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Busulfan target exposure attainment in children undergoing allogeneic hematopoietic cell transplantation: a single day versus a multiday therapeutic drug monitoring regimen

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Busulfan exposure has previously been linked to clinical outcomes, hence the need for therapeutic drug monitoring (TDM). Study objective was to evaluate the effect of day 1 TDM-guided dosing (regimen d1) versus days 1 + 2 TDM-guided dosing (regimen d1 + 2) on attaining adequate busulfan exposure. In this observational study, we included all children receiving busulfan-based allogeneic hematopoietic cell transplantation. Primary outcome was the percentage of patients achieving busulfan target attainment in both TDM regimens. Secondary outcomes were the variance in busulfan exposure and day-4 clearance (Cl_{day4}) estimates between both TDM regimens and dosing day 1 and 2. In regimen d1, 84.3% ($n = 91/108$) attained a therapeutic busulfan exposure, while in regimen d1 + 2 a proportion of 90.9% was found ($n = 30/33$, not-significant). Variance of Cl_{day4} estimate based on busulfan day 2 concentrations was significantly smaller than the variance of Cl_{day4} estimates based on day 1 concentrations ($p < 0.001$). Therefore, day 1-guided TDM (pharmacometric model-based) of busulfan may be sufficient for attaining optimal target exposure, provided that subsequent TDM is carried out if required. However, performing TDM on subsequent days may be beneficial, as measurements on day 2 seemed to reduce the variance in the estimated clearance as compared to day 1 sampling.

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INTRODUCTION

Busulfan is widely used as part of conditioning regimens in patients undergoing allogeneic hematopoietic cell transplantation (HCT). It is characterized by a narrow therapeutic window in terms of clinical efficacy/toxicity and high inter- and intra-patient pharmacokinetic variability [1]. Due to the large interpatient variability in exposure, therapeutic drug monitoring (TDM) of busulfan is warranted [1].

Previous studies have shown a clear relation between busulfan exposure and clinical outcomes for several underlying indications and age groups. In children, underexposure has been associated with graft failure and disease recurrence, whereas overexposure has been associated with toxicity, such as veno-occlusive disease/sinusoidal obstruction syndrome [1, 2]. The therapeutic busulfan exposure, expressed as a cumulative 4-day area under the concentration-time curve (AUC0-∞), is 80–100 mg*h/L [1, 2].

Currently, busulfan TDM protocols vary widely between transplant centers, with marked differences in the timing and frequency of the measurements and the models used to estimate the AUC [1, 3, 4]. Several studies have reported that performing TDM on the first day is not sufficient for an accurate estimation of the AUC0-∞, especially because of the within-patient fluctuation in clearance and volume of distribution. It has therefore been suggested that additional TDM on the second and/or third day may lead to a more accurate estimation [5, 6]. Comparing such strategies using real world data is complicated however, as it typically requires indirect comparisons between centers, which is confounded by other factors that may differ between transplant centers. Ideally, both TDM strategies should be used in the same transplant center to allow for a fair comparison, but this has not been done to date.

It remains therefore unclear whether busulfan TDM on multiple days leads to a better prediction of the actual cumulative exposure. The aim of this study was to evaluate the effect of day 1 TDM-guided dosing (regimen d1) versus days 1 + 2 TDM-guided dosing (regimen d1 + 2) on attaining adequate busulfan target exposure in children undergoing an allogeneic HCT.

