Original Research
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# Investigation of oral and general health status and IL-1β gene polymorphism as risk factors for oral mucositis in hematopoietic stem cell transplantation patients

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**Abstract:** The aim of the present study was to analyze the relationship of OM with possible risk factors such as oral health condition, immunological status and IL-1β profile in patients submitted to hematopoietic stem cell transplantation (HSCT). Fifty-four individuals submitted to HSCT were included. All patients received previous dental treatment and photobiomodulation (PBM) as the institutional OM preventive protocol. OM scores, immune status, and IL-1β levels were determined during the conditioning period and at D+3 and D+8 after HSC infusion. IL-1β gene polymorphism was also analyzed during conditioning. Possible associations of OM with risk factors were analyzed using conditional Fisher's exact test. OM was observed in 34 patients (62.9%) classified as Grade 1 (13 patients/24.1%), Grade 2 (14 patients/25.9%), Grade 3 (3 patients/5.5%), and Grade 4 (4 patients/7.4%). Allogeneic HSCT individuals exhibited a higher OM grade than autologous subjects. Moreover, an association was observed between severe OM and severe gingivitis ($p = 0.01$), neutropenia ($p = 0.03$), and leukopenia ($p = 0.04$). A significant association between OM and lower IL-1β levels was detected at three time points, *i.e.*, conditioning ($p = 0.048$), D+3 ($p = 0.01$), and D+8 ($p = 0.005$). The results showed that IL-1β gene polymorphism was not associated with OM. Our study provided important insights into the scope of OM risk factors in the setting of HSCT. Patients submitted to HSCT with severe gingivitis prior to chemotherapy and with severe neutropenia and leukopenia exhibited a higher OM grade. Further investigation will be necessary to better understand the exact role of IL-1β in the context of OM pathobiology and to validate cytokine analysis in larger cohorts.

**Keywords:** Stomatitis; Hematopoietic Stem Cell Transplantation; Risk Factors; Laser Therapy.

## Introduction

Oral mucositis (OM) represents one of the most common and painful effects of hematopoietic stem cell transplantation (HSCT).[1,2] Almost 70% of individuals receiving HSCT develop this condition, characterized by an inflammatory reaction clinically exhibiting erythematous and/or

ulcerated lesions associated with mild to intense pain.[1] Severe mucositis can lead to significant impairment of quality of life, prolongation of hospital stays, and increased re-admission rates, and can compromise the nutritional status of patients or require discontinuation of cancer therapy and, occasionally, lead to death.[1,3]

The pathobiology of OM is not fully understood and encompasses a multifactorial cascade of biological events explained by a sequence of five stages: initiation, upregulation/activation, signal amplification, ulceration, and healing.[4,5] It is thought to involve direct and indirect mechanisms. Direct mechanisms include mucosal injury caused by radiotherapy and chemotherapy, while indirect damage results from the release of therapy-induced neutropenia and oral microbiome and environment modification.[6] Although the expression rate of some proteins included in the inflammation response, such as IL-1β, is partially associated with the OM process, the presence of these proteins does not exclude others mucositis-independent inflammation events. This strongly implies the need to find biomarkers that specifically characterize the development of the mucositis process.[7] Although several aspects of OM development have been defined, accumulating data have demonstrated that this process is more complex than originally described.

The clinical manifestations of OM include signs and symptoms of an inflammatory process ranging from mild erythema, edema, and soreness to extreme pain and ulceration.[8] Severe OM (grades 3 and 4 on the WHO scale) can negatively affect daily activities such as speaking, eating, and swallowing, as well as patient prognosis, with an important economic impact resulting from costs associated with management of the symptoms and prolonged hospitalization.[9] The most important risk factors related to the development of OM in HSCT patients are the potential aggressiveness of antineoplastic agents, the therapeutic regimen, duration of treatment, dose intensity, the concomitant use of other drugs, or previous treatment.[1,10] Moreover, Shouval et al.[2] demonstrated that HSCT individuals with moderate-to-severe OM were more likely to be of younger age, to have a lower body mass index, to have been exposed to antibiotics during the month preceding admission for transplantation, and to receive myeloablative conditioning and MTX for graft versus host disease prophylaxis. A high incidence of severe oral toxicity is observed in patients with multiple myeloma (MM), non-Hodgkin's lymphoma (NHL), and acute myeloid leukemia (AML) who have received high-dose melphalan, BEAM (carmustine, etoposide, cytarabine, and melphalan), and BuCy (busulfan plus cyclophosphamide) conditioning regimens, respectively, and autologous or allogeneic HSCT.[11,12]

A preventive protocol using photobiomodulation (PBM) is currently considered to be an important tool for the reduction of the incidence and severity of OM in patients receiving high doses of chemotherapy and/or radiotherapy before HSCT.[13,14] Also, some studies have demonstrated an economic value of PBM based on the fact that this tool reduces costs that result in savings of US$5,000 per prevented OM case.[15] The high cost of OM can be explained in general by the fact that patients with severe OM need to treat pain and fungal/bacterial infections and sometimes require gastrostomy feeding tubes and prolonged hospitalization. The latest evidence-based guidelines published in 2019 by the Mucositis Study Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) have demonstrated several lines of evidence recommending PBM as OM preventive therapy.[16] However, some patients still exhibiting OM and risk factors associated with this condition need to be evaluated. Another important aspect is the hypothesis that OM pathogenesis is partly related to a microbial interaction with the oral tissues.[17] In this respect, the objective of the present study was to investigate the incidence and risk factors (oral and general health status and IL-1β gene polymorphism) for OM in patients undergoing HSCT.

