Received: 10 October 2019 | Revised: 22 July 2020 | Accepted: 7 August 2020

DOI: 10.1002/hon.2789

ORIGINAL RESEARCH ARTICLE

WILEY

Targeted-dose of busulfan: Higher risk of sinusoidal obstructive syndrome observed with systemic exposure dose above 5000 μMol·min. A historically controlled clinical trial

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Funding information
Ministério da Saúde

Abstract

Busulfan is given in the conditioning regimens preceding hematopoietic stem cell transplantation (HSCT), and plasma levels can be monitored. A targeted, individualized systemic exposure (SE) dose can be achieved by calculating the area under the plasma concentration versus time curve (AUC). The objective of this study was to determine a cutoff value for safety for the AUC for busulfan plasma levels in patients undergoing HSCT. A total of 149 consecutive HSCT patients were studied. After an oral test dose of busulfan, we set target doses of 4000, 5000, or 6000 μMol·min/day, and analyzed the AUC of oral or intravenous Bu. These patients were compared with 53 historical control subjects who had received myeloablative conditioning regimen without busulfan pharmacokinetic monitoring. Using a test dose and the administration route had no impact on the sinusoidal obstructive syndrome (SOS) incidence, transplant-related mortality or 1-year overall survival. However, patients receiving busulfan at doses set up at AUC > 5000 had an increased risk to develop SOS after HSCT (hazard ratio 3.39, $p = 0.034$, 95% CI 1.09–10.52). Adjusting the busulfan dose according to SE levels target dose during conditioning is associated with lower rates of oral severe mucositis and SOS. A cutoff of 5000 μMol·min is safe and does not impair survival.

KEYWORDS
acute toxicity, busulfan, hepatic sinusoidal obstructive syndrome, pharmacokinetics

Multicenter study conducted in São Paulo, Brazil.

1 | INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for many diseases. However, the toxicity of the conditioning regimen used in HSCT is high.[1] Veno-occlusive disease (VOD), also known as sinusoidal obstructive syndrome (SOS),[2,3] may affect up to 35% of patients.[4,5] Despite the complex etiology of SOS, there are evidences that the risk of developing SOS/VOD after busulfan-containing conditioning regimens increases with high plasma steady-state levels of busulfan.[6,7]

Plasma levels of busulfan can be monitored to reduce toxicity, with the maximum tolerated amount of drug limited by liver injury.[8,9] A pharmacokinetic analysis is made by calculating the area under the plasma concentration-versus-time curve (AUC). This technique, known as "targeted-busulfan," that is, the administration of an individualized dose of busulfan based on personalized clearance. The targeted dose can potentially allow better results,[10–17] not only related to toxicity but also the control of the underlying primary disease.[18,19]

The objective of this study was to determine a cutoff value regarding safety for plasma levels of busulfan in patients undergoing HSCT. The safety outcomes evaluated were overall regimen-related toxicity and SOS. A secondary outcome was survival. The hypothesis to be tested here was that the different target AUCs of 4000, 5000, and 6000 μMol · min/day would cause different outcomes in terms of toxicity when busulfan is given in a 4-day regimen.

2 | METHODS

2.1 | Design, setting, participants, and ethics

This is a historically controlled clinical study, conducted in three transplantation centers, one private and two public hospitals in São Paulo, Brazil. Patients were eligible for inclusion if they had malignant or non-malignant diseases and indication to undergo HSCT with autologous or allogeneic grafts, and regardless of patient age. Two study groups were compared with a third, control group. The control group underwent HSCT using busulfan-based conditioning therapy between January 2005 and July 2009, but with a busulfan dose based on bodyweight only, since they were treated before the adoption of the targeted-dose protocol in one of the hospitals. Patients in the study groups were admitted between January 2009 and July 2011. The two study groups received a test dose of busulfan (TBu) before HSCT to determine a predetermined systemic exposure-dose (targeted-busulfan).

One of the study groups received oral busulfan, and the other received intravenous (IV) busulfan in bioequivalent doses of 1 mg/kg of body weight (oral) and 32 mg/m^2 (IV). This dosage was based on a study using one-fourth of the standard daily dose as an IV test dose in children submitted to busulfan and fludarabine regimen, in up to 15 days before transplant.[20] The test dose of IV Bu provided information to adjust subsequent daily doses of IV busulfan successfully.[20] These study groups are hereafter named TBuIV and TBuOral.

