Long-term safety of photobiomodulation therapy for oral mucositis in hematopoietic cell transplantation patients: a 15-year retrospective study

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Abstract
Photobiomodulation therapy (PBMT) has demonstrated efficacy in the prevention and treatment of oral mucositis (OM) in hematopoietic cell transplantation (HCT). However, based on the cell stimulation properties, its long-term safety has been questioned, mainly in relation to risk for secondary malignancies in the oral cavity. The aim of this study was to investigate if different PBMT protocols for OM control have association with immediate and late adverse effects in HCT patients. Data on autologous and allogeneic transplantation, conditioning regimen, PBMT protocols, and OM severity were retrospectively collected from medical and dental records. Presence of secondary malignancies in the oral cavity was surveyed during a 15-year follow-up. Impact of OM on overall survival was also analyzed. Different PBMT protocols for prevention and treatment of OM were recorded over the years. Severe OM (grades 3 and 4) was infrequently observed. When present, we observed a significant decrease of the overall survival. No immediate adverse effect and secondary malignancy was associated to PBMT. In conclusion, the PBMT protocols used in the study were considered safe. The low frequency of severe OM observed encourages the implementation of this technique, with a special emphasis on the dosimetry adjustments focused on the HCT context.

Keywords Photobiomodulation therapy · Low-level laser therapy · Oral mucositis · Hematopoietic cell transplantation · Safety

Introduction
Hematopoietic cell transplantation (HCT) is a consolidation treatment for diseases in which there is a failure and/or deficiency of the hematopoietic system, including both neoplastic and non-neoplastic disorders. HCT procedure includes the reduction of bone marrow cellularity by means of conditioning with high doses of chemotherapy and/or radiotherapy, followed by an infusion with normal stem cells. The stem cells will replace the original hematopoietic cells precursors, improving the free-disease survival [1].

The HCT conditioning causes high toxicity and prolonged immunosuppression, predisposing the patient to infections and injuries in several tissues. The digestive system is particularly affected by conditioning-induced toxicity, being the oral cavity one of the most affected site. Oral mucositis (OM) is an important dose-limiting adverse event that occurs immediately after the HCT [2]. OM is a painful inflammatory condition that can impair the oral intake and compromise the patient’s quality of life. Difficulties of eating and swallowing often predispose a significant loss of body weight and a nutritional imbalance [3]. In addition, ulcerated OM favors secondary oral infections, increasing the risk of bacteremia and sepsis in the immunocompromised patients [4].

Currently, photobiomodulation therapy (PBMT) protocols have been proposed for OM prevention and treatment in
different phases of the transplantation procedure. The majority of studies have used laser devices and a great variability of the light dosimetry. Table 1 shows studies published in the last 10 years focused in HCT patients and the PBMT effect on preventing or reducing oral mucositis severity and oral pain. Despite the dosimetry variation and the type of light used, all of them showed efficacy of PBMT. However, in these studies, the frequency of immediate and late side effects was not reported, leading to questions about the safety of this therapy in the immunocompromised individual.

Some authors have raised questions about the role of PBMT on those patients exposed to mutagenic agents, such as chemotherapy and radiotherapy, based on the principle that PBMT, depending of the dose, can induce modulation of oxidative stress, cell proliferation, growth factors release, and several transcriptional pathways of activation, among other mechanisms that could favor tumor recurrence or the development of secondary malignancy [12, 13]. Systematic reviews and retrospective investigations evaluated the PBMT safety in oncologic patients, addressing mainly individuals who underwent chemotherapy and radiotherapy or radiochemotherapy for tumors of the head and neck [14–16]. These studies concluded that PBMT is safe. However, because there is a great variability in the parameters used (dosimetry, wavelength, time, and protocols of application), further clinical investigation is necessary.

To the best of our knowledge, there is no study addressing the PBMT safety for HCT patients. During the HCT procedure, the patient is exposed to high doses of chemotherapy and radiotherapy with few fractionations, inducing a high mutagenic stress in the majority of the body tissues. Moreover, these patients are immunosuppressed for a long time, increasing significantly the risk of secondary malignancies [17]. The oral cavity is one of the most affected sites by secondary tumors [18], probably due to a persistent genomic instability in the oral mucosa after the transplantation [19].

The aim of this study was to investigate the safety of PBMT in HCT patients. We analyzed different PBMT protocols for OM prevention and treatment. This single HCT center retrospective study aimed to determine whether there is an association of PBMT with immediate and late adverse effects or the development of secondary malignancies in the head and neck region. We also evaluated the development of OM in all the patients exposed to PBMT and the impact of this condition on the overall survival.