## Methodology

The present prospective cohort study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guideline for Reporting *cohort studies*. In addition, it was approved by the Institutional Human Research Ethics Committee (HCPA protocol 12-0173). Patients and/or their legal guardians gave written informed consent.

## Study population

Fifty-four consecutive patients who were admitted to the Porto Alegre Clinical Hospital (HCPA) for HSCT were enrolled in the study between June 2012 and October 2013. Exclusion criteria were patients who received recent periodontal therapy (within the previous 6 months) and edentulousness.

## Dental and periodontal assessment

Figure 1 displays the study flowchart. Upon enrollment, demographics and medical history were assessed by interview at the initial consultation. Oral health measurements including plaque index (PI), gingival index (GI), number of decayed, missing and filled teeth (DMFT), and oral mucosal surface examination were performed.[18]

Full-mouth periodontal and dental variables were assessed by two examining dentists (JJCMCB and MC). Plaque and gingival indices were recorded by assigning a 0 to 3 score to each surface and calculating the full-mouth mean score. PI, which is a measure of oral hygiene was scored on a 0 to 3 scale (0, no plaque; 1, plaque seen after probing; 2, moderate visible plaque; and 3, abundance of visible plaque).[19] GI, a measure of gingival inflammation was classified as: grade 0 (no alteration), grade 1 (mild inflammation, erythema, and edema without bleeding on probing), grade 2 (moderate inflammation, redness, edema, and shiny surface with bleeding on palpation), and grade 3 (severe inflammation, intense redness, and edema with spontaneous bleeding).[20] An analysis of all measurements was performed and the 75th percentile was considered the cut-off point. A partial-mouth method was employed for the examination of periodontitis, with the assessment of specific index teeth: firsts molars and central incisors (one superior and one inferior).

## HSCT and preventive PBMT protocol

Chemotherapy conditioning for HSCT was started 7 (D-7) to 3 (D-3) days before stem cell infusion, which occurred on day 0 (D0). All patients were evaluated daily throughout treatment. PBM was used for the prevention and treatment of OM. All patients received PBM daily from the beginning of conditioning to D+15 for autologous transplant and D+21 for allogeneic transplant. PBM was applied using a continuous wave diode laser (InGaAlP; MM Optics, São Carlos, Brazil) in contact with a wavelength of 660 nm (visible-red). The irradiation parameters (Table 1) were: output power of 40 mW (0.04W), round beam shape, spot area of 0.04 $cm^2$, spot diameter size of 0.4 cm, round beam shape, energy density of 6J/$cm^2$, 1W/$cm^2$ of irradiance at target, 6 seconds of exposure time, and 0.24 J of energy per point of application. Patients were irradiated at 33 points: 6 points on the

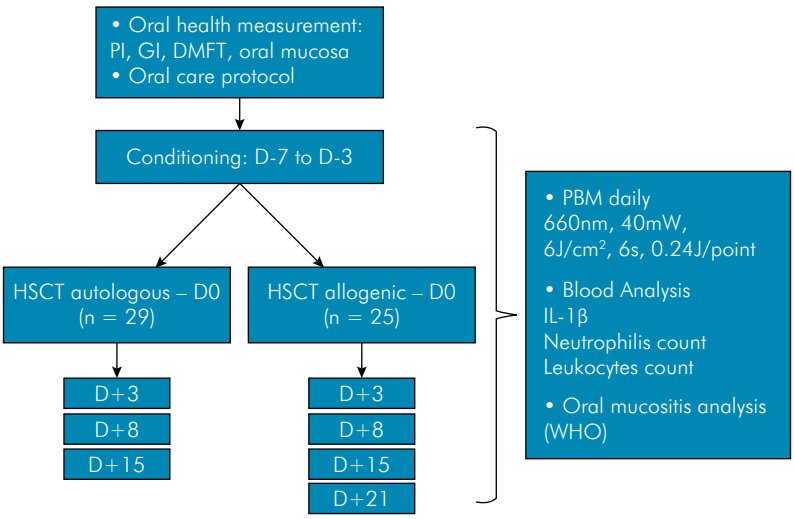


**Figure 1.** Flowchart showing subject enrollment and the evaluations performed.

lips (3 in the upper, 3 in the lower), 2 points at the labial commissure (1 on the right, 1 on the left), 8 points in the buccal mucosa (4 on the right, 4 on the left), 8 points at the lateral border of the tongue (4 on the right, 4 on the left), 5 points on the tongue center, and 4 points on the floor of the mouth.

Patient demographics and data about baseline disease, type of conditioning for transplantation, and type of HSCT (autologous or allogeneic) were collected.