The protocols of basic supportive care were very similar regarding nutrition, antibiotics use, antivirals, blood tests, and clinical evaluations in the hospitals. All patients were followed-up until Day +100 after HSCT.

At the hospitals using the target-dose, the patients were treated on a protocol of fludarabine and busulfan with a test dose given 15 days to 48 h before transplant. The correction of busulfan doses in these hospitals was usually made only after the first day of treatment with busulfan. Three main protocols were used in the institutions for immunosuppression after allo-HSCT: (1) cyclosporine and methotrexate; (2) Prograf and methotrexate; and (3) cyclophosphamide, Prograf, and mycophenolate mofetil in haploidentical allogeneic HSCT.

All patients in the study groups signed informed consent forms to participate in this study. Because the control group was a historical cohort of patients retrospectively evaluated through medical records, informed consent was waived, and anonymity was guaranteed. The study was approved by the ethics committee of the participating hospitals.

2.2 | Intervention

The intervention under analysis is the adjustment of busulfan dose after a test dose before HSCT. The impact of each dosage on acute clinical outcomes up to D + 100 was evaluated. As the individual response (pharmacokinetics) to this test dose may vary, the dosage to be administered for each patient was determined by monitoring the plasma levels of busulfan after this test dose. A dose of 32 mg/m^2, or 0.8 mg/kg/dose of IV busulfan is bioequivalent to an average of 1 mg/kg/dose of the oral formulation.[8,20]

The test dose was administered only once. This test dose was infused before HSCT to estimate the target dose (TBu) to be used during the conditioning regimen, based on pharmacokinetics. The period between the test dose and the HSCT depended on the availability of hospital beds, and it varied between 15 days and 48 h before HSCT.

After the administration of the test dose of busulfan, blood samples were collected at pre-specified time points during and after the conditioning regimen administrations (at 0 h, 30′, 45′, 60′, 90′, 120′, 180′, 240′, 300′, 360′, and 480′) for the assessment of busulfan plasma levels and pharmacokinetic profiling. A target dose was established for each patient according to the baseline disease and AUC. Different doses were administered during the conditioning regimen, as described below.

High-performance liquid chromatography (HPLC) was used for the extraction and measurement of plasma busulfan.[21] Pharmacokinetic calculations were performed and validated by PK Solutions Noncompartmental Data Analysis v. 2.0 (Summit, USA) and were

dosed according to a stipulated AUC, based on the risk of disease, performance status, and the protocol used in each institution. The systemic exposure (SE) doses used were 4000, 5000, or 6000 μMol · min in 24 h, at the attending physician's choice, according to the calculated AUC.

2.3 | Dosages of oral and intravenous busulfan

The busulfan dosage used in the conditioning regimen of patients in groups TBuIV and TBuOral were as follows. The TBuIV group received intravenous busulfan (Busilvex; Patheon Manufacturing Services LLC) at 6 mg/ml dissolved in polyethylene glycol 400 (67%, vol/vol) and dimethylacetamide (33%, vol/vol) at a dose of 32 mg/m^2. This test dose was diluted in saline or 5% dextrose in water to a concentration of about 0.5 mg/ml, infused over 45 min before HSCT to estimate the target dose (TBu) to be used during the conditioning regimen (based on pharmacokinetics). During the HSCT, the drug in the IV formulation was administered over 3 h, once a day, associated with other chemotherapeutic agents, based on the protocols used by each institution[21,22] (and described in Table S1). Patients with unrelated donors or who had had one mismatch received horse anti-thymocyte globulin (ATG), 4 mg/kg/day from D−3 to D−1.

The TBuOral group received oral busulfan (Myleran, GlaxoSmithKline) in 2 mg-coated tablets, with a single dose of 1 mg/kg before HSCT (test dose). For the conditioning regimen, the drug was administered at the same dose every 6 h, for a total of 16 doses over 4 days, associated with other chemotherapeutic agents. The two institutional protocols are described in Table S1. Patients with unrelated donors or who had a 1-antigen mismatched donor received horse ATG at a dose of 4 mg/kg/day from D−3 to D−1.

2.4 | Supportive care and prophylaxis

Patients in TBuIV group underwent oral mucositis prophylaxis with laser[23–25] at the beginning of the conditioning regimen until improvement of signs and symptoms in the oral cavity, which was observed following neutrophil engraftment.