METHODS

Setting, design, and study population

In this retrospective cohort study with prospectively collected data, we included all pediatric (<18 years) patients who received their first

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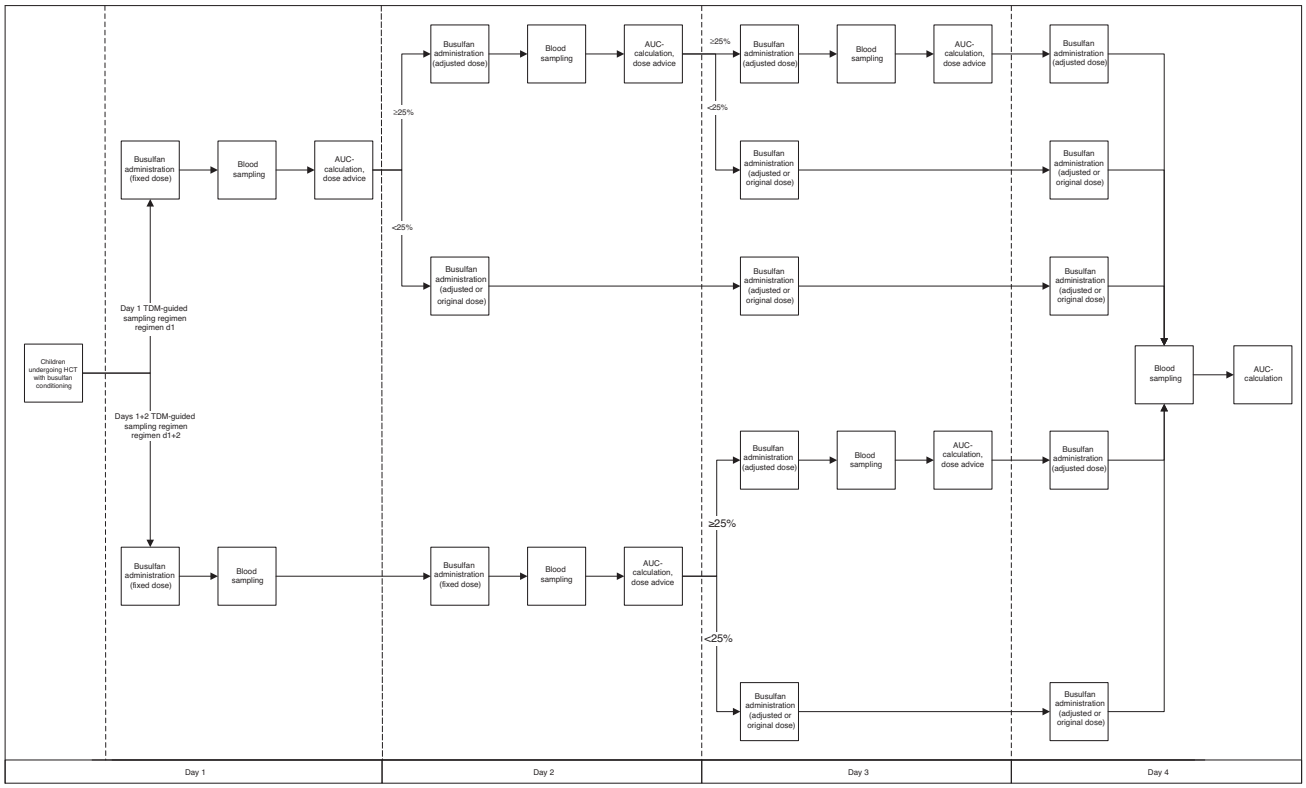

Fig. 1 Two busulfan therapeutic drug monitoring (TDM) sampling strategies were applied: either blood sampling was performed on day 1 (regimen d1), with a dose adjustment based on the day 1 AUC, or blood sampling was performed on days 1 + 2 (regimen d1 + 2), with a dose adjustment based on the days 1 + 2 AUC. Additional TDM was performed in the event of large dose adjustments (≥25%). Blood was drawn at 5 min, 1 h, 2 h, and 3 h after the end of the busulfan infusion. Blood sampling was always performed on day 4. AUC area under the curve, HCT hematopoietic cell transplantation.

allogeneic HCT with TDM-guided intravenous busulfan dosing as part of the conditioning regimen at the University Medical Centre Utrecht (UMCU) or the Princess Máxima Center for Pediatric Oncology (Máxima) between 31st July 2014 and 12th November 2021. The Medical Research Ethics Committee NedMec of the UMCU and Máxima have given permission for this study. The data were collected after patients provided written informed consent in accordance with the Helsinki Declaration. The transplantation centers registered patient-specific, demographic, medication-related, and laboratory data for at least 6 months after the start of the conditioning for the HCT. Patient-specific and demographic variables were collected from the TRIASUS database. TRIASUS is a web-based database that manages all HCT-related data from patients and their (potential) donors [7].