## OM and hematological assessment

OM, absolute neutrophil and leukocyte counts were evaluated during conditioning (D-7 or D-3) and at D+3, D+8, D+15 (recovery of marrow for autologous HSCT) and D+21 (recovery of marrow for allogeneic HSCT).

OM was clinically classified according to the World Health Organization (WHO) scale [grade 0, no OM; grade I, erythema without lesions; grade II, ulcers, but able to eat; grade III, painful ulcers but able to consume liquid food (nutrition) with analgesia for support; grade IV, parenteral or enteral support and continuous analgesia].

Blood samples were obtained at each time point for absolute neutrophil and leukocyte counts and IL-1B analysis. Neutrophil and leukocyte counts were described as mean and standard deviation and classified in grades according to the National Cancer Institute (NCI) classification (Common Toxicity Criteria, Version 2.0, 1998).

**Table 1.** Irradiation parameters and treatment standards

| Parameter | Value |
|---|---|
| Center wavelength (nm) | 660+-10 |
| Operating mode | Continuous wave |
| Spot size ($cm^2$) | 0.04 |
| Time per point (sec) | 6 |
| Energy per pulse (J) | 0.24 |
| Average power (mW) | 40 |
| Irradiance at target ($W/cm^2$) | 1 |
| Radiant exposure ($J/cm^2$) | 6 |
| Application technique | Contact |
| Number and frequency of treatment sessions | Daily once a time |

## Enzyme-Linked Immunosorbent Assay (ELISA)

IL-1β levels were quantified by ELISA using commercial kits (DuoSet Kit for the determination of human IL-1β, ELISA, DV-201, R&D) according to manufacturer's instructions. Readings of the reaction were obtained with a microplate reader (iMark Microplate Absorbance Reader with Microplate Manager Software, BioRad Laboratories, Hercules, CA, USA) at 620 nm (A620 nm). The experiment was carried out in duplicate. A five-parameter curve was constructed for IL-1β quantification.

## Analysis of single nucleotide polymorphism (SNP) by Real-Time Polymerase Chain Reaction (qPCR)

The IPrep™ Purification Instrument (Invitrogen™, Waltham, USA), as well as iPrep™ PureLink™ gDNA Blood Kit (IS-10005; Invitrogen™, Waltham, USA) were used for DNA extraction and purification. The samples were submitted to qPCR using the StepOne™ Real-Time PCR System (Applied Biosystems, Waltham, USA), from the Molecular and Protein Analysis Unit (UAMP) of the HCPA. During the reaction, the fluorescent signals were captured with high optical and thermal precision by the device. The TaqMan® SNP Genotyping Assays system (Applied Biosystems™, Waltham, USA) was used for the analysis of IL-1β polymorphism (rs139843362; +3954). The TaqMan® assay uses the 5'-3' activity of the Taq DNA polymerase nuclease to cleave a fluorogenic probe specific for one of the two alleles at the target site of the polymorphism. The qPCR used was recommended by the fluorescence manufacturer, consisting of the following parameters: 1x [95°C for 2 minutes, 62°C for 1 minute, 72°C for 1 minute], 27x [95°C for 1 minute, 62°C for 1 minute, minute], 3x [94°C for 1 minute, 62°C for 1 minute, 72°C for 5 minutes].[21]

## Cluster analysis

Cluster analysis was performed to characterize and define two groups according to clinical characteristics of the patients, taking into account three variables potentially associated with OM incidence and severity

(age, gingivitis severity, and type of transplant). The groups were formed using two-step and hierarchical methods and the Euclidean distance was used as the scores of these three items. A dendrogram based on the Ward method was used to illustrate the distribution of each case.

### Statistical analysis

Basic statistical univariate analyses were carried out using Stata software (Version 13.1, Stata Corporation, College Station, USA). We used absolute and relative (%) frequencies to describe the categorical data and the mean (ranges) for numerical data. Furthermore, we compared the severity of oral mucositis according to three different explanatory variables, i.e., neutropenia, leukopenia, and IL-1β by univariate analysis using Wilcoxon's rank sum test. Possible associations of cases (patients with OM) or controls (patients without OM) with severe gingivitis (dichotomous) were analyzed using the conditional Fisher exact test and simple logistic regression. $P < 0.05$ values for the comparison of the presence and absence of characteristics were considered to indicate statistically significant differences.

## Results

### Patient characteristics and assessment of oral outcomes

As shown in Table 2, the 54 patients submitted to HSCT ranged in age from 13 to 71 years (40.7 ± 15.19). The most frequent decade of life was the fifth (26.0%), followed by the sixth (20.3%) and third (16.6%). Seven (13.0%) individuals belong to the age group of 0 to 19 years. Males accounted for 30 cases (55.5%) and females for 24 cases (45.5%). The most frequent base diseases were multiple myeloma (31.5%) and acute myeloid leukemia (18.5%). In relation to the HSCT type, 30 (55.5%) patients were autologous, whereas 24 (45.5%) individuals were allogeneic. The most common conditioning regimens used were melphalan alone (33.4%), busulfan plus cyclophosphamide (BuCy, 22.2%), and carmustine, etoposide, cytarabine, and melphalan (BEAM, 22.2%). Of them, 30 (55.5%) were classified as reduced-intensity conditioning (RIC) and 24 (44.5%) as myeloablative. The majority of autologous HSCT