All patients received ursodeoxycholic acid (Ursacol; Zambon), in 150 mg tablets, at a dose of 10–15 mg/kg/day[26,27] before conditioning and up to D + 30. When there was a suspicion of SOS according to the Seattle–Baltimore criteria,[28–30] defibrotide was used (6.25 mg IV, four doses per day) for 21 days.[31,32]

All patients were given antiemetics before chemotherapy (IV ondansetron, 8 mg). For seizure prevention, phenytoin (15–18 mg/kg/dose), or alprazolam (0.02–0.06 mg/kg/dose) were administered, starting 24 h before busulfan administration (twice a day), and continued until 24 h after the end of busulfan infusion or oral intake. Prophylaxis of infectious diseases (viral, fungal, and bacterial) followed existing institutional protocols.

Patients received colony-stimulating factor (G-CSF: 5 μg/kg/day) up to neutrophil engraftment (3 consecutive days with a neutrophil count >500 per mm^3). Immunosuppressive agents against graft-versus-host disease (GVHD) were started according to each institutional protocol, with the following associations: calcineurin inhibitor plus methotrexate (5 mg/m^2 intravenously on D + 1, D + 3, D + 6, and D + 11) or calcineurin inhibitor, associated with mycophenolate mofetil (15 mg/12 kg/12 h) from D−3.

2.5 | Endpoints evaluated

The primary outcome of toxicity was evaluated as oral mucositis and SOS prevalence. Secondary outcomes were recurrence and event-free and overall survival. The effect of different busulfan targets of SE (AUC of 4000, 5000 e 6000 μMol · min) on these clinical outcomes was investigated.

2.6 | Area under the curve calculation

The curve of the area under the plasma concentration versus time (AUC) for the Bu dose for each patient was calculated by dividing the value of the administered drug by the final plasma clearance estimate. The plasma clearance was determined by modeling all plasma concentration versus time. From the primary parameters (distributed volume, constant of elimination rate, and microconstants), derived from model estimates, the steady-state volume, the half-lives, and the clearance were calculated.[33]

2.7 | Statistical analysis

Descriptive measurements were absolute frequencies, percentages, means, medians, with standard deviation, interquartile range, and minimum and maximum values for the variables: age, cellularity, and CD34$^+$.

Kaplan–Meier curves were used for survival analysis, and compared using the log-rank test, the cumulative incidence of disease recurrence and SOS with death as a competing event. The comparison between the different groups regarding the cumulative incidence was made using the Gray method.[34] To assess factors associated with overall and event-free survival in 100 days, Cox adjusted proportional hazards models in simple (univariate), and multiple (multivariate) approaches were calculated. The variables with p-value less than 0.10 in the simple approach were included in models of multiple analyses. p-value less than 0.05 were considered statistically significant.

The analyses were made with SPSS (SPSS Inc. Released 2008, SPSS Statistics for Windows, Version 17.0., SPSS Inc.) and R (R Core Team, 2012, http://www.R-project.org/), as well as the "cmprsk" package of R.

3 | RESULTS

3.1 | Participants' characteristics

In the study periods, 202 patients were admitted in the participating institutions to undergo HSCT, 70% adults. The control group had 53 patients treated from 2005 to 2009, all adults. Between 2009 and 2011, 149 patients underwent the TBu intervention: 83 patients in the TBuIV group (34% or 40.9% aged up to 18 years) and 66 patients in the TBuOral group (15% or 22.7% aged up to 18 years). On average, patients received the test dose seven days before the start of conditioning therapy. Table 1 shows the patients' characteristics. Table S2 shows these baseline data stratified by busulfan exposure.

Malignant diseases were more common in all groups (80.6%). The conditioning regimen used in the TBuIV group was busulfan with fludarabine in most cases (56.2%), and cyclophosphamide in the TBuOral group (57.6%) and the Control group (88.7%). Allografts were used in most patients (88.1%).

3.2 | Clinical events

Among the 202 patients, 91% had mucositis, with a median duration of 5 days, with no significant difference between the groups receiving any target of SE ($p = 0.75$; chi-squared test).