Busulfan dosing and TDM regimens

Busulfan TDM and HCT-related procedures were performed according to a harmonized UMCU and Máxima treatment protocol. Busulfan was administered once a day over 4 consecutive days as a 3-hour intravenous infusion. Blood sampling was performed at 5 min, 1 h, 2 h, and 3 h after the end of the infusion, according to the local TDM protocol. The plasma samples were analyzed with a liquid chromatography–tandem–mass spectrometry assay [8]. The analytical method was validated in accordance with the EMA guideline for bioanalytical method validation [9].

Regimen d1 was defined as busulfan TDM on the first day of therapy with day 1 AUC-guided dose adjustment on day 2. Regimen d1 + 2 was defined as busulfan TDM on the first two days of therapy with days 1 + 2 AUC-guided dose adjustment on day 3. Dose adjustment was based on the estimated AUC of the preceding dosing day(s). Additional TDM was performed in the event of large dose adjustments (≥25%). In all patients, blood sampling was performed on day 4 for evaluation. The TDM protocol is illustrated in Fig. 1. The choice of TDM regimen was solely based on pure practical reasons, namely the first day busulfan was administered (regimen d1 + 2 occurred when conditioning started on Saturday). Patients were divided into two groups based on their TDM regimen. Exposure of interest was the TDM regimen that was utilized (regimen d1 vs regimen d1 + 2).

Outcomes

The primary outcome was attainment of the therapeutic busulfan target (AUC0-∞ 80–100 mg*h/L). We estimated the AUC0-∞ using an optimized two-compartment model that accounted for intra-individual variation in busulfan clearance (Table S1; Fig. S1) [2]. This model was based on a previously validated model described elsewhere [10]. To estimate the AUC0-∞, we collected the following variables from the laboratory information system database: busulfan dose, time of busulfan administration, duration of infusion, sampling times, busulfan concentrations, and the busulfan dose advice from day 1 to day 4. The busulfan exposure was estimated using all available samples that were taken on days 1–4.

As a secondary outcome, we estimated the busulfan clearance on day 4 (CL_{day4}) with the concentrations measured on day 1 (regimen d1) and the concentrations measured on days 1 + 2 (regimen d1 + 2). In addition, we predicted the CL_{day4} in two data-subsets: one subset with the concentration-time profiles from all patients in whom the measurements were taken on day 1, a second subset from all patients in whom measurements were taken on day 2, regardless of the TDM regimen. Because samples were routinely taken on day 4, this allowed us to estimate the sampling-derived CL_{day4}. We then compared the estimated CL_{day4} with the sampling-derived CL_{day4} for both TDM regimens.

Potential confounders and effect modifiers

Biological plausibility and available literature suggest that the following determinants may influence busulfan concentrations and were therefore considered potential confounders and/or effect modifiers: sex, body weight, disease status (malignant/non-malignant), serotherapy regimen (anti-thymocyte globulin) and the conditioning regimen.

Data analysis

Demographic, donor, and transplant characteristics of patients were compared using the Chi-square test. Patients were stratified by age (0–2, 2–5, 5–12, and 12–18) and the magnitude of busulfan dose adjustment (<25% and ≥25%), and Wald tests were used to detect statistical