**Table 2.** Patient distribution according to demographic characteristics, diagnosis, type of HSCT, conditioning regimen, and oral health outcomes.

| Variable | n (%) |
|---|---|
| Number of patients | 54 (100.0) |
| Gender | |
| Male | 30 (55.5) |
| Female | 24 (45.5) |
| Mean age ± SD, min-max, in years | 40.7 ± 15.19, 13-71 |
| 10–19 | 7 (13.0) |
| 20–29 | 7 (13.0) |
| 30–39 | 9 (16.6) |
| 40–49 | 14 (26) |
| 50–59 | 11 (20.3) |
| 60–69 | 5 (9.3) |
| 70–79 | 1 (1.8) |
| Smoking status | |
| Yes | 5 (9.3) |
| No | 49 (90.7) |
| Alcohol comsumption | |
| Yes | 4 (7.4) |
| No | 50 (92.6) |
| Diagnosis | |
| Multiple myeloma | 17 (31.5) |
| Acute myeloid leukemia | 10 (18.5) |
| Non-Hodgkin lymphoma | 08 (14.8) |
| Hodgkin lymphoma | 07 (13.0) |
| Acute lymphoblastic leukemia | 06 (11.1) |
| Chronic myeloid leukemia | 02 (3.7) |
| Myelodysplastic syndrome | 02 (3.7) |
| Others | 02 (3.7) |
| HSCT type | |
| Autologous | 30 (55.5) |
| Allogeneic | 24 (45.5) |
| Conditioning regimen | |
| Reduced-intensity conditioning | |
| Melphalan | 18 (33.4) |
| Fludarabine + Melphalan | 04 (7.4) |
| Cyclophosphamide + TBI# | 08 (14.8) |
| Myeloablative | |
| BuCy* | 12 (22.2) |
| BEAM** | 12 (22.2) |
| DMFT## (mean ± SD) | 15.9 ± 7.2 |
| Plaque score | |
| Low | 40 (74.1) |
| Severe | 14 (25.9) |
| Periodontal disease | |
| No or low gingivitis | 40 (74.1) |
| Severe gingivitis | 14 (25.9) |
| Oral mucositis scores | |
| Grade 0 | 20 (37.1) |
| Grade 1 and 2 | 27 (50.0) |
| Grade 3 and 4 | 7 (12.9) |

*BuCy: Busulfan + Cyclophosphamide; **BEAM: carmustine + etoposide + cytarabine + melphalan; #TBI: total body irradiation; ## DMFT: number of decayed, missing and filled teeth.

patients used RIC (n=16/61.5%); in the allogeneic group, 50% received RIC and 50% myeloablative.

Assessment of the oral outcomes of the overall population showed 15.9±7.2 DMFT, mean PI of 0.31, and mean GI of 0.25. OM was observed in 34 patients (62.9%) and classified as grade 1 (13 patients/24.1%), grade 2 (14 patients/25.9%), grade 3 (3 patients/5.5%), and grade 4 (4 patients/7.4%) (Table 2).

Table 3 shows the distribution of the autologous and allogeneic patients according to OM score and period of evaluation. On D+8, patients submitted to allogeneic HSCT exhibited a higher grade of mucositis compared to patients of the autologous group (Figure 2).

## Severe OM is associated with severe gingivitis

Forty of the 54 patients (74.1%) exhibited no/mild gingivitis and 14 (25.9%) exhibited severe gingivitis. A significant association was detected between severe OM and severe gingivitis (p = 0.01, Fisher's exact test) (Figure 3). Simple logistic regression was also performed. The results indicated that severe gingivitis is a statistically significant predictor of severe OM (OR = 10.55; 95%CI: 1.8–63.4).

## OM development is associated with immunosuppressed status

Usually, patients submitted to HSCT receive a conditioning chemotherapy protocol leading to

**Table 3.** Distribution of autologous and allogeneic patients according to OM score and period of evaluation.

| Type of HSCT | Time of assessment | WHO OM Grade (n of events) | | | | |
|---|---|---|---|---|---|---|
| | | G0 | G1 | G2 | G3 | G4 |
| Autologous (n = 30) | Conditioning | 27 | 3 | 0 | 0 | 0 |
| | D+3 | 17 | 9 | 4 | 0 | 0 |
| | D+8 | 19 | 5 | 4 | 2 | 0 |
| | D+15 | 29 | 0 | 1 | 0 | 0 |
| Allogeneic (n = 24) | Conditioning | 21 | 3 | 0 | 0 | 0 |
| | D+3 | 14 | 7 | 2 | 1 | 0 |
| | D+8 | 9 | 2 | 8 | 1 | 4 |
| | D+21 | 19 | 2 | 1 | 1 | 1 |