The use of a Bu test dose had no effect on SOS prevalence (Figure 1), either comparing two groups (both groups with TBu vs. the Control group) (SHR 0.70 [95% CI 0.25–1.91; $p = 0.48$]), the two groups with TBu (TBuOral or TBuIV), (SHR 0.83, $p = 0.73$, 95% CI [0.30–2.31]), or even the three groups (the TBuOral group, the TBuIV group, and the Control group), (1.48, $p = 0.41$, 95% CI [0.57–3.85]). However, patients who received a Bu-SE with an AUC > 5000 μMol · min (vs. <5000 μMol · min) had a higher risk of SOS: SHR 3.39, $p = 0.034$, 95% CI 1.09–10.52, described over time in Figure 2.

In 5 years, 20% of patients had a relapse (95% CI 14%–27%). In 1 year, the prevalence was 19% (95% CI 13%–26%), and in 100 days, 9% (95% CI 5%–14%). Among those using the test dose, relapse occurred in 300 days in 22% patients (95% CI 13%–33%), while the prevalence of relapse in patients of the Control group was 16% (95% CI 8%–27%). The test dose of busulfan could not reduce the relapse rate (SHR: 0.92, $p = 0.68$, 95% CI 0.63–1.35; Figure 3).

The recurrence rate was evaluated in the first year after transplant among patients who received AUC 4000 μMol · min during HSCT (SHR: 0.42; $p = 0.08$; 95% CI 0.15–1.13) and AUC 6000 μMol · min (SHR: 1.92; $p = 0.17$; 95% CI 0.74–4.96). For patients receiving AUC < 4000, the recurrence rate was 43% (95% CI 12%–71%), and for those receiving >4000, the rate was 21% (95% CI 14%–30%; Table 2). For those with AUC < 6000, the rate was 22% (95% CI 14%–31%) and for those receiving AUC > 6000, it was 32% (95% CI 13%–52%).

3.3 | Survival analyses

Overall survival in 100 days was 80% (95% CI 74%–86%); 63% (95% CI 55%–69%) at 1 year; and 43% (95% CI 31%–54%) at 5 years.

No association was found, in the univariate or multivariate analyses, between sex, diagnosis, type of HSCT, conditioning regimen, or the target dose of busulfan and overall survival at D + 100. Dosage modification based on the AUC for busulfan did not affect survival (Table 3).

SOS increased the risk of death in both univariate (HR: 5.21; 95% CI [2.53–10.7]) and multivariate analyses (HR: 5.27; 95% CI [2.46–11.27]), with $p < 0.001$. In the univariate analysis, recurrence significantly increased the risk of death ($p = 0.006$), but the result was not significant in the multivariate analysis.

Survival at 1 year for patients receiving the test dose was 68% (95% CI 54%–78%) and 60% (95% CI 46%–62%) for patients in the control group. Using the test dose of busulfan before HSCT had no significant effect on overall survival (HR: 0.83, $p = 0.52$, 95% CI [0.46–1.47]; Figure 4). Survival was similar for patients receiving different target dosages (Table 2).

4 | DISCUSSION

In this study, the plasma level of busulfan was monitored after a single test dose before HSCT, and the target dose was adjusted individually during the conditioning regimen. The study demonstrated that although dosage modification based on the AUC has shown no direct effect on survival, patients who received protocols of AUC > 5000 μMol · min (vs. <5000 μMol · min) were at higher risk of SOS, and SOS increased the risk of death. This is an important take-home message, as no other study has established a cutoff dose for AUC for busulfan in HSCT.

One might think that it is enough to administer busulfan intravenously to reduce the SOS rate. Kashyap et al.[9] have shown that the oral administration of busulfan can be itself a predictive factor for the development of SOS related to the hepatic first-pass effect. However, the authors did not monitor busulfan plasma levels. And in the present study, busulfan route of administration did not affect the onset of this clinical complication.

In an observational, retrospective study, the adjustment of the dose using a test dose of 0.9 mg/kg IV 1 week before transplant increased the targeted stable busulfan concentration, without elevating SOS or mucositis risks significantly. However, the study was small (60 patients), and it was not able to evaluate a cutoff of a target, safe dose.[17] The test dose of busulfan in the first day of conditioning can help to predict the optimum IV dose.[20] In the present prospective study, it was possible not only to confirm the importance of the target dose but also to provide a reference value of 5000 μMol · min as a safe target dosage for young patients, something to be tested in randomized controlled trials.