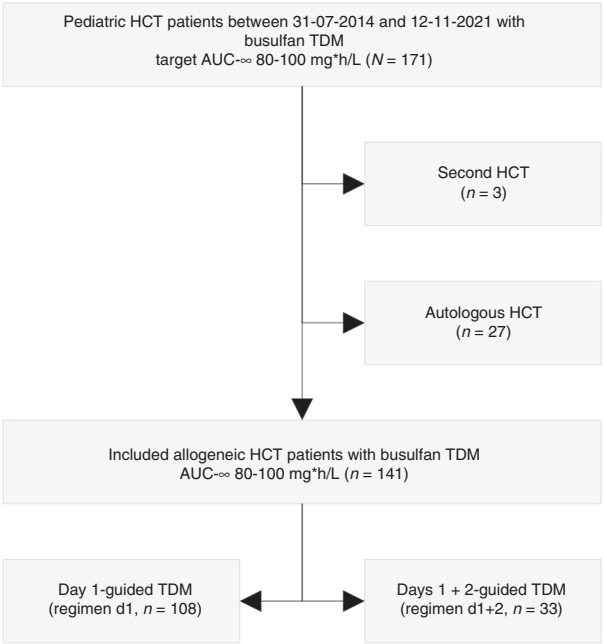

Fig. 2 Overview of the reasons for patient exclusion and the number of patients that were included in the study in the regimen d1 group (day 1-guided therapeutic drug monitoring (TDM)) and the TDM regimen d1 + 2 group (days 1 + 2-guided TDM). HCT hematopoietic cell transplantation, AUC area under the curve.

interaction. The target attainment was calculated by stratum and in the total population. The target attainment in both TDM regimens was compared using descriptive statistics and a propensity score-adjusted logistic regression model (SAS institute, version 9.4). The propensity score was calculated with the following covariates: gender, body weight, disease status (malignant/non-malignant), serotherapy regimen (anti-thymocyte globulin), and the conditioning regimen. The variance of the AUC0–∞ between TDM regimens was compared using the *F*-test (SAS institute, version 9.4)

RESULTS

A total of 141 children underwent an allogeneic HCT with intravenous busulfan as part of their conditioning regimen in the seven-year study period (Fig. 2). The median age was 6.7 years (range 0.2–17.8 years) and the median body weight was 24.9 kg (range 3.8–114 kg, Table 1). The most frequently used conditioning regimens were busulfan/fludarabine/clofarabine (55.3%, $n = 78$) and busulfan/fludarabine (44.0%, $n = 62$).

Patient characteristics were equally distributed between both TDM regimen groups (Table 1). In total, 76.6% ($n = 108$) of patients started with regimen d1. Overall, 49.6% of the patients received subsequent TDM (on day 2 and/or day 3) of busulfan due to a large dose adjustment (≥25%) that was based on the AUC of the previous sampling day. Within the regimen d1 cohort, 51.9% ($n = 56$) of the patients were monitored on day 1 only, 38.9% ($n = 42$) on days 1 and 2, and 9.3% ($n = 10$) on days 1, 2 and 3. In total, 23.4% of patients ($n = 33$) started with regimen d1 + 2. Within the regimen d1 + 2 cohort, 45.5% ($n = 15$) of the patients were monitored on days 1 + 2 only, and 54.5% ($n = 18$) on days 1, 2 and 3. The blood of all patients was drawn on day 4, according to protocol. The mean number of blood samples drawn from each patient was 10.2 in the regimen d1 group and 13.8 in the regimen d1 + 2 group. The mean number of TDM occasions was 2.6 in the regimen d1 group and 3.5 in the regimen d1 + 2 group.

Target attainment of busulfan

In total, 85.8% ($n = 121$) of patients attained therapeutic busulfan exposure (AUC0–∞ 80–100 mg*h/L). The busulfan exposure was estimated using all available samples taken on days 1–4. For all patients, at least days 1 and 4 plasma levels were available. We found that 84.3% ($n = 91$) of patients attained their target with regimen d1 and 90.9% ($n = 30$) with regimen d1 + 2 (Table 2 and Fig. 3). In the regimen d1 group, 15.7% ($n = 17$) of patients were underexposed or overexposed to busulfan, while 9.1% ($n = 3$) were in the regimen d1 + 2 group (odds ratio [OR] = 0.46, 95% confidence interval [CI] 0.12–1.72). There was no significant difference in target attainment between both TDM regimens in the total population and various age groups (Table 2).

