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ORIGINAL ARTICLE

Retrospective study of the digestive tract mucositis derived from myeloablative and non-myeloablative/reduced-intensity conditionings with busulfan in hematopoietic cell transplantation patient

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Abstract

Busulfan is a major component of chemotherapy conditioning in hematopoietic cell transplantation (HCT). This alkylating agent is highly toxic at myeloablative doses, exposing HCT patients to risks of mortality. Non-myeloablative (NMA) and reduced-intensity conditioning (RIC) using busulfan have shown impaired toxicity. However, the toxicity of NMA/RIC in the digestive tract is poorly described. This study aimed to characterize the mucositis in the oral cavity (OM), oropharynx/esophagus, and gastrointestinal tract derived from conditionings with myeloablative and non-myeloablative doses of busulfan. We retrospectively retrieved clinical data of HCT patients ($n = 100$) who underwent myeloablative conditioning (MAC) or NMA/RIC with busulfan. Frequency and time duration of mucositis in the oral cavity and oropharynx/esophagus, diarrhea, and prescription of total parenteral nutrition (TPN) and opioids were also collected. OM severity ($p = 0.009$) and time duration of mucositis in oropharynx/esophagus ($p = 0.022$) were frequently higher in MAC than NMA/RIC. A myeloablative dose of busulfan was a risk factor for OM grade ≥ 2 (OR = 4.8, $p = 0.002$) and for mucositis in oropharynx/esophagus ≥ 5 days (OR = 2.64, $p = 0.035$). A longer duration of mucositis in the oropharynx/esophagus was also associated with an increase in the prescription of opioids (OR = 7.10, $p < 0.001$).Overall survival (OS) in MAC was significantly higher than that in NMA/RIC ($p = 0.017$). No variables related to mucositis interfere significantly in OS. In conclusion, myelosuppression in busulfan-based regimens are predisposed to a high risk for severe OM and to prolonged mucositis in the oropharynx/esophagus.

Keywords Oral mucositis · Gastrointestinal mucositis · Hematopoietic cell transplantation · Busulfan

Introduction

Hematopoietic cell transplantation (HCT) is an important therapeutic option for several hematological disorders. Busulfan is an alkylating agent used as a major component of chemotherapy conditioning prior to HCT. Even under high doses, the myelosuppressive potential of busulfan is limited, requiring a complementation with lymphocytotoxic agents (e.g., cyclophosphamide and fludarabine) or other antitumor potent agents (e.g., melphalan and thiotepa) [1]. However, busulfan is highly cytotoxic, especially when combined with cyclophosphamide, exposing the patient to various injuries in the central nervous system, digestive tract, and liver. High non-relapse mortality (NRM) is a major concern in myeloablative regimens using busulfan and cyclophosphamide, mainly in elderly patients or in patients with a high co-morbidity index [2]. The replacement of cyclophosphamide by fludarabine in busulfan-based myeloablative conditioning (MAC) has minimized the toxicity without efficacy prejudice [2].

Besides the use of less toxic agents combined with busulfan at myeloablative doses, non-myeloablative (NMA) or reduced-intensity conditioning (RIC) have been tested with

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busulfan for some hematological conditions, such as acute myeloid leukemia (AML) [3, 4], myelodysplastic syndrome [5, 6], and in high-risk non-Hodgkin lymphoma [7]. RIC induces a higher level of myeloablation than NMA but is less myeloablative than MAC, involving intermediate doses of alkylating agents. The antitumor potential is maintained in RIC with lower risk of NRM; besides, the graft-versus-tumor effect mediated by donors' immunocompetent cells is preserved in allogeneic HCT. However, the risk of disease recurrence is high, sometimes decreasing the overall survival (OS) and the disease-free survival (DFS) [4, 8].

One of the main toxicities with early impacts during HCT is mucositis in the digestive tract. Gastrointestinal mucositis can be responsible for nutritional imbalance and important body weight loss during allogeneic HCT [9], whereas oral mucositis (OM) has been related to improvements in hospitalization days and costs [10–12]. In autologous HCT, mucositis tends to be less severe when compared with OM in allogeneic HCT [9]. One of the factors associated with toxicity in the digestive tract of allogeneic patients is the graft-versus-host disease (GVHD) prophylaxis and treatment, which can determine an increased risk for mucositis, particularly when methotrexate is used in the GVHD protocol [13].

Despite the high morbidity, the effect of NMA and RIC on mucositis is poorly described in the literature. Clinical studies comparing mucositis in MAC and NMA/RIC regimens in HCT are restricted to OM and its impact on total parenteral nutritional (TPN), opiod prescriptions, and days of hospitalization [14–16]. In addition, these investigations address various types of regimens, with discrepant results concerning the differences of OM frequency and severity. Other studies have described mucositis in MAC and RIC regimens using busulfan [4, 17–20], but these analyses are not detailed because the primary endpoints to generally describe the treatment efficacy were GVHD and relapse incidences, as well as survival data.

