Hematol Oncol Stem Cell Ther (2017) 10, 70–78

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ORIGINAL RESEARCH REPORT

Therapeutic drug monitoring-guided dosing of busulfan differs from weight-based dosing in hematopoietic stem cell transplant patients

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Received 27 June 2016; accepted 14 March 2017
Available online 6 April 2017

KEYWORDS
Busulfan;
Pharmacokinetics;
Drug monitoring;
Hematopoietic stem cell transplantation

Abstract
Busulfan (Bu)-based preparative regimens in hematopoietic stem cell transplantation are commonly used. Previous studies have shown that Bu at a fixed dose of 3.2 mg/kg/day (FBD) given intravenously decreases variability in drug pharmacokinetics and this decreases the dependency on therapeutic drug monitoring (TDM) of Bu. We compared the Bu dose given using TDM with the FBD of 3.2 mg/kg/day. Seventy-three patients with acute leukemia, myelodysplasia, chronic myeloid leukemia, thalassemia major, and sickle cell disease were included. The mean age at transplant was 15 years (range 2—55 years) with 57% adults. Indication for transplantation was leukemia/myelodysplastic syndrome in 46% of the patients, while the remaining 54% were transplanted for inherited blood disorders. We found that the median FBD was lower than the median TDM dose by 39 mg/day with a statistically significant difference ($p < 0.001$) even after adjusting for the weight (median total FBD of 349 mg, median TDM dose of 494 mg, $p < 0.0001$). Age and underlying condition (malignant vs. nonmalignant) were the main factors affecting Bu clearance ($p < 0.001$ and $p < 0.07$, respectively). TDM remains an important tool for the appropriate dosing of Bu in preparative regimens of hematopoietic stem cell transplantation, especially in populations with genetic admixture.

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http://dx.doi.org/10.1016/j.hemonc.2017.03.003

Background

Busulfan (Bu) is a bifunctional DNA alkylating agent of the alkyl sulfonate type [1]. It is one of the most frequently used chemotherapeutic agents in preparative chemotherapy combination regimens in patients undergoing hematopoietic stem cell transplantation (HSCT) for various malignant and nonmalignant diseases. Bu pharmacokinetic (PK) profile is best described as a single-compartment model [2]. Absorption is rapid with maximum concentration (Cmax) achieved at around 1 h with a highly variable oral bioavailability of approximately 70–90%. Bu is predominantly metabolized in the liver and excreted in the urine mainly as its metabolites, with very minimal amount (<2%) of the parent compound recovered. The terminal half-life of Bu was found to be 2–3 h [2].

Several investigators demonstrated a relation between Bu exposure and clinical outcome [2]. It was found that Bu has a narrow therapeutic window. Studies have shown that myeloablative doses of Bu are one of the factors that may contribute to enhanced toxicity in HSCT, such as the development of acute graft-versus-host disease and veno-occlusive disease (VOD) of the liver, whereas underexposure to Bu may be one of the predictors of graft rejection or relapse [2]. Therapeutic drug monitoring (TDM) strategy was developed for Bu to allow patients to reach and maintain Bu concentration within the therapeutic window. TDM for Bu using steady state concentration (Css) and area under the curve (AUC) was found to correlate with the incidence of graft failure, transplant-related mortality, and relapse of the primary disease [1]. For example, the incidence of graft rejection was reduced with a target Css > 600 ng/mL or AUC > 900 μM/min. Similarly, the incidence of VOD and severe toxicities increases when Bu Css exceeds a threshold value of 1025 ng/mL (AUC > 1500 μM/min) [1,2].

Recent studies of Bu drug exposure and clinical outcomes have suggested that Bu dose targeting can be eliminated as the fixed-dose intravenous (IV) Bu (FBD) regimens are as safe and effective as targeted doses based on AUC or Css, and at least 80% of the patients achieved the therapeutic window, close to the threshold values [3]. This needs to be proven for populations with genetic admixture and to include patients with benign and malignant indications for transplant.

Therefore, the primary objective of this study was to estimate the difference in the total Bu dose between TDM-based and the calculated weight-based Bu dosing methods. Secondary objectives included assessment of the impact of patients' age and diagnosis on the difference in the total Bu actual TDM dose versus FBD.

Patients and methods

Patients and study design

This was a retrospective study of patients who received IV Bu as part of their preparative regimen prior to HSCT at Sultan Qaboos University Hospital (Muscat, Oman) from 2003 to 2014.