Materials and methods

This was a single center retrospective, observational study carried out using data collected from medical and dental records of patients treated at the Bone Marrow Transplant Center at Hospital Israelita Albert Einstein (HIAE). The methodology described below was previously approved by the Research Ethics Committee of our institution (Project #3471–18) 98904918.4.0000.0071.

Oral care protocol

The study was conducted by dental professionals with expertise in oncology working in the oral oncology section of the Bone Marrow Transplant Center. Prior to start conditioning, all patients were evaluated by the oral oncologists and treated as needed for stabilization of oral disease. Dental and periodontal infection, elimination of areas that could produce trauma to the oral tissues, extraction of hopeless teeth, and implementation of the institutional oral care protocol were conducted. Patients were educated about the importance of maintaining oral hygiene and the procedures associated with PBMT protocol, including patient acceptance.

Eligibility criteria

All available medical and dental records of patients who underwent HCT during the period of January 2004 to December 2019 were surveyed by two calibrated investigators (FPE and LMB). Inclusion criteria were as follows: any age; both sexes; autologous or allogeneic HSCT; prescription of oral care protocols in the pre-, trans-, and post-transplantation periods until marrow engraftment; prescription of PBMT; description of frequency and severity of oral mucositis throughout transplantation; description of the conditioning regimen; and graft-versus-host disease (GVHD) prophylaxis. Records must have had a description of the patient’s general systemic condition. Exclusion criteria were as follows: medical records of patients who did not adhere to the protocol of oral care, absence or insufficient information about the oral conditions, PBMT protocol, oral mucositis, death before the neutrophil engraftment, and graft failure.

Data collection

We collected data on age, sex, primary diagnosis, transplantation type, conditioning regimen, GVHD prophylaxis, and day of neutrophil engraftment (> 500 neutrophils/mm³). The conditioning regimen was classified according with the risk for oral mucositis as follows: high risk - BEAM (carmustine, etoposide, cytarabine, and melphalan); R-(rituximab)-BEAM; regimens containing busulfan, total body irradiation (TBI), and melphalan; low risk - other regimens. The GVHD prophylaxis with methotrexate was considered high risk for OM. The medical informatics service of HIAE provided data for secondary malignancies, date of transplantation, date of death, and the date of last follow-up.

Data about oral mucositis, oral care protocol prescription, and type of PBMT protocol were collected from the
| Patients (number and age range) | Type of HCT             | PBMT protocol                                                                 | Outcomes for oral mucositis severity                                      | Outcomes for oral pain                                      | Reference |
|--------------------------------|-------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------|-----------|
| N = 49 (24 laser and 25 control) | Autologous and allogeneic | InGaAlP laser, 660 nm, 10 mW, 10s irradiation per point, 2.5 J/cm²              | Reduction, but not significant, on the frequency of severe oral mucositis in the laser group | Significant reduction of oral pain in the laser group              | Jaguar et al. [5] |
| N = 70 (23 laser 650 nm, 23 laser 780 nm, 24 control) | Autologous and allogeneic | InGaAlP laser, 650 nm, 40 mW, and InGaAlP laser, 780 nm, 60 mW; both arms with 2 J/cm²; irradiation time per point and spot area not informed | Significant reduction on the frequency of severe oral mucositis in the 650 nm laser group | Significant reduction of oral pain in the 650 nm laser group | Schubert et al. [6] |
| N = 42 (21 laser and 21 control) | Autologous and allogeneic | InGaAlP laser, 660 nm, 0.04 cm² spot area, 40 mW, 0.16 J per point, 4-s irradiation per point, 4 J/cm² | Significant reduction on the frequency of severe oral mucositis in the laser group | Not evaluated                                                  | Santos et al. [7] |
| N = 80 (40 laser and 40 control) | Autologous and allogeneic | LED, 670 nm, 50 mW/cm², 80-s irradiation per point, 4 J/cm², extraoral application (cheeks and throat region) | Reduction, but not significant, on the frequency of severe oral mucositis in the laser group | Significant reduction of pain in the laser group                 | Hodgson et al. [8] |
| N = 24 (12 laser and 12 control) | Autologous and allogeneic | InGaAlP laser, 685 nm, 35 mW, 0.35 J per point, 10-s irradiation per point, energy density not informed | Significant reduction on the frequency of severe oral mucositis in the laser group | Not evaluated                                                  | Silva et al. [9] |
| N = 35 (17 laser and 18 sham) | Autologous and allogeneic | InGaAlP laser, 650 nm, 0.028 cm² spot area, 100 mW, 2 J per point, 20-s irradiation per point, 70 J/cm² | Significant reduction on the frequency of severe oral mucositis in the laser group | Significant reduction of oral pain in the laser group              | Ferreira et al. [10] |
| N = 68 (34 laser and 34 control) | Autologous and allogeneic | InGaAlP laser, 660 nm, 0.04 cm² spot area, 40 mW, 0.16 J per point, 4-s irradiation per point, 4 J/cm² | Significant reduction on the frequency of severe oral mucositis in the laser group | Not evaluated                                                  | Salvador et al. [11] |