In this study, we aimed to analyze whether a MAC using busulfan is a risk factor for OM and mucositis in the oropharynx/esophagus and gastrointestinal tract. We also analyzed the impact of mucositis in OS.

Patients and methods

This study was previously approved by our institution's ethical committee (process number 2902-16).

Patients

This a retrospective study of consecutive adult HCT patients who attended the Hospital Israelita Albert Einstein (HIAE), Brazil, between 2012 and 2016. We selected medical records from patients who underwent autologous and allogeneic HCT using intravenous busulfan in conditioning regimens prior to HCT. Exclusion criteria were patients younger than 18 years old; conditioning regimen without busulfan; medical records without adequate description of toxicity, especially mucositis; patients who refused to participate from the oral care protocol before, during, and after transplantation periods; and patients who died during the HCT.

Conditioning regimen

The patients were divided in two groups in accordance with their conditioning regimen: (a) the myeloablative conditioning (MAC) group included busulfan (AUC 4500–5000 μM/L*min) during 4 days plus cyclophosphamide (50-120 mg/kg) with or without thiotepa (250 mg/m^2); busulfan (AUC 4500–5000 μM/L*min) during 4 days combined with fludarabine (25–40 mg/m^2/day) with or without antithymocyte globulin (ATG) (2.5–5.0 mg/kg/day); and busulfan (AUC 4000 μM/L*min) plus melphalan (120 mg/m^2) with or without gemcitabine (2700 mg/m^2); (b) the non-myeloablative/reduced-intensity conditioning (NMA/RIC) group included busulfan (AUC 4000 μM/L*min) plus fludarabine (25-40 mg/m^2/day) for 4 days with or without ATG (1.5 mg/kg/day); we also have an experimental protocol using busulfan (AUC 4500 μM/L*min) plus fludarabine (30 mg/m^2) and total marrow irradiation (two times a day, 6Gy total dose) that has lesser toxicity than protocols using total body irradiation (TBI).

Therapeutic monitoring of busulfan

In all patients, plasmatic busulfan was measured after a standard dose of intravenous busulfan, as described previously [21]. Dose adjustments were performed for each patient based on their individual curve of busulfan pharmacokinetics.

Infection prevention

A protocol for infection prevention was adopted for all patients, which included intravenous administration of acyclovir, fluconazole, levofloxacin, and sulfamethoxazole.

Oral care protocol

Before the conditioning regimen, the oral cavity was examined by a dental professional, who removed all the infection foci. During transplantation, a dental professional monitored the oral hygiene and prescribed alcohol-free antimicrobial mouthwashes with enzymatic action, manual tooth-brushing, cocoa butter lip balm, vitamin E in the case of dry lips, and dry mouth moisturizing in the case of xerostomia. Low-level laser therapy (once a day) was indicated for all patients from the first day of the conditioning regimen to the day of marrow engraftment [10]. In the case of melphalan conditioning,

cryotherapy during melphalan infusion was also adopted to reduce the risk of OM [9].

Assessment of mucositis in the gastrointestinal tract

All patients were prospectively examined by a dental professional, who classified the severity of OM daily using the following World Health Organization criteria: grade 0 = none; grade I = soreness and erythema; grade II = erythema and ulcers without difficulty to swallow solid food; grade III = ulceration, requiring only a liquid diet; and grade IV = introduction of TPN. The number of OM days was also recorded in the medical records.

In addition to OM, mucositis in the pharynx and esophagus was recorded based on the patient's report regarding pain and discomfort with swallowing. The dental professional collected this information in detail in order to distinguish the presence of mucositis in the oral cavity from mucositis in the pharynx and esophagus. The frequency and length of diarrhea were also recorded, and negative antimicrobial culture and nauseas/vomiting were used to infer gastrointestinal mucositis. Diarrhea was defined as two or more episodes of liquid evacuation. This information was collected from the first day of conditioning to the time when the patient was discharged, by reading the clinical description made by the physicians and nurses.

Data collection

We collected the following data from the HCT patients: gender, age, type of HCT, primary disease, disease status, comorbidities, conditioning regimen, GVHD prophylaxis, number of days of neutropenia (< 550 neutrophils/mm^3), and length of stay (number of days counting from the first to the last day of hospitalization in the Bone Marrow Transplantation Center of HIAE). The age-adjusted Charlson comorbidity index was calculated, and "2" was the minimal score. Data on gastrointestinal mucositis and related variables included the grade of OM, frequency/number of days of OM, mucositis in oropharynx/esophagus, mucositis-related diarrhea, opioid prescription, and total parenteral nutrition (TPN).