We included male and female patients, from all age groups, undergoing identical sibling or matched (8/8) related donor allogeneic stem cell transplant who received IV TDM-based Bu for any of the following conditions: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), beta-thalassemia major (β-TM), and sickle cell disease (SCD). Patients with missing outcome and PK data were excluded. Patients 13 years and older were taken care of by an adult hematologist and were analyzed under the adult group for the purpose of this study.

Conditioning regimens

For patients transplanted in 2003–2004, the preparative regimen consisted of targeted IV Bu given from Day −9 to Day −6 and IV cyclophosphamide (Cy) 50 mg/kg from Day −5 to Day −2. One patient received IV Bu with melphalan and antithymocyte globulin (ATG). The preparative regimen was changed in September 2004, when fludarabine (Flu) replaced Cy. Patients with acute ALL, AML or MDS, aged < 50 years old, received myeloablative conditioning which consisted of TDM-based IV Bu and Flu 40 mg/m^2 from Day −6 to Day −3, inclusive. Bu was administered as a single daily dose (SDD). The same regimen was also used in patients with β-TM with the addition of ATG-F (Fresenius) 10 mg/kg from Day −4 to Day −1. Patients older than 50 years of age with AML or MDS received a reduced-intensity conditioning (RIC) regimen that consisted of SDD, TDM-based IV Bu for 2 days (Day −6 and Day −5), IV Flu 30 mg/m^2 (Day −10 to Day −5), and ATG-F 10 mg/kg (Day −4 to Day −1). Patients with SCD received the same RIC with the 6-hourly Bu regimen. The target Css for RIC and myeloablative conditioning were 800 ng/mL and 900 ng/mL, respectively.

Dose and administration of Bu

Patients received a test dose of 0.5 mg/kg 48 h prior to conditioning. Dose 1 was adjusted if needed linearly to reach the target Css according to the Css achieved after the test dose. Similarly, dose adjustments were made possible for Dose 5, Dose 9, and Dose 13. When using the SDD regimen, adjustments were only possible for Dose 3 given the time needed to get the results of Bu PK. The total TDM-based dosing was calculated by adding the actual doses given to the patients, which were retrieved from the chemotherapy request forms sent to the pharmacy for the preparation of IV Bu doses. Bu was administered IV via a central venous catheter as a 2-h infusion for multiple daily doses (MDD) or 3-h infusion for SDD.

We calculated the FBD using 0.8 mg/kg administered every 6 h for MDD or 3.2 mg/kg for SDD. The total dose was the sum of calculated doses according to the number of days as per protocol used. This dose was not actually administered to the patient and comparison was done based on theoretical measures. All doses were calculated according to actual body weight.

Bu blood concentration measurement and PK analysis

Heparinised blood samples (2 mL) were drawn in conjugation with the administration of the test dose and Dose 1,

Dose 5, Dose 9, and Dose 13 of IV Bu with the MDD regimen. Samples of the test dose were collected immediately before drug infusion and at 5 min, 1 h, 2 h, 4 h, and 6 h after the end of the 20 min infusion ($n = 6$ samples). For the rest of the doses, collection was done immediately before drug administration and at 2 h, 3 h, 4 h, and 6 h after the start of the 2 h infusion. With SDD, Bu was infused over 3 h and five samples were collected before each dose (at times 0 h, 3 h, 6 h, 12 h, 18 h, and 24 h from the start of the infusion). Because IV Bu was administered through a central venous catheter, all blood samples for PK studies were collected from a peripheral IV catheter to avoid contamination caused by the proximity between the lumens of the catheter used for infusion. Samples were immediately placed in ice and sent to the laboratory. Samples were then centrifuged for 10 min at +4 °C at 3000 rpm (1781 g). The plasma was separated in labeled cryogenic tubes and frozen at −30 °C for the PK study.

The plasma concentration of Bu was measured by validated liquid chromatography-tandem mass spectrometry using an API 3200 triple quadruple mass spectrometer equipped with an electrospray ion source (AB SCIEX, Framingham, MA, USA) in a method similar to that described by dos Reis et al. [4].

PK modeling was performed using the Macro function in Microsoft Excel (Microsoft Office Excel 2007, Microsoft Windows XP Professional V5.1: Microsoft Corporation, Santa Rosa, California, USA; or earlier versions starting 2003) with a program using the standard formulae. The plasma concentration-time curve was obtained by measuring the plasma concentration at several time points. The AUC at 6 h was calculated from the Bu concentration-time curve starting from time 0 according to the trapezoidal rule. The elimination rate constant was estimated according to a one-compartment model using the semilogarithmic plot of the concentrations at 2 h, 4 h, and 6 h and finding the slope of the resulting straight line. This constant was used to extrapolate the AUC (at infinity) from the following relationship: AUC (0−∞) (ng/mL/h) = Bu AUC at 6 h/elimination rate constant. This AUC was then used to find the concentration at steady state using the following formula: Css (ng/mL) = AUC (0−∞)/dosing interval (6 h). Clearance was calculated using the primary parameters by the following formula: clearance (L/h) = dose/AUC (0−∞) * 1000 [5].