Only studies with control group and information about the photobiomodulation therapy were included.
dental records. The data on the oral care protocol and the use of PBMT protocols was collected by the same team of dental professionals who have been performing patient care since 2004 when the oral oncology service was implemented at the HCT center at HIAE. Therefore, the information obtained on oral health status and oral mucositis, as well as the utilization of the PBMT protocol, was standardized. This team also inquired the patient about adverse effects immediately after the PBMT procedure, such as oral discomfort, tingling in the irradiated site, and burning sensation. Absence or presence of these events was registered in the dental records.

Oral mucositis was classified in accordance with WHO classification [20] as follows: 0, without lesions; 1, oral soreness (only erythema); 2, oral erythema and ulcers (but solid diet is tolerated); 3, oral ulcers (only liquid diet is tolerated); and 4, oral ulcers (artificial nutrition is needed). The oral oncology team collected oral mucositis grade daily. After healing occurred, only the highest degree of OM was considered, and the number of days with oral mucositis was also recorded.

Statistical analysis

Categorical data is presented in absolute and relative (%) frequencies. Numerical data is shown in median and minimum/maximum values. Overall survival was calculated using Kaplan-Meier curve. The follow-up was from the first day of transplantation to the last day of contact with the patient (censored cases) or the death day. The impact of oral mucositis on overall survival was calculated by means of Cox proportional hazards regression. For this, oral mucositis degrees were classified as absent (grade 0), mild (grade 1), moderate (grade 2), and severe (grades 3 and 4). The level of significance was set as 5%.

Results

Medical records selection

From 2004 to 2019, 841 medical records of patients who underwent HCT were surveyed. Of these, 148 were excluded due to patient’s death before the engraftment (n = 18), graft failure (n = 27), and absence of enough medical and dental data (n = 103) (Fig. 1).

Patient and transplantation characteristics

A total of 693 records were reviewed. The majority of patients were male (59.0%), with a quite variable age frequency, including 3–11-year-old children (12.0%) and 31–60-year-old adults (42.5%). A significant frequency of >60-year-old patients (24.5%) was also included. Leukemias (28.1%), lymphomas (21.6%), and multiple myeloma (18.2%) were the most common primary disease groups (Table 2).

Patients received autologous (42.7%) and allogeneic (57.3%) HCT; in allogeneic HCT, there was a predominance of matched unrelated donor transplantation (26.7%). Conditioning regimens of high risk for oral mucositis were the most frequent, mainly melphalan alone (30.4%) and regimens containing busulfan (30.6%) and TBI (16.2%). For allogeneic transplantation, GVHD prophylaxis with methotrexate was prescribed with high frequency (52.6%) (Table 1).

Photobiomodulation therapy protocols

All patients included in the study were treated with PBMT. The same professional applied the PBMT therapy and the oral care protocol. During the years of patient care, the laser machine and the PBMT protocols were modified. Different parameters were used due to the modifications required by the laser machine. Four time periods were established.
in accordance with the PBMT protocol characteristics: 2004–2006; 2007–2014; 2015–2016; and 2017–2019. In addition, two different basic protocols of PBMT application were used, one for prevention of oral mucositis and other for the treatment when lesions developed. Table 3 describes the information of the various PBMT protocols used throughout the 15 years of patient follow-up.

### Oral mucositis prevention protocol

Laser parameters used for PBMT prevention started on the first or second day of the HCT conditioning and ended at neutrophil engraftment. The oral mucosal tissues had to be clear of any abnormalities or any suggestion of OM (oral mucositis grade 0). Prevention protocol was done using red lasers (650 nm or 660 nm), with a lower energy density. The entire oral mucosa was irradiated (right and left buccal mucosa, upper and lower lip mucosa, lateral borders and ventral surface of the tongue, floor of mouth, and soft palate), with exception of tongue dorsum and hard palate, sites considered of low risk for oral mucositis. Comparing 2004–2006 with 2007–2014 period, the laser power increased from 0.04 to 0.1 W. This higher power was maintained in the following periods. The time of irradiation also varied from 2 s in 2004–2006 to 10 s in 2017–2020. These variations in the laser parameters changed significantly the power and energy densities per point in the subsequent periods (Table 3).