Clinical endpoints and statistical analysis

The primary endpoints were mucositis in the oral cavity, oropharynx/esophagus, and gastrointestinal tract (diarrhea), as well as prescriptions of TPN and opioids. The second endpoint was OS in MAC and NMA/RIC regimens. We used absolute and relative (%) frequencies to describe the categorical data and medians (ranges) for numerical data. We compared MAC and NAM/RIC groups by means of univariate using chi-squared test and Wilcoxon's rank sum test. We performed multivariate analyses using logistic regression, considering OM, mucositis in the oropharynx/esophagus, and diarrhea as dependent variables. We dichotomized the variables related to signs and symptoms of mucositis based on their median results, as follows: OM ≥ grade 2, OM ≥ 10 days, mucositis oropharynx/esophagus ≥ 5 days, and diarrhea ≥4 days. Dichotomic independent variables were age ≥ 50 years, Charlson index ≥ 3, 2nd remission or worst, matched-unrelated donor transplant, myelosuppression, conditioning with fludarabine-ATG, conditioning with cyclophosphamide and melphalan, GVHD prophylaxis with methotrexate, and neutropenia ≥ 14 days. Only variables with $p > 0.100$ were inserted in the regression models. OS was calculated from the first day of conditioning until death and was estimated using Kaplan-Meier methods. OS comparisons were performed using the log-rank test. The Cox-proportional hazards regression was adopted to determine the factors interfering with OS. The significance level was set to 5%. We used R statistical software package [16] for all analyses.

Results

Figure 1 shows a flow chart of the HCT patients who attended our institution from 2012 to 2016. In this period, 355 patients underwent HCT. We excluded 85 patients due age < 18 years and 153 patients who underwent a conditioning regimen without busulfan. After reading the medical records from 117 patients, we also excluded more 17 patients due to inconsistency in the clinical register, including absence of a clear information about busulfan regimen, not allowing the conditioning classification. At the end, 100 patients composed the sample addressed in the present study, 66 in MAC group and 34 in NMA/RIC group.

Table 1 shows the patient's characteristics and the transplantation conditions in the MAC and NMA/RIC regimens using busulfan. As expected, there were more aging patients in the NMA/RIC group. In both groups, the most frequent primary disease was AML; in the NMA/RIC group, myelodysplastic syndrome was the most frequent. Median Charlson comorbidity indexes were 3 and 4 in the MAC and NMA/RIC groups, respectively. Regarding disease status, first remission had a high frequency in both groups; patients with active disease were more frequent in the NMA/RIC group. Most patients underwent allogeneic match-related and unrelated donor transplantations, but in the MAC group, there was an important proportion of autologous transplantation. Busulfan-based conditioning regimens in the MAC group included fludarabine with or without ATG (30.0 and 42.0%, respectively), fludarabine-thiotepa (3.0%), cyclophosphamide-thiotepa (5.0%), only cyclophosphamide (6.0%), and melphalan with or without gemcitabine (6.0 and 8.0%, respectively). In the non-MAC/RIC group, busulfan

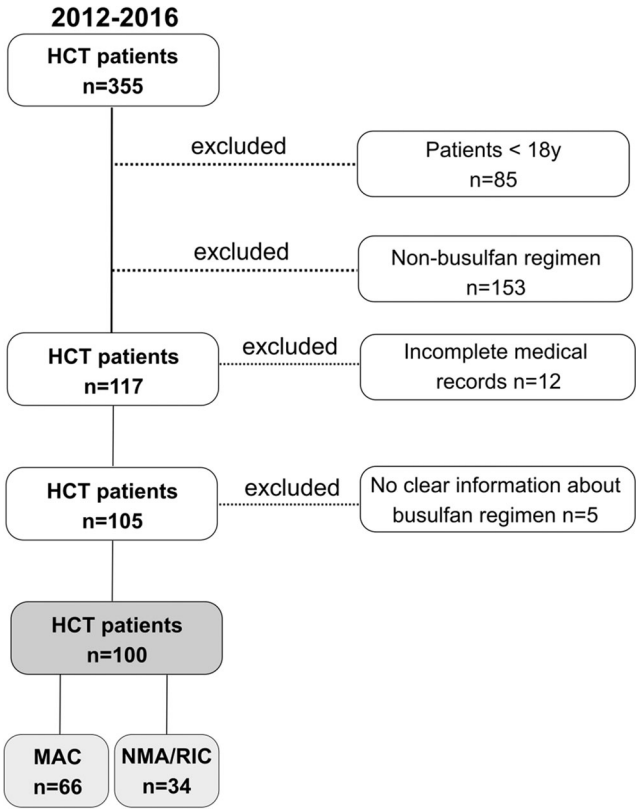

Fig. 1 Flow chart of HCT patients attended at 2012–2016, and the number of patients selected after the application of the exclusion criteria. HCT hematopoietic cell transplantation, MAC myeloablative conditioning, NMA/RIC non-myeloablative/reduced-intensity conditioning