Variance of the busulfan exposure

The busulfan AUC0–∞ varied considerably (range 68.1–114.6 mg*h/L, mean = 88.7, standard deviation [SD] = 7.0). The variance of the AUC0–∞ in the regimen d1 group (range 71.1–114.6 mg*h/L, mean = 88.9, SD = 7.2) did not significantly differ from the variance of the AUC0–∞ in the regimen d1 + 2 group (range 68.1–102.5 mg*h/L, mean = 88.1, SD = 6.5, $p = 0.54$). In addition, in the various age groups, the variance of the AUC0-∞ did not significantly differ between TDM regimens (Fig. 3, data not shown).

Estimation of the clearance on day 4

We estimated the CL_{day4} in both TDM regimen groups, using the concentrations measured on day 1 (regimen d1) and the concentrations measured on days 1 + 2 (regimen d1 + 2). We found considerable variation in the difference between the estimated busulfan CL_{day4} and day 4 sampling-derived CL_{day4} in the regimen d1 group (mean = 5.6%, SD = 15.9%) and the regimen d1 + 2 group (mean = −1.0%, SD = 13.9%, Fig. 4). This variance did not vary significantly between the regimen d1 and the regimen d1 + 2 groups ($p = 0.39$).

In addition, we also estimated the CL_{day4} with the concentrations from all patients with all measurements that were taken on day 1 and all measurements that were taken on day 2, regardless of the TDM regimen. The difference of the variation in the estimated busulfan CL_{day4} and day 4 sampling-derived CL_{day4} varied significantly between all patients with busulfan concentrations measured on day 1 (mean = 4.6%, SD = 15.5%) and all patients with busulfan concentrations measured on day 2 (mean = −2.0%, SD = 10.8%, $p < 0.001$, Fig. 4).

Intra-individual variability of the clearance

Between day 1 and day 4, 62.4% ($n = 88$) of patients experienced a decrease in the clearance of busulfan, with a median decrease of 5.8%. In addition, 37.6% ($n = 53$) experienced an increase in clearance, with a median increase of 2.6%. Overall, the clearance increased and decreased considerably (median −2.1%, minimum −65.1%, maximum 40.1%).

DISCUSSION

In this study, we compared the target attainment (exposure target AUC0–∞ 80–100 mg*h/L) of busulfan between HCT patients with day 1-guided TDM (regimen d1) and days 1 + 2-guided TDM (regimen d1 + 2). The AUC0-∞ was estimated using nonlinear mixed-effects modeling with an optimized model that adjusted for (inter-occasion) variability in clearance. The busulfan dose was adjusted accordingly on the remaining days of therapy and additional TDM was performed in the event of large dose adjustments (≥25%). There was no significant difference in the busulfan target attainment between both TDM regimens. The target attainment was 84.3% in the regimen d1 group, compared to 90.9% in the regimen d1 + 2 group. Busulfan blood concentrations taken on day 2 result in a significantly smaller variation in the

Table 1. Patient characteristics of both therapeutic drug monitoring regimens at the start of conditioning (Chi-squared test).