### Oral mucositis treatment protocol

The protocol for oral mucositis treatment was implemented when the oral mucosal tissues started to show early signs of OM such as erythema (oral mucositis grade 1). While the development of OM continued with atrophy, erosion, or ulceration (oral mucositis grades 2, 3, and 4), the PBMT continued to be used. PBMT was also used when the patient reported oral discomfort or oral pain mainly during mastication and swallowing. The PBMT treatment protocol was applied only in areas with lesions. Areas around the lesions and the rest of the oral mucosa received parameters of prevention protocol. The laser parameters for oral mucositis treatment included both red and infrared wavelengths (650 nm, 660 nm, 780 nm, and 808 nm depending on the time of application (Table 3).

Laser equipment, power, spot area, and power density were the same used in the protocol for oral mucositis prevention. Irradiation time, energy per point, and energy density per point had higher values, in general double values (Table 3). The number of irradiation points was established in accordance with the size of each lesion. Starting in 2015, infrared lasers (808 nm) were adopted for analgesia induction. During the 2015–2016 period, lesions were first irradiated with 660 nm and then with 808 nm (consecutive irradiation). Starting in 2017, the laser machine was enhanced, allowing a 660 nm and 808 nm irradiation at the same time (simultaneous irradiation). This laser setting was indicated when patient reported extreme pain and discomfort. When the two wavelengths were used, the irradiated point received double energy, i.e., 2 J derived from the 660 nm and 2 J derived from 808 nm.

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**Table 2 Main clinical characteristics of the patients and transplantation**

| Sex          | N   | %  |
|--------------|-----|----|
| Male         | 409 | 59.0 |
| Female       | 284 | 41.0 |

| Age          | N   | %  |
|--------------|-----|----|
| 0–2 years    | 47  | 6.8 |
| 3–11 years   | 83  | 12.0 |
| 12–20 years  | 46  | 6.6 |
| 21–30 years  | 52  | 7.5 |
| 31–40 years  | 80  | 11.5 |
| 41–50 years  | 92  | 13.3 |
| 51–60 years  | 123 | 17.7 |
| 61–70 years  | 136 | 19.6 |
| 71–76 years  | 34  | 4.9 |

| Primary disease group | N | %  |
|-----------------------|---|----|
| Anemias               | 33 | 4.8 |
| Autoimmune diseases   | 31 | 4.5 |
| Genetic syndromes     | 20 | 2.9 |
| Immunodeficiencies     | 53 | 7.6 |
| Leukemias             | 195| 28.1 |
| Lymphomas             | 150| 21.6 |
| Multiple myeloma       | 126| 18.2 |
| Other myeloproliferative disorders | 57 | 8.2 |
| Solid tumors          | 28 | 4.0 |

| Type of transplantation | N | %  |
|-------------------------|---|----|
| Autologous              | 296| 42.7 |
| Allogeneic              | 397| 57.3 |
| Matched related donor   | 115| 16.6 |
| Matched unrelated donor | 185| 26.7 |
| Haploidentical          | 97 | 14.0 |

| Conditioning regimen    | N | %  |
|-------------------------|---|----|
| BEAM or R-BEAM          | 73 | 10.5 |
| Melphalan               | 211| 30.4 |
| Containing busulfan     | 210| 30.3 |
| Containing total body irradiation | 112 | 16.2 |
| Other                   | 87 | 12.6 |

| Graft-versus-host disease prophylaxis | N | %  |
|--------------------------------------|---|----|
| Containing methotrexate              | 209| 52.6 |
| Other                                | 188| 47.4 |

| Neutrophil engraftment (day +) | Median | Range |
|--------------------------------|--------|-------|
|                                | 11     | 7–43  |

**BEAM** (carmustine, etoposide, cytarabine, and melphalan); **R** rituximab
used for patients with dysphagia, using infrared laser and a higher energy density (100 J/cm² per point). The irradiation was performed in 12 points on the skin of the neck and in the region around the pharynx and esophagus (Fig. 2).

There was no difference in the laser settings with regard to the patient’s age, type of conditioning and transplantation, as well as type of GVHD prophylaxis. Particularly to the pediatric patients with age < 2 years, some changes in the irradiation technique were implemented, such as performance of laser irradiation with the patient positioned on the mother’s lap. These adaptations were previously published [21].