was combined with fludarabine with or without ATG (50.0 and 42.0%, respectively) and fludarabine-TMI (8.0%). Autologous HCT patients were mainly submitted to diathe MAC regimen with busulfan and melphalan, with or without gemcitabine (58.9%), and busulfan with cyclophosphamide (35.3%). One patient (5.8%) received a NMA/RIC regimen with busulfan and fludarabine (5.8%). Prophylaxis GVHD was performed mainly with tacrolimus-methotrexate in both groups. The groups had similar medians for the number of days of neutropenia (< 550 neutrophils/mm^3), but the median length of stay in the NMA/RIC group was significantly higher than in the MAC group. Complications during transplantation included important systemic infections in both regimens; some patients who underwent MAC also exhibited hepatic and renal disturbances, but at low frequency. Acute GVHD was reported in few patients (Table 1).

Mucositis in the digestive tract and prescriptions of TPN and opioids are described in Table 2. The frequency and time duration of OM were similar between the two groups. However, the severity of OM in the MAC group was higher than in the NMA/RIC group, mainly of OM grade 3 ($p = 0.009$). The number of days with mucositis in the oropharynx/esophagus was also higher in the MAC group than with the NMA/RIC group ($p = 0.022$). There were no significant differences between the two groups in relation to diarrhea or prescriptions of TPN and opioids. Prescription of TPN was mainly due to pain in the oral cavity and inappetence/nauseas, which leads to reduction of oral intake. In relation to opioids, although there were significant differences in relation to motifs of prescriptions between the MAC and NMA/RIC groups ($p = 0.043$), pain in the oropharynx and pain oral cavity are the most frequent in both groups. There is no significant association of OM, mucositis in the oropharynx/esophagus, and diarrhea with TPN prescriptions. Long duration and high severity of OM was significantly associated to opioid prescriptions ($p = 0.011$ and $p = 0.010$, respectively).

The variables related to toxicity in the oral cavity and in the digestive tract were compared with those related to patient and transplantation characteristics, as shown in Table 3. As the number of patients was relatively small in each group, mainly in the NMA/RIC group, we decided to analyze all patients in conjunction to verify the impact of busulfan on the mucositis independent of the dose.

Myelosuppression (OR = 4.87, $p = 0.002$) and 2nd remission or worst (OR = 2.89, $p = 0.018$) showed significant odds ratios for OM ≥ grade 2 and OM ≥ 10 days, respectively (Table 4). Mucositis in oropharynx/esophagus ≥ 5 days was significantly associated to myelosuppression ($p = 0.032$) (Table 3). Besides this variable, we added Charlson index ≥ 3 and combination of busulfan with cyclophosphamide as well as melphalan to perform a multivariate logistic regression. After model adjustments, only myelosuppression was a significant risk factor for mucositis in the oropharynx/esophagus (OR = 2.64, $p = 0.035$). Prescription of opioids was also significantly associated with mucositis in the oropharynx/esophagus ≥ 5 days ($p < 0.001$) (Table 3). Analyzing opioids as a dependent variable, the odds ratio was significant (OR = 7.10, $p < 0.001$) (Table 4). Diarrhea ≥ 4 days was significantly associated with Charlson index ≥ 3 and methotrexate ($p = 0.011$) (Table 3). Besides these variables, we also added match-unrelated donor to perform a multivariate logistic regression. Only methotrexate was a significant risk factor for persistent diarrhea (OR = 2.61, $p = 0.031$).