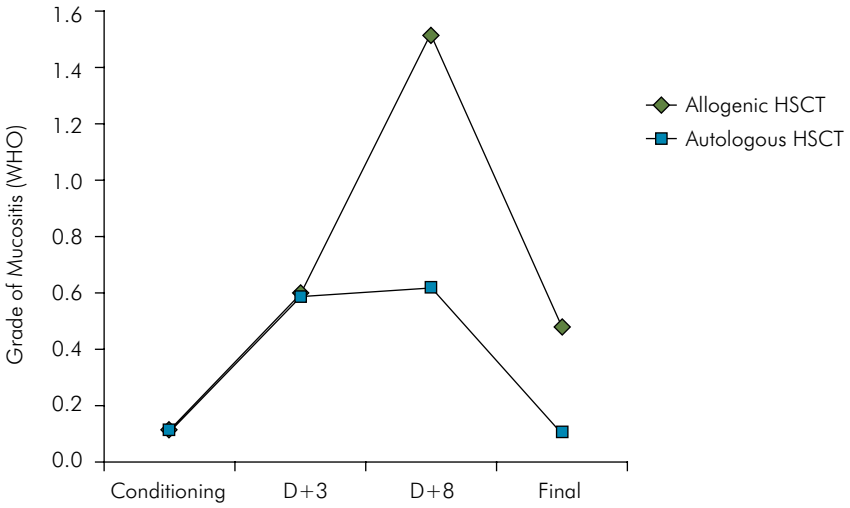


**Figure 2.** Oral mucositis scores of allogeneic and autologous patients from conditioning to the end of treatment.

NADIR after 7–12 days, which represents the "lowest point" of platelet and white blood cell counts.[9,10] In our patients, the mean neutrophil count was 4,861 cell/mm$^3$ during the conditioning period and, as expected, was significantly reduced on D+3 (433 cell/mm$^3$) and D+8 (130.90 cell/mm$^3$). On D+3, patients with OM had a lower neutrophil count (417 cells/mm$^3$) than patients without OM (813 cells/mm$^3$). A significant association was observed between the presence of OM and severe neutropenia ($p = 0.03$). On D+8 all patients showed severe neutropenia, indicating the NADIR period.

The analysis of neutrophil demonstrated a difference between autologous and allogeneic HSCT. Patients submitted to allogeneic HSCT showed the lowest neutrophil levels on D+3 ($p = 0.01$) and D+8 ($p < 0.001$) compared to autologous group. Neutrophil recovery in autologous HSCT patients was observed on D+15 (mean of 2,929 cells/mm$^3$). In allogeneic HSCT patients was observed an increasing of neutrophils on D+15 (mean of 1,096 cells/mm$^3$) and D+21 (2,969 cells/mm$^3$).

Regarding leukocytes, patients submitted to allogeneic HSCT had significantly lower levels than patients in the autologous program on Day +3 ($p = 0.01$), D+8 ($p < 0.01$), and D+15 ($p < 0.01$). Allogeneic patients showed the lowest values of leukocytes with 6,947.46 cells/mm$^3$ during the conditioning period, followed by a significant reduction on D+3 (546 cells/mm$^3$) and D+8 (126 cells/mm$^3$) and increased values on D+15 (1,096 cells/mm$^3$) and D+21 (2,069 cells/mm$^3$).

On D+3, patients with OM showed lower leukocyte counts (292 cells/mm$^3$) than patients without OM (801 cells/mm$^3$). A significant association was observed between the presence of OM and severe leukopenia ($p = 0.04$). On D+8 all patients exhibited severe leukopenia, indicating the NADIR period. No significant association of OM and neutropenia and leukopenia on D+15 and D+21 was observed.

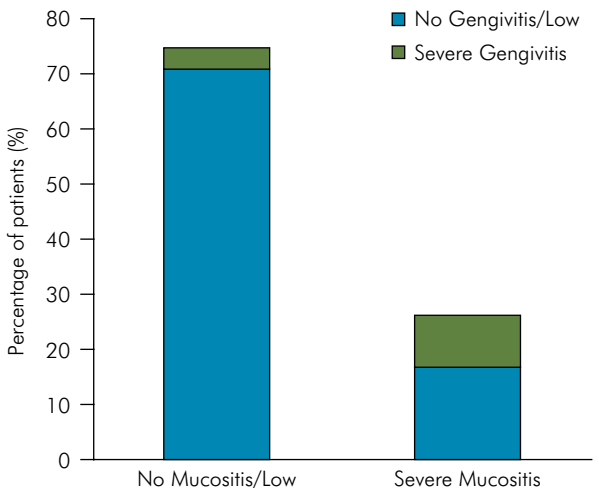


**Figure 3.** Analysis of mucositis on Day +8 (percentage) and gingivitis in patients submitted to HSCT ($p = 0.01$, Fisher's exact test).

## Cluster analysis

Cluster analysis was performed using the three clinical variables potentially associated with OM - age, gingivitis severity, and type of transplant. The clinical characteristics of the groups, created with two-step and hierarchical cluster analyses, are described in Figure 4. A dendrogram, generated to illustrate the distribution of each case according to the proximity of the variables investigated, is presented in Figure 5. The k-means clustering clearly showed that there is an influence of gingivitis, age, and type of transplant on the OM outcome.