Supportive care

For acute graft-versus-host disease prophylaxis, all patients were given cyclosporine 2.5 mg/kg/dose IV every 12 h starting Day −1 and short course methotrexate. Methotrexate was given at a dose of 15 mg/m^2 on Day +1 and 10 mg/m^2 on Day +3, Day +6, and Day +11. Folinic acid 15 mg IV twice daily for two doses was initiated 24 h after each dose of methotrexate. Phenytoin was used as seizure prophylaxis in all patients receiving Bu. Prophylactic defibrotide (10 mg/kg/day) was used in heavily pretreated patients, those with chronic iron overload and if the baseline liver function tests were deranged, as these patients were considered high risk for VOD. Defibrotide was started 1 day before initiation of Bu until Day +20. Prophylactic antimicrobials included IV fluconazole and acyclovir starting on the first day post-transplant and stopping when absolute neutrophil count (ANC) > 1.0 × 10^9/L. Phenoxymethylpenicillin and oral acyclovir were started at discharge and continued for 2 years post-transplant. Prophylactic cotrimoxazole was started at first follow-up and continued for 1 year. Platelets were given to keep platelet counts above 10 × 10^9/L at all times unless a higher level otherwise indicated. Blood transfusion was given to keep Hb > 8 g/dL at all times. All patients were cared for in high-efficiency, particle-free air-filtered positive-pressure isolation rooms.

Statistical analysis

Continuous variables were presented as means with standard deviation (SD) if normally distributed and otherwise as medians with interquartile range (IQR). For categorical variables, the frequencies and the percentages were reported.

To estimate the difference in the total Bu dose between the two methods (TDM-guided vs. FBD), paired t test was used. To assess the impact of patient's age on the total dose difference, a univariable linear regression model was used with gender as a predictor. Weight was adjusted for using multivariate linear regression.

The impact of the following demographic factors on clearance was analyzed: gender, age, weight, and body surface area (BSA). The effect of gender and age on clearance was analyzed using Student's t test if clearances were normally distributed and otherwise by the Mann−Whitney test. For weight and BSA, Pearson's correlation coefficient was computed to assess the impact of these continuous variables on clearance.

All analyses were carried out using STATA version 13 (StataCorp, 2013, Stata Statistical Software: Release 13; StataCorp LP, College Station, TX, USA).

The study was approved by the Ethics Committee at the College of Medicine and Health Science at Sultan Qaboos University.

Results

Baseline characteristics

We included 73 patients who fulfilled the inclusion criteria. The baseline characteristics of the included patients are detailed in Table 1. Females constituted 52% of the study population. The median age at transplant was 15.5 years (IQR 9.1−24.3; range 2.2−55.9). Adults comprised 57% of the study population. The median and the IQR for weight, height, and serum bilirubin were 39.6 kg (IQR: 23−61.5), 152 cm (IQR: 130−163), and 10 μmol/L (IQR: 4−17.5), respectively. Indication for transplantation was ALL, AML, and MDS in 11, 14, and five patients, respectively. The indication was SCD in 23 patients (31%), β-TM in 16 patients (22%), and CML in four patients (5%) (Table 1). For those with acute leukemia, five of the 11 ALL patients were in their first complete remission (CR1), whereas 10 of the 14 AML patients were in CR1. The graft source was peripheral blood stem cells in 71% of patients and bone marrow in 27% of patients. In one patient, both sources were used to

Table 1 Patient demographics and conditioning regimens.

