinovic M. Can the pharmacogenetics of GST gene polymorphisms predict the dose of busulfan in pediatric hematopoietic stem cell transplantation? *Pharmacogenomics*. 2009;10(11):1729–1732.

23. Ansari M, Lauzon-Joset JF, Vachon MF, et al. Influence of GST gene polymorphisms on busulfan pharmacokinetics in children. *Bone Marrow Transplant*. 2010;45(2):261–267.
24. Choi B, Kim MG, Han N, et al. Population pharmacokinetics and pharmacodynamics of busulfan with GSTA1 polymorphisms in patients undergoing allogeneic hematopoietic stem cell transplantation. *Pharmacogenomics*. 2015;16(14):1585–1594.
25. Kim SD, Lee JH, Hur EH, et al. Influence of GST gene polymorphisms on the clearance of intravenous busulfan in adult patients undergoing hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2011;17(8):1222–1230.
26. Yin J, Xiao Y, Zheng H, et al. Once-daily i.v. BU-based conditioning regimen before allogeneic hematopoietic SCT: A study of influence of GST gene polymorphisms on BU pharmacokinetics and clinical outcomes in Chinese patients. *Bone Marrow Transplant*. 2015;50(5):696–705.
27. Zwaveling J, Press RR, Bredius RGM, et al. Glutathione S-transferase polymorphisms are not associated with population pharmacokinetic parameters of busulfan in pediatric patients. *Ther Drug Monit*. 2008;30(4):504–510.
28. Srivastava A, Poonkuzhali B, Shaji RV, et al. Glutathione S-transferase M1 polymorphism: A risk factor for hepatic venoocclusive disease in bone marrow transplantation. *Blood*. 2004;104(5):1574–1577.
29. Langenhorst JB, Boss J, van Kesteren C, et al. A semi-mechanistic model based on glutathione depletion to describe intra-individual reduction in busulfan clearance. *Br J Clin Pharmacol*. 2020;86(8):1499–1509.
30. Gibbs JP, Murray G, Risler L, et al. Age-dependent tetrahydrothiophenium ion formation in young children and adults receiving high-dose busulfan. *Cancer Res*. 1997;57(24):5509–5516.
31. Gibbs JP, Liacouras CA, Baldassano RN, et al. Up-regulation of glutathione S-transferase activity in enterocytes of young children. *Drug Metab Dispos*. 1999;27(12):1466–1469.
32. Szmit Z, Gorczynska E, Król A, et al. Introduction of new pediatric EBMT criteria for VOD diagnosis: is it time-saving or money-wasting?: Prospective evaluation of pediatric EBMT criteria for VOD. *Bone Marrow Transplant*. 2020;55(11):2138–2146.