**ANOVA**

| | *Cluster* | | *Error* | | | |
|---|---|---|---|---|---|---|
| | Mean Square | gl | Mean Square | gl | F | Sig. |
| **Gingivitis** | 1.131 | 1 | .178 | 52 | 6.368 | .015 |
| **Transplant** | 4.264 | 1 | .174 | 52 | 24.444 | .000 |
| **Age** | 3.293 | 1 | .068 | 52 | 48.611 | .000 |

**Figure 4.** Clinical characteristics of the groups, created with two-step and hierarchical cluster analyses.

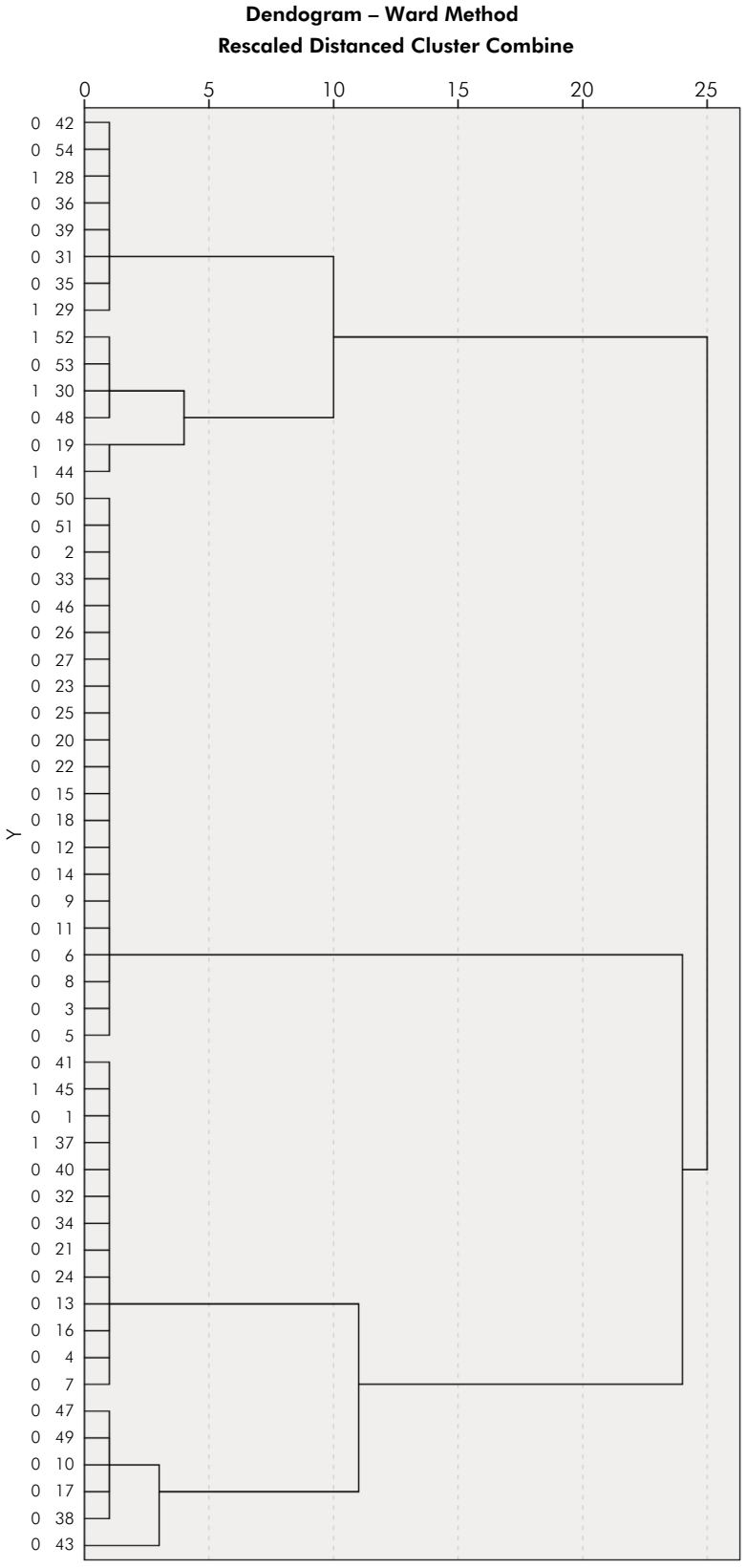


**Figure 5.** Cluster analysis. A dendrogram illustrating the distribution of each case according to the proximity of the three variables used in the present study.

### OM development is associated with a reduction of blood IL-1β levels

We determined the mean IL-1β levels of all patients during the conditioning period and on D+3 and D+8. Separate analysis of patients with and without OM revealed that the group without OM had similar high mean IL-1β levels at all time points (conditioning, D+3, and D+8) (Huynh-Feldt, test, p = 0.57), while patients with OM showed a significant reduction of IL-1β from conditioning through D+8 (Table 4). An important association between OM and lower IL-1β levels was observed during the conditioning period (p = 0.048) and on D+3 (p = 0.01) and D+8 (p = 0.005). These results indicate that patients with OM exhibited lower IL-1β levels. Moreover, a simple logistic regression analysis also revealed that the maintenance of basal IL-1β levels during treatment was correlated with protection against the development of severe oral mucositis on D+3 (OR 0.76; 95%CI: 0.58–0.98) and D+8 (OR 0.66; 95%CI: 0.44–0.96).