In the MAC group, OS for 1 year was 85% (CI 73–91%), 3 years was 68% (CI 54–78%), and 5 years was 63% (49–74%). In the NMA/RIC group, OS for 1 year was 76% (CI 54–88%), 3 years was 47% (26–65%), and 5 years was 41% (22–60%). Using the log-rank test, 5 year-OS was found to be significantly higher in the MAC group than in the NMA/RIC group ($\chi 2 = 5.6$, $p = 0.017$) (Fig. 2a). No variables related to mucositis in the digestive tract significantly impacted OS of the MAC or NMA/RIC patients. Because the number of patients in each group was relatively reduced, we included all patients to compare the OS curves in accordance with the variables listed in Table 4. Using the log-rank test, diarrhea ≥ 4 days, days of neutropenia ≥ 14 days, Charlson index ≥ 3,

Table 1 Patient characteristics, transplantation conditions, and systemic complications during the transplantation in the myeloablative conditioning (MAC) and non-myeloablative/reduced-intensity conditioning (NMA/RIC) groups

Variable	MAC ($n = 66$)	NMA/RIC ($n = 34$)	P value
Gender—*N* (%)			
Male	35 (53.0)	20 (58.8)	0.734
Female	31 (47.0)	14 (41.2)	
Age—median (range)	49.5 (20–75)	59 (18–74)	0.001
Primary disease—*N* (%)			
Acute lymphocytic leukemia	6 (9.1)	1 (2.9)	0.059
Chronic lymphocytic leukemia	0 (0.0)	1 (2.9)	
Acute myeloid leukemia	36 (51.5)	9 (29.4)	
Chronic myeloid leukemia	0 (0.0)	1 (2.9)	
Acute myelomonocitic leukemia	2 (3.0)	1 (2.9)	
Hodgkin's lymphoma	3 (4.5)	1 (2.9)	
Non-Hodgkin lymphoma	4 (6.1)	1 (2.9)	
Myelofibrosis	3 (4.5)	6 (17.6)	
Multiple myeloma	3 (4.5)	0 (0.0)	
Myelodysplastic syndrome	7 (10.6)	8 (26.5)	
Other[a]	4 (6.1)	3 (8.8)	
Charlson's index—median (range)	3 (2–7)	4 (2–7)	0.183
Disease status—*N* (%)			
1st remission	30 (45.5)	9 (26.5)	0.011
2nd-4th remission	14 (21.2)	3 (8.8)	
Recurrence	11 (16.7)	5 (14.7)	
Active disease	6 (9.1)	9 (26.5)	
Without information	4 (7.6)	8 (23.5)	
Transplant type—*N* (%)			
Allogeneic			
Matched-related donor	21 (31.8)	6 (17.6)	<0.001
Matched-unrelated donor	20 (30.3)	25 (73.5)	
Haploidentical	9 (13.6)	2 (5.9)	
Autologous	16 (24.2)	1 (2.9)	
GVHD Prophylaxis—*N* (%)			
CSA-MMF	4 (8.0)	3 (9.1)	0.964
Tacrolimus	11 (22.0)	7 (21.2)	
Tacrolimus-MTX-ATG	7 (14.0)	5 (15.2)	
Tacrolimus-MTX	19 (38.0)	14 (42.4)	
Tacrolimus-MMF-CSA	5 (10.0)	3 (9.1)	
MTX-CSA	4 (8.0)	1 (3.0)	
Days number of neutropenia—medium (range)	13 (9–29)	14 (10–28)	0.063
Length of stay (days)—median (range)	29.5 (19–189)	39 (24–138)	0.001
Systemic complications during the transplantation—*N* (%)			
Important systemic infection	15 (22.7)	11 (32.4)	0.576
Cardiac/vascular disturbances	2 (3.0)	2 (5.9)	0.607
Renal disturbances	3 (4.5)	0 (0.0)	0.549
Hepatic disturbances	4 (6.1)	0 (0.0)	0.300
CNS disturbances	2 (3.0)	2 (5.9)	0.607
Allergic reaction during conditioning	2 (3.0)	0 (0.0)	0.551

GVHD graft versus host disease, *MTX* methotrexate, *MMF* mycophenolate mofetil, *CSA* cyclosporine, *CNS* central nervous system

[a] Granulocytic sarcoma (2), falciform anemia (2), plasma cell leucemia (1), chronic myeloproliferative disease (1), biphenotypic leukemia (1)

Table 2 Frequency of mucositis in the digestive tract, and prescription of total parenteral nutrition and opioids