TABLE 1 Characteristics of patients undergoing hematopoietic stem cell transplantation (HSCT) per group: the TBuOral group, receiving the oral busulfan target dose, the TBuIV group, receiving the intravenous busulfan target dose, and the Control patients, receiving busulfan without the calculation of a target dose

		TBuIV		TBuOral		Control	
		n	%	*n*	%	*n*	%
Age (years)	0–18 years	34	40.9	26	39.4	2	3.8
	>19 years	49	59.1	40	60.6	51	96.2
Sex	Female	36	43.4	30	45.5	27	50.9
	Male	47	56.6	38	57.5	26	49.1
Disease status at HSCT	1CR	24	28.9	25	37.8	6	14.3
	>2CR	13	15.6	19	28.8	8	19.0
	Active disease	41	49.4	15	22.7	12	28.6
	Chronic disease	2	2.4	1	1.5	16	38.1
	Prior HSCT	5	6	0	0.0	0	0.0
Diagnosis category	Malignant disease	56	67.5	61	92.4	46	86.9
	Non-malignant disease	27	32.5	5	7.6	7	13.2
Diagnosis	AML/MDS	36	43.4	31	46.9	20	37.7
	ALL	9	10.9	9	13.6	2	3.8
	CML	0	0.0	4	6.06	16	30.2
	CLPD	2	2.4	4	6.06	7	13.2
	SCID, osteopetrosis, adrenoleukodystrophy, hemophagocytic lymphohistiocytosis, and Chediak–Higashi syndrome	22	26.5	0	0.0	1	1.9
	Thalassemia/Sickle cell anemia	5	6.02	0	0.0	0	0.0
	Solid tumors	2	2.4	11	16.6	0	0.0
	Aplastic anemia	0	0.0	5	7.6	6	11.3
	Myelofibrosis, MPD, JMML, and CMML	7	8.4	2	3	1	1.9
Type of HSCT	Autologous	5	6.02	17	20.5	2	3.8
	Allograft from related donor	27	22.9	38	44.6	51	96.2
	Allograft from unrelated donor	51	61.4	11	13.3	0	0.0
Graft source	Peripheral blood	21	25.3	40	57.5	29	54.7
	Cord blood	16	48.2	1	1.51	0	0.0
	Bone marrow	46	55.4	25	37.8	24	45.3
Conditioning regimen	Bu Flu	47	56.2	8	12.1	6	11.3
	Bu Cy	14	16.8	38	57.6	47	88.7
	Bu Mel	4	4.81	15	22.7	0	0.0
	Bu Flu Thiotepa	13	15.6	0	0.0	0	0.0
	Bu Flu Mel	1	1.2	0	0.0	0	0.0
	Bu Flu Clo	2	2.4	0	0.0	0	0.0
	Bu Cy Mel	1	1.2	5	7.6	0	0.0
	Bu Clo Thiotepa	1	1.2	0	0.0	0	0.0
	Total	3	100	66	100	53	100

Note: For patients receiving allografts from unrelated donors, horse ATG was associated.

Abbreviations: 1CR, first complete remission; 2CR, second complete remission; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; Bu, busulfan; Clo, clofarabine; CLPD, chronic lymphoproliferative disease; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; Cy, cyclophosphamide; Flu, fludarabine; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; Mel, melphalan; MMF, mycophenolate mofetil; MPD, myeloproliferative syndrome; MTX, methotrexate; SCID, severe combined immunodeficiency.