### IL-1β polymorphisms are not associated with OM

When IL-1β SNP was determined (rs139843362; +3954), the CC genotype was detected in all patients, indicating that OM was not related to this IL-1β SNP.

## Discussion

OM is a very frequent debilitating adverse effect related to toxicity and myelosuppression stemming from cancer treatment, such as chemotherapy, head and neck radiotherapy, or conditioning for HSCT. Knowledge of the risk factors for OM and of the pathobiology of the condition is necessary for better treatment and prevention. Thus, the aim of the present study was to analyze the relationship of OM with possible risk factors such as oral health condition and immunosuppressant and IL-1β profile in patients submitted to HSCT. Our main results indicated a lower incidence (62.9%) and lower severity of OM. It is important to highlight that, among the individuals younger than 20 years (seven patients), six developed some degree of OM. However, only one was classified as severe. Moreover, our findings showed that the development of OM, especially the severe grade, was associated with the presence of severe gingivitis prior to HSCT, severe immunosuppression (neutropenia and leukopenia), and lower blood levels of IL-1β on D+3 after HSCT.

**Table 4.** Mean IL-1β levels (pg/dL) in patients with and without OM during conditioning and at D+3 and D+8.

| Variable | Conditioning | D+3 | D+8 | p-value |
|---|---|---|---|---|
| OM Absence | 6.3 | 6.2 | 6.05 | 0.86 |
| OM Presence | 2.4 | 0.55 | 0.15 | 0.57 |
| p-value | 0.048 | 0.01 | 0.005 | |

In the present study, OM was observed in 34 patients (62.9%) and was severe in only 7 patients (12.9%), whereas Shouval et al.[2] detected a higher rate of moderate-to-severe OM (83%). In our sample, there was a large number of autologous HSCT cases, which are usually related to reduced intensity conditioning regimes. In this sense, it probably explains the lower incidence and severity of OM. Moreover, these lower rates detected in our sample were expected based on the fact that all patients received oral care before chemotherapy and daily PBM as a preventive treatment protocol for OM. Our service has been routinely applying this protocol since 2010 for all patients submitted to HSCT based on its positive effects described in the literature.[22,23] However, in another study we compared two protocols that differ only in session frequency, either daily or three times a week. The results showed that PBM is effective in preventing OM in patients undergoing HSCT even when administered three times a week.[24] According to Sabater-Sabater Recolons et al.,[23] the maintenance of a healthy oral cavity during oncological treatment, with no or low level plaque or gingival inflammation, is a factor that would lead to a lower and less serious incidence of mucositis. In parallel, several clinical studies have shown the positive effect of PBM in preventing and reducing OM severity.[25] Based on these studies, the MASCC/ISOO (Multinational Association of Supportive Care) clinical practice guideline recommends PBM to prevent OM in patients receiving high doses of chemotherapy or chemoradiotherapy before HSCT.[16]

The incidence and severity of OM in patients undergoing HSCT depends on several factors

such as HSCT type (allogeneic or autologous), conditioning regimen, and the use of methotrexate (MTX) for prophylaxis of graft versus host disease (GVHD).[10] Conditioning may involve chemotherapy, radiotherapy, or both modalities. Conditioning regimens for allogeneic HSCT are usually associated with higher toxicity than regimens for autologous HSCT. In the present study, in agreement with the literature, allogeneic HSCT patients exhibited more intensive mucositis than autologous recipients.[26] In fact, the more intense conditioning used for allogeneic HSCT is intentional due to the need to induce bone marrow aplasia and patient immunosuppression, prerequisites for the success of this treatment modality. This can be explained by the more intensive conditioning regimens in the allogeneic setting that use BuCy or cyclophosphamide + total body irradiation (TBI), or fludarabine + melphalan.[12] According to Zerbe et al. (1992), patients that receive TBI as conditioning for HSCT have higher OM scores, more prolonged time of morphine infusion, and additional days of parenteral nutrition than patients receiving busulfan.[27] Sobecks et al.,[28] showed that 94% of the patients receiving TBI in combination with high doses of etoposide developed severe OM. BuCy is known to cause severe acute and chronic toxicity, including OM. Lee et al.,[29] showed a 68% incidence of grades 2, 3, and 4 OM in patients receiving BuCy drugs for HSCT. Some studies have reported severe OM rates of about 60% for patients treated with MEL and of 50% for those treated with the BEAM protocol.

In addition to the reduction in OM with the oral care and PBM protocols used, our study describes interesting findings regarding OM and periodontal diseases. We observed an important association between the occurrence of OM and gingivitis, indicating that patients who experience severe OM during the HSCT period had severe gingivitis before chemotherapy. It was also confirmed by the cluster analysis, which demonstrated that there is an influence of gingivitis on the OM outcome. Periodontal diseases are also inflammatory responses related to bacterial challenges and represent a local barrier damage due to open sores in the mucosa and consequent infiltration by pathogens, bacterial endotoxins, and proinflammatory cytokines.[17] Therefore, we may assume that OM and periodontal disease may be correlated since they represent a dysregulation of the inflammatory response, as suggested by the "two-hit" model for other inflammation-related systemic conditions.[17] Patients submitted to HSCT experience an important immunosuppressive condition induced by chemotherapy and by the presence of some degree of periodontal disease prior to or during treatment that can act as source of microorganisms and stimulate mucosal breakdown leading to OM.[30] Our study confirms these ideas, establishing that healthy gingival status before conditioning is associated with lower OM severity and supporting the fact that gingivitis may aggravate oral mucosa inflammation based on the hyperinflammatory status that this condition stimulates in oral tissues.