		Regimen d1		Regimen d1 + 2		
		%	*N* = 108	%	*N* = 33	*P*
Patient demographics						
Gender	Male	47.2	51	48.5	16	0.90
	Female	52.8	57	51.5	17	
Age (years)	<2	26.9	29	15.2	5	0.37
	2–5	16.7	18	18.2	6	
	5–12	25.9	28	39.4	13	
	12–18	30.6	33	27.3	9	
Weight (kg)	<10	15.7	17	9.1	3	0.59
	10–20	30.6	33	27.3	9	
	20–30	15.7	17	24.2	8	
	>30	38.0	41	39.4	13	
BMI (kg/m2)	0–18.5	63.0	68	63.6	21	0.62
	18.5–25	32.4	35	27.3	9	
	25–30	2.8	3	3.0	1	
	>30	1.9	2	6.1	2	
Donor characteristics						
Diagnosis	Malignant	62.0	67	60.6	20	0.88
	Non-malignant	38.0	41	39.4	13	
Donor	Family	15.7	17	18.2	6	0.86
	Unrelated	82.4	89	78.8	26	
	Missing	1.9	2	3.0	1	
Matching status	Matched	55.6	60	63.6	21	0.41
	Mismatch	44.4	48	36.4	12	
Donor source	Bone marrow	29.6	32	51.5	17	0.053
	Peripheral blood	2.8	3	0.0	0	
	Cord blood	67.6	73	48.5	16	
Hematopoietic cell transplantation characteristics						
Conditioning regimen	Busulfan/fludarabine	42.6	46	48.5	16	0.73
	Busulfan/cyclophosphamide/melphalan	0.9	1	0.0	0	
	Busulfan/fludarabine/clofarabine	56.5	61	51.5	17	
Serotherapy	Antithymocyte globulin	66.7	72	84.8	28	0.13
	Campath (alemtuzumab)	0.9	1	0.0	0	
	Missing/other	32.4	35	15.2	5	

BMI body mass index, *BSA* body surface area.

prediction of clearance on day 4 (CL_{day4}) compared to blood concentrations taken on day 1 and may therefore provide a better estimate.

Performing TDM on an additional day (regimen d1 + 2, with subsequent TDM if required) did not significantly increase target attainment, which is not in line with the findings of Marsit et al. and Alsutan et al., who found that additional TDM increased target attainment [5, 6]. However, these results can only be compared cautiously because these studies were designed differently. First, these studies used a different busulfan dosing regimen and timing of blood sampling [5, 6]. Second, Marsit et al. also included patients receiving an autologous HCT with various conditioning regimens, which contained melphalan and cyclophosphamide. These drugs further potentiate busulfan hepatoxicity, which may hypothetically influence busulfan clearance [11, 12]. Third, they used different pharmacometric models to estimate busulfan exposure [5, 6]. We used a model that accounted for the intra-individual variability in clearance well, as shown by the high level of busulfan target attainment (85.8%). If the models used in the aforementioned studies only partially accounted for this variability, the estimate based on only day 1 concentrations may be less precise, which would have made repeated TDM necessary. Additionally, our approach allows for subsequent TDM if the patients pharmacokinetics differ from the estimations of the pharmacometric model (e.g. in patients with a large dose adjustment), which may have further improved target attainment.

The variance in the estimated busulfan CL_{day4} was significantly smaller if the CL_{day4} was based on day 2 concentrations instead of day 1 concentrations (Fig. 4). Considering that busulfan clearance often decreases on day 2 or 3 of therapy [4–6, 13–15], presumably due to intracellular glutathione depletion [13], this implies that clearance estimates based on day 1 concentrations may not hold true over the entire therapy. In line with this, we observed a decrease in busulfan clearance in 62.4% of patients, with a 5.8% median decrease between day 1 and day 4. These findings have important implications for busulfan TDM, because day 2-based estimates may be more accurate than day 1-based estimates in calculating the $AUC0-\infty$, warranting sampling on day 2 instead

Table 2. Target attainment of busulfan for both therapeutic drug monitoring (TDM) regimens, stratified for age (0–2, 2–5, 5–12, and 12–18 years).