	MAC ($n = 66$)	NMA/RIC ($n = 34$)	P value
Mucositis in oral cavity			
Frequency—*n* (%)	66 (100.0)	34 (100.0)	1.000
Number of days—median (range)	11 (1–25)	9 (1–26)	0.158
Maximum degree—*n* (%)			
0	0 (0.0)	0 (0.0)	0.009
1	8 (12.1)	13 (39.4)	
2	27 (42.4)	11 (36.4)	
3	18 (27.3)	3 (9.1)	
4	12 (18.2)	5 (15.2)	
Mucositis in oropharynx/esophagus			
Frequency—n (%)	64 (97.0)	27 (79.4)	0.627
Number of days—median (range)	8 (0–19)	5 (0–21)	0.022
Diarrhea			
Frequency—*n* (%)	53 (80.3)	23 (67.4)	0.717
Number of days—median (range)	5 (0–14)	4 (0–17)	0.154
Total parenteral nutrition			
Frequency—*n* (%)	21 (31.8)	13 (38.2)	0.811
Number of days—median (range)	0 (0–15)	0 (0–43)	0.937
Motifs of prescription—*n* (%)			
Pain in oral cavity	11 (16.7)	5 (14.7)	0.663
Pain in oropharynx	4 (6.1)	1 (2.9)	
Abdominal pain	0 (0.0)	1 (2.9)	
Diarrhea	3 (4.5)	1 (2.9)	
Inappetence/nauseas	8 (12.1)	5 (14.7)	
Desnutrition	1 (1.5)	0 (0.0)	
Opiods			
Frequency—*n* (%)	55 (83.3)	27 (79.4)	0.995
Number of days—median (range)	4 (0–15)	4.5 (0–26)	0.602
Motifs of prescription—*n* (%)			
Pain in oral cavity	24 (36.4)	7 (20.6)	0.043
Pain in oropharynx	27 (40.9)	10 (29.4)	
Pain in anus	6 (9.1)	2 (5.9)	
Pain in abdomen	12 (18.2)	3 (8.8)	
Pain in muscle/bone	3 (4.5)	4 (11.8)	
Headache	0 (0.0)	3 (8.8)	

and the use of methotrexate significantly interfered in OS. All of these variables were included in a model analyzed using Cox's proportional hazard regression, and only Charlson index ≥ 3 was significant (HR = 2.7, CI 1.1–6.5, $p = 0.02$) (Fig. 2b).

Discussion

In this study, we aimed to investigate whether a myeloablative dose of busulfan is a risk factor for mucositis in the digestive tract, considering OM, mucositis in the oropharynx/esophagus, and diarrhea as distinct variables. Identification of the site of mucositis is particularly important in the present study, because we performed a rigorous prophylactic protocol (i.e., laser therapy, oral mouthwashes, and oral hygiene control) for mucositis localized in the oral cavity, which was limited to the tonsils. The region of the oropharynx and esophagus and the remaining mucosa in the digestive tract did not receive any specific prophylaxis against cytotoxicity. Therefore, we aimed to detect the impact of MAC and NMA/RIC regimens in regions under (or not) previous protection. We found differences in the mucositis at different anatomical sites, as discussed below.

Several investigations have been conducted to evaluate the efficacy of NMA/RIC compared to MAC in HCT [3, 4, 8, 22],

Table 3 Association of mucositis in the digestive tract with variables related to patient co-morbidities, disease status, conditioning regimen, characteristics of transplantation, GVHD prophylaxis, and opioids and total parenteral nutrition prescription

	Signs and symptoms of mucositis			
	Oral mucositis ≥ grade 2	Oral mucositis ≥ 10 days	Mucositis in oropharynx/esophagus ≥ 5 days	Diarrhea ≥ 4 days
Age ≥ 50 years	0.310	0.542	0.107	0.678
Charlson index ≥ 3	0.129	0.143	0.086	0.047
2nd, 3rd, 4th remissions or active disease	0.355	0.016	0.283	0.121
Matched-unrelated donor	0.148	0.257	0.482	0.070
Busulfan + ATG	0.685	0.475	0.664	0.409
Busulfan plus Cy, and Mel	0.302	0.388	0.095	0.197
Myelosuppression	0.001	0.388	0.032	0.772
GVHD prophylaxis with methotrexate	0.465	0.435	0.761	0.011
Neutropenia ≥ 14 days	0.914	0.973	0.199	0.428
Opioids prescription	0.054	0.113	<0.001	0.440
Total parenteral nutrition prescription	0.794	0.081	0.811	0.828

ATG antithymocyte globulin, *Cy* cyclophosphamide, *Mel* melphalan, *GVHD* graft versus host disease

but few have addressed the impact of these regimens specifically in mucositis. A study comparing the OM in MAC and RIC regimens demonstrated that there were no significant differences in frequency and severity of OM, but the regimens included various chemotherapy agents and TBI, and busulfan was only presented in the RIC regimen [14]. Recently, an elegant systematic review about mucositis in different HCT conditioning regimens also concluded that there were no significant differences in severity and frequency of oral mucositis comparing MAC and RIC regimens, with caveats in relation to different transplantation protocols, diverse populations involved, and non-standardized OM classification systems described in the literature [13]. In addition, a retrospective study involving busulfan-based MAC and RIC regimens did not find differences in mucositis [4].