Characteristic(s)	*n* = 73
Sex, *n* (%)	
Male	35 (48)
Female	38 (52)
Age at transplant (y)	
All patients	
Range	2.2—55.9
<13 y, *n* (%)	31 (42)
13—49 y, *n* (%)	41 (56)
≥50 y, *n* (%)	1 (1)
Median (IQR)	15.5 (9.1—24.3)
Malignant diseases	
Median (IQR)	20 (9.1—36)
Nonmalignant diseases	
Median (IQR)	13.5 (10—21.4)
Weight (kg)	
Range	11.6—102
Median (IQR)	39.6 (23—61.5)
Height (cm)	
Range	87—176
Median (IQR)	152 (130—163)
BMI (kg/m^2)	
Range	13—42
Median (IQR)	18 (15—25)
>30 kg/m^2, *n* (%)	5/56 (8%)
Diagnosis, n (%) — (no. of patients < 13 y)	
ALL	11 (15)—(5)
AML	14 (19)—(5)
MDS	5 (7)—(3)
CML	4 (5)—(0)
SCD	23 (31)—(6)
TM	16 (23)—(12)
Disease stage, n	
ALL	
CR1	5/11
CR2	6/11
AML	
CR1	10/14
CR2	4/14
Graft source, n (%)	
PBSC	52 (71)
BM	20 (27)
Both	1 (1)
Bu regimen, n (%)	
Myeloablative	47 (64)
MDD for 4 d	
Pediatrics	13
Adults	8
SDD for 4 d	
Pediatrics	12
Adults	14
Nonmyeloablative	26 (36)

(*continued on next page*)

Table 1 (*continued*)

Characteristic(s)	*n* = 73
MDD for 2 d	
Pediatrics	6
Adults	20
Baseline LFTs	
ALT (IU/L)	
Median (IQR)	22.5 (15.5—48.5)
AST (U/L)	
Median (IQR)	23 (17.5—36.5)
Serum bilirubin (micromole/L)	
Range	2—88
Median (IQR)	10 (4—17.5)
DF use, *n* (%)	*n* = 63
Yes	33 (52)
Pediatrics	13 (39)
Adults	20 (61)
DF regimen, *n* (%)	*n* = 31
Prophylactic dose	10 (32.3)
Therapeutic dose	20 (64.5)
Prophylactic then changed to therapeutic	1 (3.2)
Follow-up time (mo)	
Range	2—143
Median (IQR)	35 (16—57)

ALL = acute lymphoblastic leukemia; ALT = alanine transferase; AML = acute myeloid leukemia; AST = aspartate transferase; BM = bone marrow; BMI = body mass index; CML = chronic myeloid leukemia; CR1 = first complete remission; CR2 = second complete remission; DF = defibrotide; IQR = interquartile range; LFTs = liver function tests; MDD = multiple daily dose; MDS = myelodysplastic syndrome, PBSC = peripheral blood stem cells; SCD = sickle cell disease; SD = standard deviation; SDD = single daily dose; TM = thalassemia major.

obtain sufficient cell count. The median follow-up time for this cohort was 35 months.

Fixed dose versus TDM dose

The median total TDM Bu dose was 494 mg (IQR: 376—678 mg) equivalent to 15 mg/kg while the median total FBD dose of 12.8 mg/kg was 349 mg (IQR: 246—614 mg). The difference in the doses between the two methods was statistically significant ($p < .0001$). The FBD was lower than the TDM dose by a median of 38 mg/day (IQR 20—62, range 60—158 mg/day). The correlation between the fixed and the calculated dose was high and statistically significant ($r = .8995$, $p < .0001$). The box plot of the difference between the doses is shown in Fig. 1.

The impact of age and diagnosis on Bu dose

The median difference between Bu doses calculated by the two dosing methods in pediatrics and adults was found to be 38 mg/day (IQR 24—56 mg/day) and 42 mg/day (IQR 10—63 mg/day), respectively, which was not statistically significant ($p = .88$). However, when we adjusted for the weight, the difference between FBD and TDM dose between pediatrics and adults was statistically significant. The FBD was lower than the TDM dose by a median of 6.3 mg/kg (IQR: −10.8 to −3.3) in pediatric patients compared to 2.3 mg/kg in adults (IQR: −3.6 to −0.1; $p < .0001$) (Fig. 2). Even when changing the definition of adults to include only patients 18 years and above, the differences between the doses did not change significantly. The median difference was 37 mg/day (IQR 23—55) in pediatrics and 44 mg/day (IQR 4—78) in adults ($p = .8788$). When adjusting for the weight, FBD was lower than TDM dose by 4.5 mg/kg in pediatrics (IQR: −9.5 to −2.4) compared to 2.3 mg/kg in adults > 18 years (IQR: −3.6 to −0.1) with a p value = .0001.

The mean difference between the TDM-guided and the FBD in patients with nonmalignant and malignant conditions was 48 mg/day (SD of 31 mg/day) and 29 mg/day (SD of 42 mg/day), respectively, which was found to be statistically significant ($p = .0328$) (Fig. 3). On the multivariable analysis, none of the predictors, i.e., age, weight, and diagnosis showed a statistically significant difference between the two dosing methods ($p = .47$, $p = .16$, and $p = .07$, respectively).