			Therapeutic AUC (mg*h/L)		Non-therapeutic AUC (mg*h/L)			
			80–100		<80 or >100			
Stratum		***N* patients**	**%**	***n***	**%**	***n***	**adjOR**	**95% CI**
Total population	Regimen d1	108	84.3%	91	15.7%	17	Ref	
	Regimen d1 + 2	33	90.9%	30	9.1%	3	0.46	0.12–1.72
0–2 (years)	Regimen d1	29	79.3%	23	20.7%	6	Ref	
	Regimen d1 + 2	5	100.0%	5	0.0%	0	NE	
2–5 (years)	Regimen d1	18	77.8%	14	22.2%	4	Ref	
	Regimen d1 + 2	6	83.3%	5	16.7%	1	0.38	0.02–6.00
5–12 (years)	Regimen d1	28	92.9%	26	7.1%	2	Ref	
	Regimen d1 + 2	13	84.6%	11	15.4%	2	2.55	0.28–23.08
12–18 (years)	Regimen d1	33	84.8%	28	15.2%	5	Ref	
	Regimen d1 + 2	9	100.0%	9	0.0%	0	NE	

adjOR odds ratio adjusted for gender, body weight, disease status (malignant/non-malignant), serotherapy regimen (anti-thymocyte globulin) and the conditioning regimen, *AUC* area under the curve, *CI* confidence interval, *NE* not estimable, *Ref* reference.

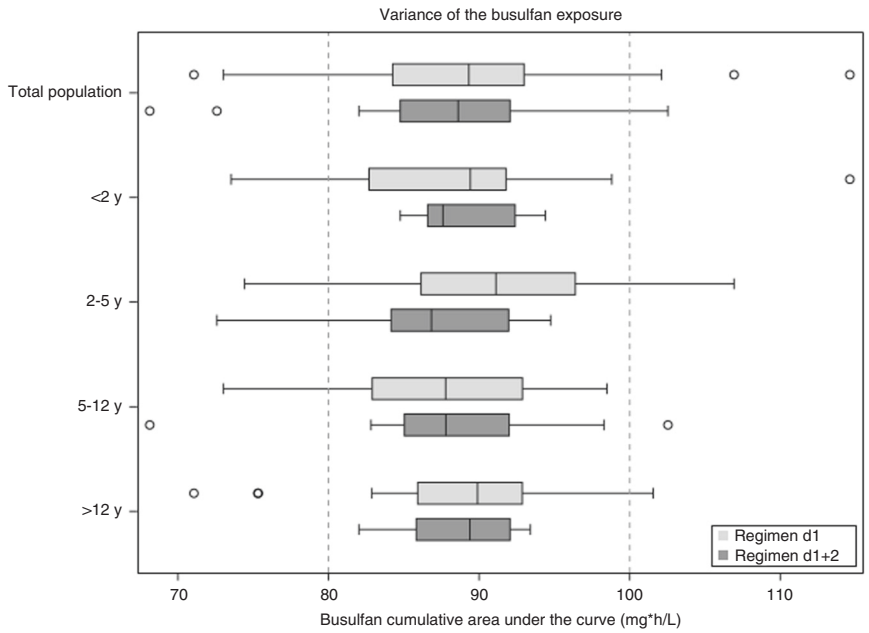

Fig. 3 The variance of the busulfan cumulative exposure (target cumulative area under the curve = 80–100 mg*h/L) for both therapeutic drug monitoring (TDM) regimens, stratified for age (0–2, 2–5, 5–12, and 12–18 years). The variance did not differ significantly between TDM regimens (*F*-test).

of day 1. However, caution must be exercised when applying these results to current clinical practice, because in our total patient population, the clearance of busulfan increased and decreased considerably throughout therapy (minimum −65.1%, maximum 40.1%). This further complicates the estimation of the total exposure, which may necessitate performing TDM several times over the course of therapy.

Interestingly, 37.6% of patients tended to have an increased clearance of busulfan throughout treatment (median increase of 2.6%). The current findings appear to be inconsistent with previous studies, which reported an 8–15% decrease [15–21] or no change in busulfan clearance [22, 23]. However, it should be noted that some patients in these studies also exhibited a significant increase in clearance, similar to what has been observed in this study. The reason for this increase in clearance is not clear and may have multiple potential explanations. First, a small number of studies have shown that busulfan can induce its metabolism by increasing glutathione synthesis and/or glutathione transferase (GST) activity [12, 24, 25]. Second, interacting medication can induce GST or CYP450 enzymes by which busulfan is metabolized [12, 14]. However, this effect can be mitigated to some extent by TDM-guided dose adjustments, but may still be relevant on the final day of busulfan therapy, on which TDM cannot be applied. Unfortunately, we did not collect data on medication co-administered during busulfan therapy.