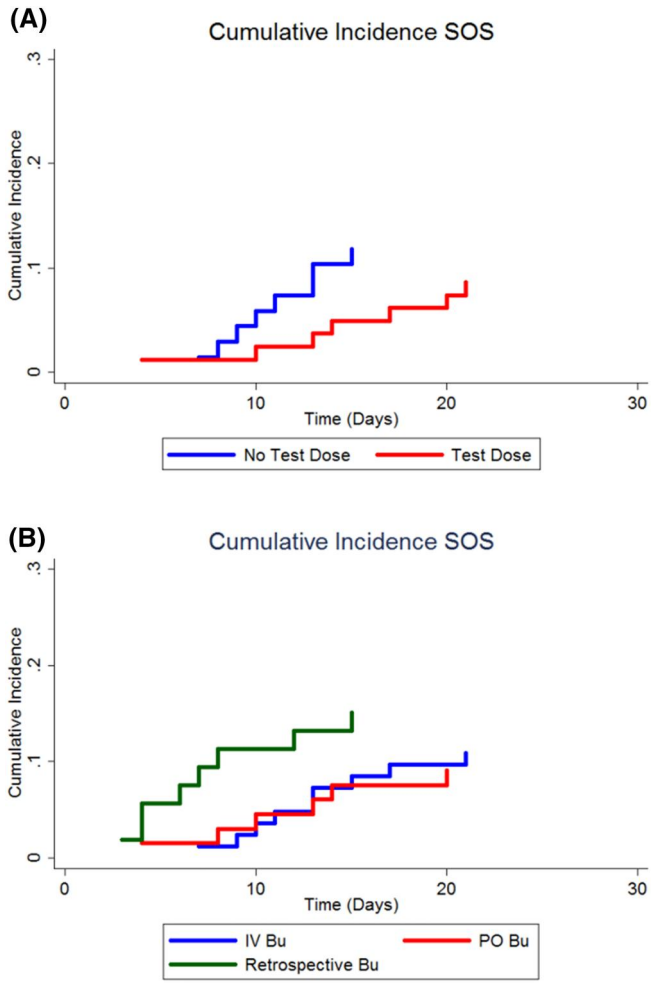

FIGURE 1 Effect of the use of the calculation of a target of SE of busulfan (TBu) using a test dose before HSCT on the prevalence of SOS. In (A) comparison between two groups (both groups with TBu vs. the control group), and in (B) comparison between the three groups (the TBuOral group, the TBuIV group, and the Control group). HSCT, hematopoietic stem cell transplantation; SE, systemic exposure; SOS, sinusoidal obstructive syndrome

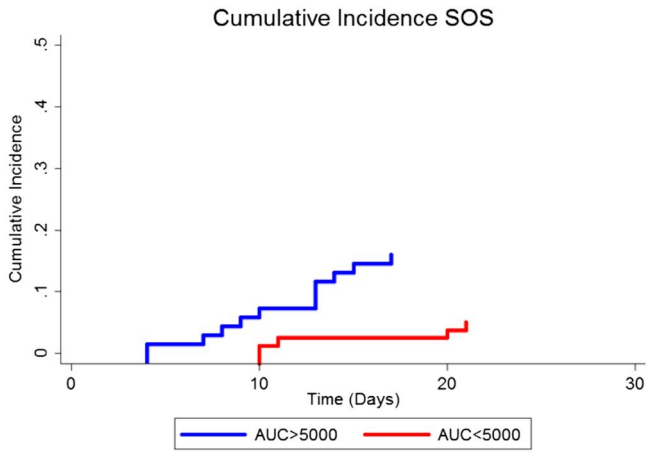

FIGURE 2 Effect of the calculation of a target of SE of busulfan (TBu) using a test dose before HSCT, of less than or more than 5000 μMol · min per hour, on the prevalence of SOS. HSCT, hematopoietic stem cell transplantation; SE, systemic exposure; SOS, sinusoidal obstructive syndrome

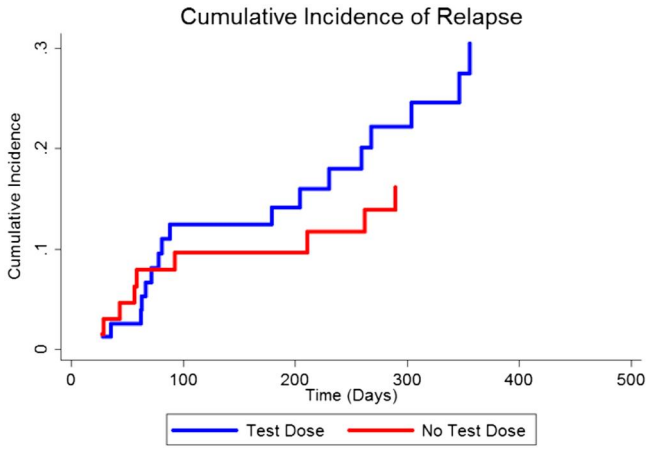

FIGURE 3 Disease recurrence in patients undergoing the calculation of a target of SE of busulfan (TBu) using a test dose before HSCT. HSCT, hematopoietic stem cell transplantation; SE, systemic exposure

SOS, a severe complication after HSCT, follows the damage to the hepatic vascular endothelium and the creation of a hypercoagulable state, with excessive production of thrombin.[35,36] In the present study, SOS prevalence was 11.4% and similar between the groups with and without the test dose. However, patients receiving a target AUC > 5000 μMol · min had a significantly higher rate of SOS than those receiving an exposure dose <5000 μMol · min ($p = 0.034$). SOS was associated with mortality at D + 100 after HSCT ($p < 0.001$) both in univariate and multivariate analyses. This finding can be partly explained by the fact that, we transplanted a large fraction of patients with an active disease requiring target doses between 5000 and 6000 μMol · min of AUC for better control of their disease.