Bu clearance and its predictors

The median Bu clearance after all the tested doses in the study population was found to be 3.72 mL/min/kg (IQR: 3.18—4.48). No significant change in Bu clearance was observed from the test dose to Dose 9 (equivalent to Dose 3 with the SDD). However, Bu clearance was significantly higher after Dose 13 (Dose 4 with SDD) with a median of 4.28 mL/min/kg (IQR: 3.43—4.87, $p = .0330$) (Fig. 4).

We used the test dose clearance to assess the impact of demographic characteristics on Bu clearance. Gender did not predict the variability in clearance; both males and females had a median clearance of 3.7 mL/min/kg (p = .8062). Pediatric patients had a higher clearance (median of 4.36 mL/min/kg [IQR: 3.67—5.21]) compared to adults (median of 3.36 mL/min/kg [IQR: 3.18—3.75]), which was statistically significant (p = .0001). Bu clearance normalized to body weight was found to correlate negatively with weight and BSA (r = —.5561 and r = —.6318, respectively). The correlation (r) of the weight with clearance was —.534 and —.147 for pediatric and adult patients, respectively. Similarly, the correlation (r) of BSA with clearance was —.561 and —.154 for pediatric and adult patients, respectively (Figs. 5 and 6). Patients transplanted for a malignant condition had lower test dose clearance (median of 3.38 mL/min/kg [IQR 2.92—4.14]) than those transplanted for a benign condition (median of 3.7 mL/min/kg [IQR 3.55—4.57]); however, the difference did not reach statistical significance (p = .0738). On the multivariable model, BSA was the only significant predictor for the difference in clearance (p = .049).

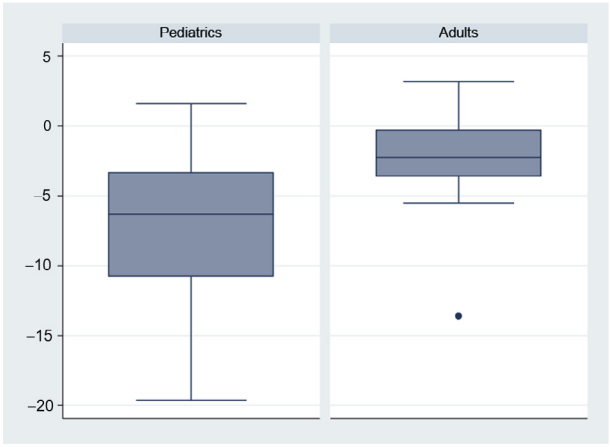

Fig. 2 Difference between the fixed busulfan dose (FBD) dose and therapeutic drug monitoring (TDM) dose in pediatrics and adults. The upper and lower bars (whiskers) of the box plot represent the 90th and 10th percentiles, respectively. The horizontal bar at the center of the box plot represents the median value, and the top and bottom of the box plot represent the 75th and 25th percentiles, respectively.

Discussion

Bu is a key agent in conditioning regimens for HSCT in both adults and pediatrics. Its use has significantly improved the HSCT outcome. However, Bu shows considerable variation in efficacy and toxicity mainly due to its wide interindividual and intraindividual variability in PK parameters such as clearance [1].

The high prevalence of potentially curable inherited diseases of the blood in the country, such as SCD and β-TM, makes our study population different from most studies, as the number of transplants performed for benign conditions is high. Studies such as de Lima [6] and Choe et al. [7] only considered Bu in patients with hematological malignancies. Only few included patients with hemoglobinopathies were in studies by Dedeken et al. [8] and Bernaudin et al. [9]. The primary distribution of the background conditions in our study might therefore have influenced the observed outcomes when compared to other studies. Another unique characteristic of our study participants is the relatively younger transplant population, even with malignant diseases, where the median age was 20 years (IQR 9—36). We had only one patient older than 50 years

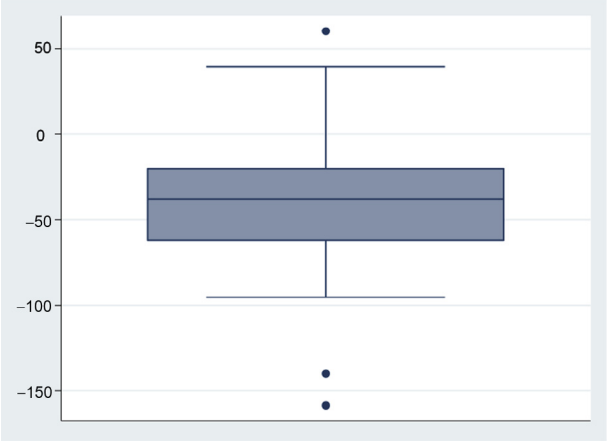

Fig. 1 Difference between the total fixed busulfan dose (FBD) (3.2 mg/kg* busulfan [Bu] duration) and the total therapeutic drug monitoring (TDM) dose (over the duration of Bu administration) divided by the number of days over which Bu was administered. The upper and lower bars (whiskers) of the box plot represent the 90th and 10th percentiles, respectively. The horizontal bar at the center of the box plot represents the median value, and the top and bottom of the box plot represent the 75th and 25th percentiles, respectively.