Finally, several limitations need to be considered. First, various studies have demonstrated the influence of GST genotypes on busulfan clearance, with various genotypes showing a marked reduction in clearance [26–28]. Therefore, the observed differences in target attainment between patients may be attributed to

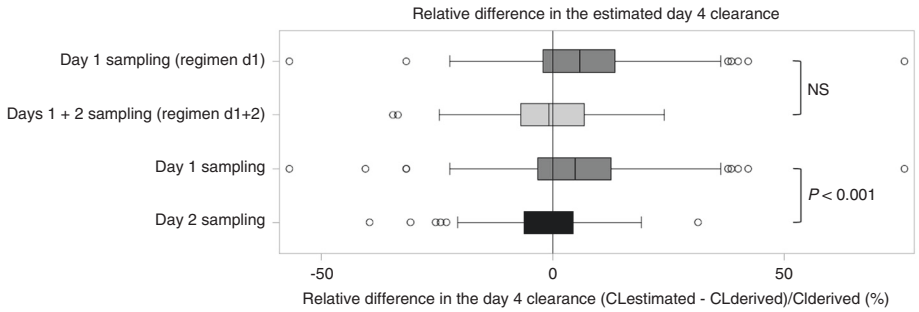

Fig. 4 The agreement between the estimated and derived day 4 busulfan clearance (CL_{day4}) for both TDM regimens and concentrations that were measured on day 1 and day 2. In regimen d1, the CL_{day4} was estimated based on the concentrations that were measured on day 1, whereas in regimen d1 + 2 regimen, the CL_{day4} was estimated based on the concentrations measured on days 1 and 2. The CL_{day4} was also estimated using the day 1 or day 2 concentrations, regardless of the TDM regimen. The derived CL_{day4} was calculated using the concentrations that were measured on day 4. NS not significant.

variations between GST genotypes, but unfortunately, we do not have data on this. Second, the number of TDM occasions and the number of blood samples are greater in the regimen d1 + 2 group than in the regimen d1 group. Therefore, the estimates of the busulfan AUC0-∞ may be more accurate in the regimen d1 + 2 group than in the regimen d1 group due to having more busulfan concentrations available. However, it is worth noting that the higher number of TDM occasions is an aspect of the intervention in the regimen d1 + 2 group and should not be misconstrued as bias.

Many centers send samples out to external laboratories for plasma busulfan testing, which logistically complicates performing additional TDM on the subsequent dosing day, e.g. in patients with a large dose adjustment. Our TDM strategy may therefore not be feasible to implement in centers where additional timely TDM is not possible. Alternative approaches, such as day 2 sampling (instead of day 1), might be more informative (as suggested by our results), but more thorough research is needed for this approach.

In conclusion, the results of this study suggest that TDM on the first day of therapy may be sufficient for attaining the optimal busulfan target in children receiving busulfan as part of the HCT conditioning regimen, provided a valid pharmacometric model is used and 'as needed' TDM on subsequent days is performed based on previous pharmacokinetic data. In some patients however, performing TDM on subsequent days may be beneficial, as sampling on day 2 seemed to reduce the variance in the estimated clearance as compared to day 1 sampling.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, TB, upon reasonable request.

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AUTHOR CONTRIBUTIONS

TB, JSK, EHS, KCME, CTMK, CAL, ACGE, IHB and AL designed the research and participated in the manuscript. TB, JSK and CL collected the data. IHB performed the pharmacometric analysis of the data. TB, ACGE and AL performed the statistical analysis. TB, ACGE, IHB and AL wrote the manuscript. All authors read and approved the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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