Although it is not fully understood, the pathogenesis of oral mucositis is thought to comprise direct and indirect mechanisms that lead to a series of dynamic interactions among molecular and cellular events involving all elements of the mucosa (epithelium, connective tissue, and oral microbial communities).[31] Some indirect stomatotoxic effects have been postulated to contribute to the development of OM, such as release of inflammatory mediators, loss of protective salivary constituents, and therapy-induced immunosuppression. Regarding immunosuppression, some studies have pointed out that leukopenia and neutropenia reduce the humoral and cellular immune defenses and play a significant role in various infectious complications, thus aggravating various inflammatory processes such as OM.[32] In the present study, at D+3 we observed a significant association between the presence of OM and severe neutropenia and leukopenia. Patients with lower levels of both cells exhibited OM. In addition, at D+3 and D+8, patients submitted to allogeneic HSCT showed significantly lower levels of neutrophils and leukocytes than patients submitted to autologous HSCT. These findings indicate that lower levels of neutrophils and leukocytes could be risk factors for the development of oral mucositis and should be monitored. The relationship between neutrophil and leukocyte counts and the occurrence of mucositis is

not completely understood. However, it is well known that healing of mucositis is associated with neutrophil recovery.[33] Although some investigators have failed to find a link between OM and neutropenia, Rapoport[34] noted that persistence of neutropenia was a risk factor for OM severity. Similarly, McCann et al.[26] observed that severe OM duration was positively correlated with time to neutrophil engraftment. Various studies have shown ambivalent neutrophil recovery using granulocyte colony-stimulating factor (G-CSF, filgrastim) or granulocyte-macrophage colony-stimulating factor (GM-CSF, molgramostim) administered systemically or orally for OM reduction. More studies are necessary in order to improve our knowledge about the relationship of neutropenia and leukopenia with OM.

Another important aspect of the pathobiology of OM is the involvement of some cytokines such as IL-1β, TNF-α, and IL-6 during the five overlapping phases described for this disease.[14,35] Local tissue levels of IL-1β have been shown to increase markedly in animal models of induced OM.[36] However, previous studies about the synthesis of proinflammatory cytokines during mucositis in humans using blood serum are scarce.[10,37] Here, we analyzed the serum level of IL-1β at the beginning of conditioning chemotherapy and on D+3 and D+8 in HSCT patients. We observed that patients without OM had higher and constant IL-1β levels than patients with OM. Patients with OM had lower IL-1β levels at the beginning of conditioning, which gradually decreased up to D+8. These results were quite striking and unexpected since clinical evidence from patients undergoing CT suggests that increases in cytokines levels occur prior to the development of clinical manifestations such as ulceration.[10,37] The interpretation of these findings is challenging. IL-1β is a multifunctional cytokine that influences a wide variety of cell types as well as interacting with many other cytokines. This cytokine was found to be critical in the initial phase of OM and was also reported to have a protective effect on the oral mucosa against radiation by increasing mucosal cell proliferation.[10,35,37,38,39] Thus, elevated serum IL-1β levels may indicate a protective factor against OM development by stimulation of epithelial proliferation.[37] Another aspect that could be considered to justify this cytokine profile in our study is the influence of some SNP of IL-1β resulting in different expression of this protein. However, all patients exhibited homozygosity (CC) for the IL-1β gene, demonstrating that these SNP are not associated with cytokine variations and consequently are not associated with OM. In our study, simple logistic regression also showed that maintenance of basal IL-1β levels during treatment was correlated with protection against the development of severe oral mucositis on D+3 (OR 0.76; 95%CI: 0.58–0.98) and D+8 (OR 0.66; 95%CI: 0.44–0.96). According to Hamblin (2019), PBM is able to regulate cell oxidative stress, thus reducing pro-inflammatory markers in inflammatory cells.[40]

Our study had some limitations. The presence of only seven cases of severe OM (stage 4) reduced the power for the examination of associations and for multivariate analyses. Our results showed a significant association of OM with some risk factors in univariate analyses. However, this sample size was insufficient to demonstrate an association between the two conditions while controlling confounding variables in a multivariate model. Even so, the severity of oral mucositis did not appear to correlate significantly with patient age, gender, smoking status, or alcohol consumption.

## Conclusion

In conclusion, our analysis of prospectively collected data has provided important insights into the extent of OM risk factors in the setting of HSCT. It should be emphasized that patients submitted to HSCT who had severe gingivitis prior to chemotherapy as well as severe neutropenia and leukopenia exhibited higher grades of OM. Further investigation will be necessary to better understand the exact role of IL-1β within the context of OM pathobiology and to validate the cytokine analysis using larger patient cohorts.

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