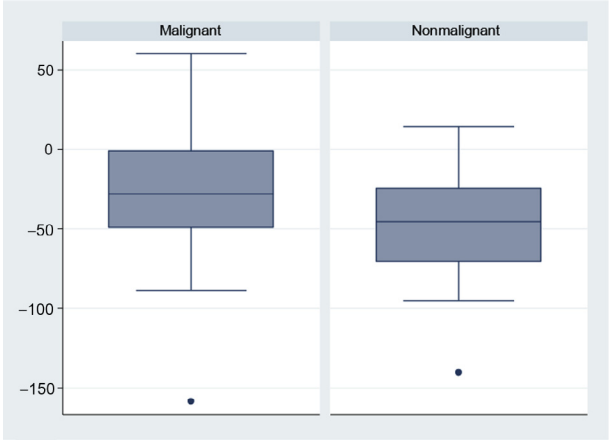

Fig. 3 Difference between the fixed busulfan dose (FBD) and therapeutic drug monitoring (TDM) dose in nonmalignant and malignant conditions. The upper and lower bars (whiskers) of the box plot represent the 90th and 10th percentiles, respectively. The horizontal bar at the center of the box plot represents the median value, and the top and bottom of the box plot represent the 75th and 25th percentiles, respectively.

who was transplanted for MDS. For most of the studies, the median age was around the forties [6,7]. The younger age of our sample is probably a reflection of the distribution of the general Omani population [10]. This observation has also been seen in the distribution of solid tumors and in reports from the regional centers [11].

We found that weight-based dosing, using 0.8 mg/kg every 6 h or 3.2 mg/kg/day, yielded significantly lower doses in both pediatric and adult patients compared to TDM-guided dosing. As the IV formulation shows consistent PK across the therapeutic dose range [1], we did not expect the difference between the two dosing methods to be clinically significant. However, in several clinical trials, higher doses were used in children and adults to achieve the target AUC [12]. Children are known to metabolize Bu faster due to relatively larger liver size translating into higher metabolizing enzyme activity compared to adults and require relatively larger doses to achieve comparable exposures [12]. Nguyen et al. suggested a new fixed dosing strategy in children based on body weight. According to their simulation, the following doses were suggested: <9 kg: 1 mg/kg; 9—<16 kg: 1.2 mg/kg; 16—23 kg: 1.1 mg/kg; >23—34 kg: 0.95 mg/kg and >34 kg: 0.8 mg/kg (similar to adults) [13]. This dosing strategy was prospectively evaluated by Michel et al. [14] and found that 78% of children achieved the target AUC. Another study by Bartelink et al. [15] found that around 50% of pediatric patients who received a starting dose of 4 mg/kg Bu required dose increment to achieve the target AUC. The need of higher doses to achieve the target exposure was also demonstrated in adults by Yeh et al. [16] who showed that only 11% of patients who received an FBD of 3.2 mg/kg/day achieved the desired Bu concentration of 800—1000 ng/mL. By contrast, a prospective PK

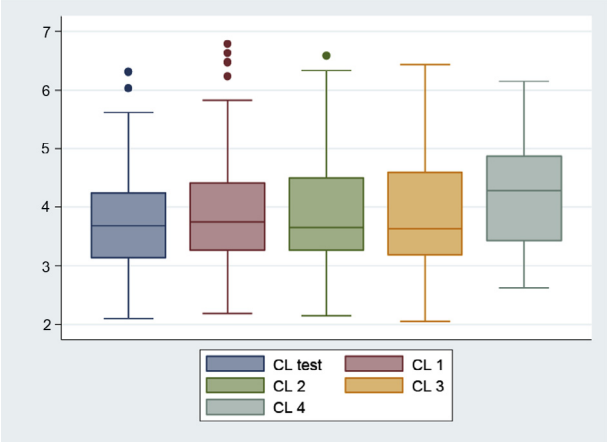

Fig. 4 Busulfan (Bu) clearance obtained after different doses. CLt is clearance after the test dose. For the four times daily (QID) regimen, CL1, CL2, CL3, and CL4 are clearances after Dose 1, Dose 5, Dose 9, and Dose 13, respectively. For once daily (OD) regimen, CL1, CL2, CL3, and CL4 are clearances after Dose 1, Dose 2, Dose 3, and Dose 4, respectively. The upper and lower bars (whiskers) of the box plot represent the 90th and 10th percentiles, respectively. The horizontal bar at the center of the box plot represents the median value, and the top (Q3) and bottom (Q1) of the box plot represent the 75th and 25th percentiles, respectively.