Different from the above studies, we found that OM was more severe, and mucositis in the oropharynx/esophagus was prevalent longer in the MAC group than in the NMA/RIC group. In addition, a myeloablative regimen was a significant risk factor for high severity of OM and prolonged mucositis in the oropharynx/esophagus. These results agree with a previous study that demonstrated that MAC as a whole and busulfan as an individual chemotherapy agent were significantly correlated with high degrees of OM [15]. The trend of high frequency and severity of OM in MAC was also previously described in a prospective study [19]. A same trend was described in another study comparing MAC and RIC regimens, but the conditioning protocols were not addressed [16]. Low frequency of high-degree OM has been also reported in studies involving only RIC busulfan-based regimens [17, 18]. We must mention that the majority of the cited studies considered the OM grades 0 to 2 as mild severity, and OM grade 3 and 4 as high severity. In the present study, we addressed the OM grade 2 in conjunction with the grades 3 and 4, because we noted that although the patients with lesions compatible with grade 2 in the oral mucosa were able to ingest solid food, they diminished the quantity of food ingested and reported severe taste sensations. We believed that these alterations had some impact on the nutritional status of the patients, and we decided to maintain the OM grade 2 as a high-severity condition.

Table 4 Logistic regression for dependent variables related to mucositis in the digestive tract and independent variables related to patient co-morbidities, conditioning regimen, and GVHD prophylaxis

Variables	OR	95%CI OR	*P* value
Oral mucositis ≥ 2 and myelosuppression	4.87	1.78–14.08	0.002
Oral mucositis ≥ 10 days and 2nd remission or worst	2.89	1.22–7.25	0.018
Mucositis in oropharynx/esophagus ≥ 5 days and myelosuppression	2.64	1.07–6.54	0.035
Mucositis in oropharynx/esophagus ≥ 5 days and opioids prescription	7.10	2.28–22.10	< 0.001
Diarrhea ≥ 4 days and methotrexate	2.61	1.10–6.40	0.031

OR odds ratio, *CI* confidence interval

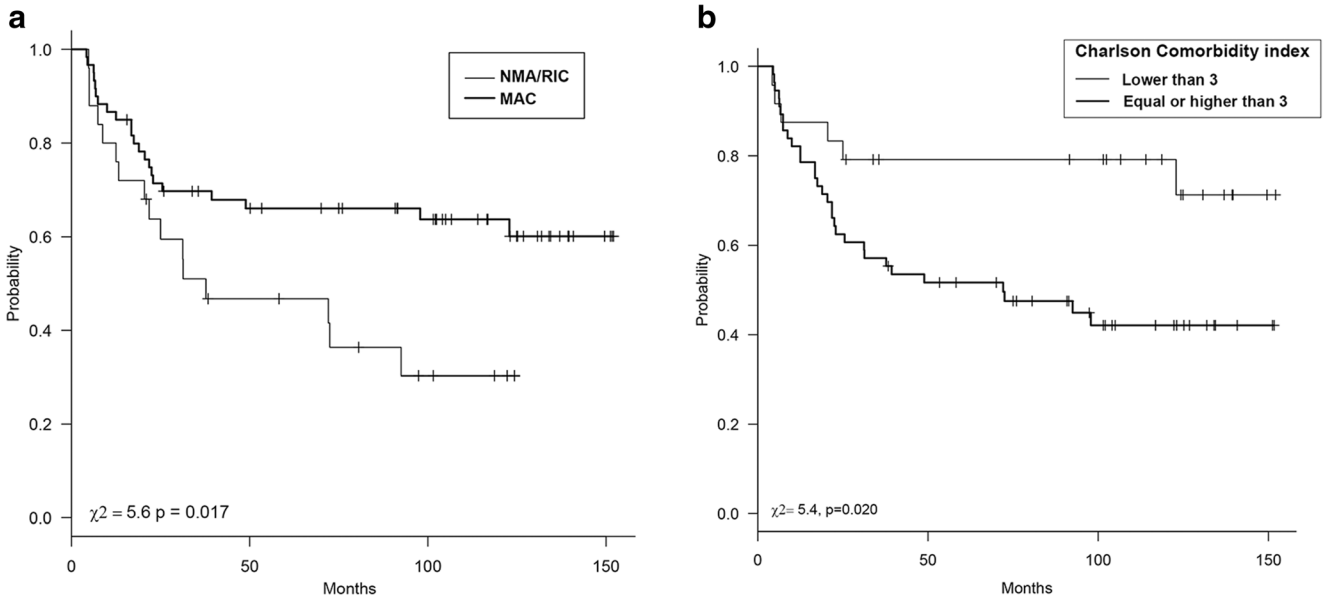

Fig. 2 **a** Overall survival for the myeloablative conditioning (MAC) and non-myeloablative/reduced-intensity conditioning (NMA/RIC) regimens with busulfan. **b** Overall survival for all patients with regard to Charlson Comorbidity index < 3 or ≥ 3

It is important to mention that all of these studies have important differences regarding patient age, type of disease, disease status, cytogenetic risk, and conditioning regimens. These variables directly impact variations in the toxicity caused by MAC and RIC in HCT. In the present study, we did not adopt severe inclusion criteria, and we did not restrict our sample to specific conditions; these facts may have influenced our results and therefore limit comprehensive comparisons with other studies.