A limitation of the present study (or maybe a pragmatic feature of it) is that the participants' sample is highly heterogeneous. It consisted of patients with both malignant and non-malignant diseases of different stages, with different preceding chemotherapy history, undergoing many different conditioning variant-regimens, and with autologous and allogeneic donors. Some of these patients had active disease. A multicenter Brazilian study, including 16 treatment centers, has shown that, in the moment of HSCT, 36% of patients with acute myeloid leukemia and 31% of those with acute lymphoid leukemia had advanced disease.[37] These patients are expected to have a lower survival rate due to advanced disease. To overcome this limitation, we divided our patients according to the disease status, recurrence rate and other clinical features that could interfere with the final results. However, no significant differences were found in the mucositis prevalence, even considering only the severe forms or comparing oral and IV administration.

In a recent study, we observed that the pharmacokinetic and clearance of the oral administration of busulfan are extremely variable, and this can be detrimental to the clinical results. Oral busulfan

TABLE 2 One-year survival in patients receiving different targets of SE of busulfan

	Survival in 1 year	Comparison
AUC < 4000 μMol · min	58% (CI 29%–80%)	HR: 0.65, $p = 0.33$, CI 0.27–1.54
AUC > 4000 μMol · min	65% (CI 55%–74%)	
AUC < 5000 μMol · min	66% (CI 51%–78%)	HR: 1.20, $p = 0.52$, CI 0.67–2.14
AUC > 5000 μMol · min	61% (CI 48%–73%)	
AUC < 6000 μMol · min	67% (CI 56%–75%)	HR 1.44, $p = 0.32$, CI 0.69–2.99
AUC > 6000 μMol · min	48% (CI 23%–70%)	

Abbreviations: AUC, area under the curve; CI, 95% confidence interval; HR, hazard ratio; SE, systemic exposure.

TABLE 3 Univariate and multivariate analyses of variables that could affect overall survival at D + 100

	Univariate				Multivariate			
		95% CI				95% CI		
	HR	Inferior	Superior	*p*	HR	Inferior	Superior	*p*
Male sex	1.52	0.75	3.07	0.245	-	-	-	-
Age (>19 years)	1.12	0.53	2.34	0.766	-	-	-	-
SOS (yes)	5.21	2.53	10.70	**<0.001**	5.27	2.46	11.27	**<0.001**
Groups								
Intravenous	1.27	0.36	4.51	0.710	-	-	-	-
Oral	1.52	0.51	4.53	0.450	-	-	-	-
Control group	2.15	0.82	5.65	0.121	-	-	-	-
Relapsed malignant disease in 100 days (yes)	3.21	1.40	7.39	**0.006**	2.31	0.93	5.72	**0.070**
Dosage modification based on the AUC (yes)	0.68	0.28	1.65	0.398	-	-		

Abbreviations: AUC, area under curve for time; CI, 95% confidence interval; HR, hazard ratio; SOS, sinusoidal obstructive syndrome.
p-values in bold are significant (< 0.05).

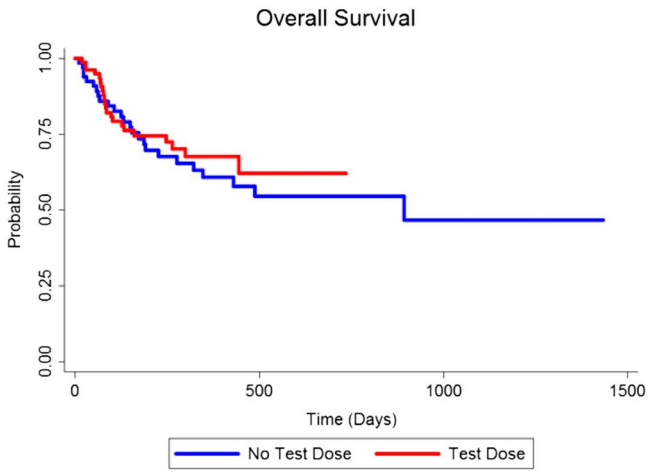

FIGURE 4 Effect of the calculation of a target of SE of busulfan (TBu) using a test dose before HSCT on overall survival. HSCT, hematopoietic stem cell transplantation

was associated with early onset of mucositis. Furthermore, the economic savings on the purchase of the oral compared with the IV busulfan should be balanced with the cost of care with gastrointestinal problems, due to the lower tolerability of drugs that need hepatic metabolization.[38]