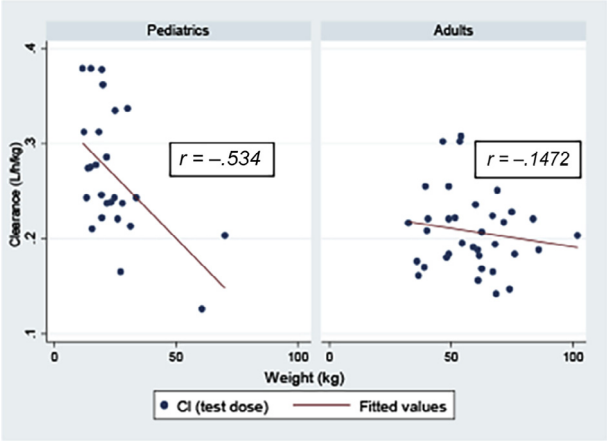

Fig. 5 The correlation of clearance with weight in pediatrics and adults.

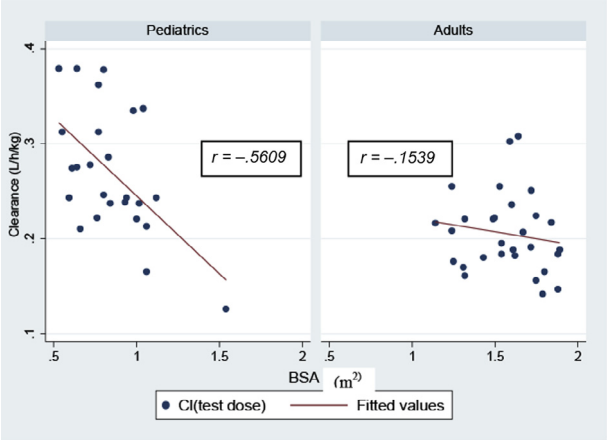

Fig. 6 The correlation of clearance with body surface area (BSA) in pediatrics and adults.

study of Bu in adult patients receiving IV Bu/Cy regimen found that 86% of patients who received 0.8 mg/kg every 6 h for 4 days maintained an AUC within the target range [17]. This was also proven with an SDD of 3.2 mg/kg/day of IV Bu [3]. The PK variability of IV Bu, although less than oral, is still considered to be clinically significant [2]. It is suggested that the interindividual variability in Bu metabolism and clearance is at least partially explained by the genetic background of the recipient [1,18]. It is worth noting that the presence of genetic polymorphisms such as the glutathione S-transferase (GST) M1 null genotype, which is known to be associated with altered Bu metabolism, was found in 38% of the healthy Omani population [19]. Srivastava [18] found that the presence of GST M1 null genotype was associated with higher Bu clearance and lower Css. Interestingly, we found that the median clearance in both adults and pediatrics was somewhat higher than that reported by some investigators. Russell et al. [20] reported that the mean clearance in 70 adult patients receiving once daily IV Bu/Flu regimen was 2.5 mL/min/kg compared to 3.36 mL/min/kg in our study. Additionally, according to the study by Vassal et al. [21] in 55 pediatric patients, the

mean clearance when using IV Bu 0.8 mg/kg every 6 h was 2.57 mL/min/kg compared to 4.36 mL/min/kg in our pediatric patients. The higher clearance may have accounted for the requirement of higher TDM-guided doses in our cohort.

The difference between the TDM dose and FBD was found to be higher in pediatric patients compared to adults when body weight was adjusted for. As was found by many investigators, the weight-adjusted total Bu clearance is higher in children than adults; this finding was not surprising. Tse et al. [22] found that younger children had less predictable PK profiles and dose adjustment was less likely to achieve the target AUC than older patients. In addition, Schechter et al. [23] found that younger children required higher weight-adjusted Bu doses. In our cohort, the median difference of around 6.3 mg/kg between the FBD and TDM dose was observed in pediatric patients, compared to 2.3 mg/kg in adults. Additionally, it was suggested that it is more difficult with children to achieve desired blood concentrations when Bu is administered as a standardized mg/kg dose compared to adults [24].