High frequency and severity of OM have been found to lead to an increase in frequency of TPN prescription in MAC and RIC regimens [19]. Conversely, we did not find differences in the frequency of TPN between MAC and NMA/RIC groups nor an association with mucositis. We believe that no association was found because we collected the motifs for TPN from medical records, which the majority of other studies did not do. It is interesting to note that TPN was prescribed in some patients who reported inappetence and nausea, but they did not exhibit important mucositis in the oral cavity and oropharynx. However, long duration of mucositis in oropharynx/esophagus was significantly associated with opioids prescriptions irrespective of the conditioning regimen. In another study, opioid prescriptions had a lower frequency in RIC regimen compared with the MAC regimen, although the mucositis characteristics were similar between the two regimens [14]. We also found that an expressive frequency of opioid prescriptions was related to other situations than mucositis, such as pain in bone/muscles and in abdomen. We believe that a detailed description of opioids and TPN prescriptions in HCT patients is necessary, mainly due to the complexity of signs and symptoms experienced by these patients in different phases of the transplantation. Although we collected information about the reason for prescribing opioids, it was not possible to obtain data about the regimen of this prescription (whether as a continuous medication or "when required"), but we did not discard the possibility that the type of conditioning could influence the opioid regimens.

One important characteristic of the busulfan-related regimens described in the present study was that all of the patients received intravenous busulfan and underwent individual therapeutic monitoring with dose adjustments. This procedure seems to minimize the general toxicity, mainly hepatic veno-occlusive disease [23, 24], although the effect of this dose individualization on mucositis is poorly described in the literature. Therefore, the present results are limited to conditions in which the intravenous busulfan dose was adjusted, which probably influenced the impact of MAC and RIC on mucositis in the digestive tract.

Another aspect related to conditioning was that, in the present study, ATG was frequently associated to busulfan-based regimens. ATG has been used for in vivo T-depletion for avoiding chronic GVHD, but its indication and the ideal dose are controversial due to the risk of infection and disease recurrence. Some studies have demonstrated that the administration of ATG has increased the disease-free survival without persistent GVHD [25]. However, the role of ATG in the frequency or time duration of mucositis had not been addressed yet. We did not find any association between busulfan-fludarabine-ATG and mucositis in the oral cavity and oropharynx in any situation.

Although methotrexate is frequently related to high-degree OM in allogeneic transplantation [26], we did not observe any association of this drug with mucositis in the oral cavity and oropharynx. However, in the present study, methotrexate was

a significant risk factor for long duration of diarrhea without distinction between MAC or NMA/RIC. There are few clinical reports regarding the effect of methotrexate on gastrointestinal mucosa, but some evidence has emerged regarding the association of gene polymorphism and high risk of methotrexate injury to the gastrointestinal tract [27]. Further investigations must be conducted to elucidate the role of low-dose methotrexate in mucositis presented in MAC and NMA/RIC regimens during allogeneic HCT.

OS was significantly higher in the MAC group than in the NMA/RIC group. This result is in accordance with other studies analyzing MAC and RIC with busulfan [4, 8]. However, this fact is not a consensus, because various investigations have demonstrated an absence of OS differences between RIC and MAC and have shown better outcomes with RIC [3, 15]. No variables related to mucositis showed differences regarding OS. Only Charlson index ≥ 3 was an OS predictor in the present study when analyzing all patients in conjunction. Although this comorbidity index was not sensitive for HCT, it has been useful as a rapid register of comorbidity [28]. In addition, other past studies demonstrated that higher Charlson index values predicted higher NRM in MAC and RIC HCT regimens [29, 30].

In addition to the restrictions on the results described above, other limitations of this study were the reduced sample for NMA/RIC group when compared with the MAC group, and the absence of information about acute and chronic GVHD, which prevented a better characterization of the patient's toxicity level and survival.

In conclusion, myelosuppression in regimens with busulfan are predisposed to a high risk of severe OM and to prolonged mucositis in the oropharynx/esophagus. OS was higher in the MAC group compared with the NMA/RIC group, but mucositis during transplantation was not associated with OS.

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Compliance with ethical standards

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

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