Because of the heterogeneity of conditioning regimen, disease status, source of grafts, type of donors, and immunosuppression after HSCT in the sample, many variables could be implicated in GVHD. Therefore, due to the possible confounding factors, it was decided to forego the evaluation of any possible association of the target dose determination and GVHD in this study.

Performing a test dose before starting conditioning, in fact, did not improve the overall survival results or the recurrence rates. What did work was to set a target, ideal dose: setting the dose below 5000 μMol · min is safer for patients. One possible reason why the test dose did not affect the outcomes could be that, when the test dose was administered, 15 days to 48 h before starting the conditioning regimen, the individual's metabolism was dependent on other variables perhaps and not yet suffering from the impact of chemotherapy. The analysis of the pharmacokinetics during conditioning probably shows other medications that potentially compete/alter the liver enzyme profile, causing the dose of Bu to be reduced or increased. Therefore, in these situations, the adjustment of the Bu dose during conditioning based on the pharmacokinetic profile of the

test dose taken before conditioning has not always followed a rule or linear correlation. Our experience with busulfan levels monitoring and with a target dose setup was presented to the Brazilian Ministry of Health, which offered financial assistance for this study, and after that many local hospitals started to use intravenous busulfan and to monitor the oral dosage in patients receiving HSCT. After analyzing the results of this study, we stopped using the test dose routinely before HSCT. We take the dosage on the first day of conditioning to calculate the target dose, with adjustments in the second day. In the following years, the results of this initiative could be further evaluated, with larger and more homogeneous samples of patients, hopefully from multicenter collaborations.

5 | CONCLUSION

The adjustment of busulfan dose according to SE levels after a target dose of 5000 μMol · min/day is associated with lower rates of oral mucositis and SOS. Different target doses of busulfan (AUC 4000, 5000 e 6000 μMol · min) have significantly different impacts on SOS; and this study suggests that patients undergoing conditioning with >5000 μMol · min of AUC had a higher prevalence of SOS. We have, therefore, shown that the cutoff of 5000 μMol · min is safe regarding toxicity and it does not impair survival.

ACKNOWLEDGMENTS

The authors wish to thank the Brazilian Ministry of Health for the financial assistance for this study. This study was funded by the Brazilian Ministry of Health (PROADI).

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Iracema Esteves, Fabio Pires Souza Santos, Nelson Hamerschlak, and Fabio Rodrigues Kerbauy conceptualized and designed the study; Iracema Esteves, Fabio Pires Souza Santos, Andreza Alice Feitosa Ribeiro, Adriana Seber, Eduardo Kinio Sugawara, Jairo José do Nascimento Sobrinho, José Carlos Barros, José Salvador Rodrigues Oliveira, Juliana Folloni Fernandes, Nelson Hamerschlak, Borje S. Andersson, Marcos de Lima, and Fabio Rodrigues Kerbauy collected and analyzed data; Iracema Esteves and Fabio Pires Souza Santos performed statistical analysis; Iracema Esteves wrote the manuscript; Nelson Hamerschlak, Borje S. Andersson, Marcos de Lima, and Fabio Rodrigues Kerbauy revised the manuscript critically. All authors approved the final manuscript.

ETHICAL APPROVAL

This study is in accordance with the Declaration of Helsinki. All patients in the study groups signed informed consent forms to participate in this study. Because the control group was a historical cohort of patients retrospectively evaluated through medical records, informed consent was waived, and anonymity was guaranteed. The study was approved by the ethics committees of all the participating hospitals.

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PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/hon.2789.

Correction added on 01 October 2020, after first online publication: Peer review history statement has been added.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Esteves I, Santos FPS, Ribeiro AAF, et al. Targeted-dose of busulfan: Higher risk of sinusoidal obstructive syndrome observed with systemic exposure dose above 5000 μMol · min. A historically controlled clinical trial. *Hematological Oncology*. 2020;38:773–781. https://doi.org/10.1002/hon.2789