Interestingly, we found that patients with nonmalignant conditions required significantly higher TDM-based doses than patients with malignant diseases. As the prevalence of hereditary blood disorders in our cohort was high (57%), this might have affected the results with the FBD. The requirement for higher TDM-based doses in patients with hemoglobinopathies may be partly explained by the higher Bu clearance compared to malignant conditions. In our study, we found that the mean Bu clearance was significantly higher in patients with hemoglobinopathies.

This finding was also reported with oral Bu [25]. Although this has not been prospectively evaluated with IV Bu, Gaziev et al. [26] reported a high IV Bu clearance in patients with β-TM especially after the first dose. One possible explanation for this could be the high hepatic GST activity and high plasma GST A1 levels in patients with β-TM compared with controls and age-matched leukemic patients [25].

We observed that Bu had predictable intraindividual PK with minimal interdose variability in clearance. The minimal interdose variability in Bu clearance could reduce the number of doses that need TDM if the test dose TDM is reliable. Andersson et al. [27] also found that clearance did not change significantly from Dose 1 to steady state (Dose 5 or Dose 9) when a fixed infusion time was used. Nevertheless, Bleyzac et al. [28] evaluated the usefulness of test dose monitoring in predicting individual PKs. He found that 17% of patients had significantly different PK behavior with later doses. We found that the fourth dose (SDD) or Dose 13 (MDD) clearances were significantly higher than earlier doses' clearances. This is contrary to the finding by Gaziev et al. [26] where the clearance of the first dose IV Bu was significantly higher than later doses. Yeh et al. [16] and Lee et al. [29] showed that 35% and 27% of the patients, respectively, experienced an increase in clearance on Day 4, which was considered as potentially clinically significant. One reason for this might be the concurrent use of prophylactic phenytoin which is started earlier in the regimen to accommodate the test dose strategy. Phenytoin was found to increase oral Bu clearance by 15% or more through modification of liver GST activity [12]. Apart from phenytoin, metabolic pathways of Bu may also be induced by other drugs which may be started toward the end of conditioning as complications start to appear. Examples of such drugs are antibiotics, antiemetics, and pain medications [12]. Similar to the suggestion by Bleyzac et al. [28], the variability of Bu clearance with subsequent doses may necessitate TDM-guided dosing.

This study had several limitations. Firstly, due to the retrospective nature of the study, selection bias is a risk especially given the small sample size. Additionally, the study population included a heterogeneous sample of age groups and transplant conditions. In this study, the comparator group was a hypothetical group and the doses were calculated using fixed dose per weight strategy. Although that allowed us to compare doses, we could not evaluate major clinical outcomes of the two dosing strategies. Moreover, patients were included over a prolonged duration of 11 years. Over these years, protocols changed and advances in Bu sampling technique and PK analysis were seen. Finally, as the study was conducted in a single center, the generalizability of the results may be limited.

Despite these limitations, to the best of our knowledge, this is the first study to assess IV Bu clearance and the factors that influence it in the Omani population. Also, contrary to most studies, we used Bu clearance instead of AUC as a marker for Bu exposure. Although both parameters (clearance and AUC) are interrelated, clearance is endogenous to the patient and might be used to develop PK models of the population of interest, which will help in dose individualization and sampling frequency [30]. Additionally, all the patients included had complete PK data and TDM monitoring was done to the best standards known, including the analytical methods used. We currently use liquid chromatography-tandem mass spectrometry for Bu sampling, which is the preferred method due to the many advantages associated and high degree of accuracy in the test results [1].

Based on the results of this study, shifting to FBD cannot be recommended and TDM should remain as the standard of care. However, these data may be used in the future to develop a population PK model. One interesting finding that might impact Bu dosing in the Omani population is the higher clearance of Bu, which should be investigated again in the future to ensure the reproducibility of the results. Additionally, pharmacogenetic analysis may also be integrated with the current findings. The impact of disease on IV Bu disposition and clearance is currently not fully elucidated and further research in this field is needed.

In conclusion, this study showed that there is a statistically significant difference between the TDM-guided Bu dose and weight-based dosing in the Omani population. Age and underlying condition were the main factors affecting Bu clearance and dosing. As TDM dosing ensures that the target concentrations are achieved, shifting to FBD cannot be recommended without a larger prospective study evaluating the effect of different dosing strategies on HSCT outcomes.

Conflict of interest

The authors of the paper certify that they have no affiliations with or involvement in any organization or entity with any financial interest.

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