Table 3 Laser parameters indicated for oral mucositis prevention and treatment in accordance with different periods of the HCT patient survey

| Indication for oral mucositis | 2004–2006 | 2007–2014 | 2015–2016 | 2017–2020 |
|------------------------------|-----------|-----------|-----------|-----------|
| **Prevention**               |           |           |           |           |
| Equipment                    | Twin laser (MMO, São Carlos, SP, Brazil) | Therapy (DMC, São Carlos, SP, Brazil) | Therapy XT (DMC, São Carlos, SP, Brazil) | Therapy EC (DMC, São Carlos, SP, Brazil) |
| Type                         | Diode     | Diode     | Diode     | Diode     |
| Power (W)                    | 0.04      | 0.1       | 0.1       | 0.1       |
| Wavelength (nm)              | 650 nm    | 660 nm    | 660 nm    | 660 nm    |
| Spot area (cm²)              | 0.04      | 0.04      | 0.04      | 0.09      |
| Mode                         | Punctual  | Punctual  | Punctual  | Punctual  |
| Energy (J) per point         | 0.08      | 0.3       | 0.4       | 1         |
| Irradiation time (s) per point | 2         | 3         | 4         | 10        |
| Power density (W/cm²)        | 1         | 2.5       | 2.5       | 1.1       |
| Energy density (J/cm²)       | 2         | 8         | 10        | 11.1      |
| Therapy frequency            | Daily     | Daily     | Daily     | Daily     |
| Beginning and ending of the therapy | From first day of conditioning to neutrophil engraftment | Second day of conditioning; neutrophil engraftment | |

| **Treatment**                |           |           |           |           |
| Equipment                    | Twin laser (MMO, São Carlos, SP, Brazil) | Therapy (DMC, São Carlos, SP, Brazil) | Therapy XT (DMC, São Carlos, SP, Brazil) | Therapy EC (DMC, São Carlos, SP, Brazil) |
| Number of points in the oral mucosa | In accordance with the lesion area; 1-cm distance between each point | | | |
| Type                         | Diode     | Diode     | Diode     | Diode     |
| Power (W)                    | 0.04 and 0.06 | 0.1       | 0.1       | 0.1       |
| Wavelength (nm)              | 650 nm and 780 nm | 660 nm and 808 nm | 660 nm and 808 nm | 660 nm and 808 nm |
| Spot area (cm²)              | 0.04      | 0.04      | 0.04      | 0.09      |
| Mode                         | Punctual  | Punctual  | Punctual  | Punctual  |
| Energy (J) per point         | 0.08      | 0.5       | 2         | 2         |
| Irradiation time (s) per point | 2         | 5         | 8         | 20        |
| Power density (W/cm²)        | 1         | 2.5       | 2.5       | 1.1       |
| Energy density (J/cm²)       | 2         | 12.5      | 20        | 22.2      |
| Therapy frequency            | Daily     | Daily     | Daily     | Daily     |
| Beginning and ending of the therapy | From first day of clinical sign and symptoms onset to 2 days after the clinical sign and symptoms disappearance | | |

The description of dose parameters was individual for each wavelength.

From 2010, it was adopted an extracutaneous PBMT protocol for patients with dysphagia, performed in the neck region around the pharynx and esophagus with the following parameters: 808 nm, 0.1 W, punctual, 40 s, 0.04 spot area, 2.5 W/cm², 4 J per point, 100 J/cm² per point.

According to the manufacturer, the spot area for Therapy XT and Therapy EC was dependent on the fabrication year and lot number. The spot area described in the table considered these two conditions.
Oral care protocol

The PBMT was adjuvant to a standardized oral care protocol, which was described in all the eligible medical records. All patients underwent dental and radiographic examination prior to transplantation. Oral infectious foci and traumatic surfaces were eliminated before the start of conditioning. From all the enrolled patients, 58% underwent some dental intervention in the pre-transplantation period, which included dental plaque prophylaxis, caries removal, scaling and root planning, occlusal adjustment, dental extractions, and prosthesis adjustments. During HCT, a dental professional performed daily oral examination to confirm the maintenance of oral health status and the absence of infection and to monitor the quality of oral hygiene. When oral fungal infections were suspected, a topical application of nystatin suspension 4 times/day was prescribed. When other opportunistic infections were suspected, the dentist performed appropriate diagnostic test and instituted indicated treatment. For the patients who received melphalan conditioning, an oral cryotherapy protocol was adopted during the conditioning, as previously described [22] in order to decrease the incidence of OM.

Adverse effects related to photobiomodulation therapy

No undesired adverse reactions could be seen during or after PBMT. The medical records reviewed did not reveal any documentation of adverse reactions. In addition, in the analyzed period, the department of health quality and safety at HIAE did not identify any adverse event related to the PBMT.

Oral mucositis severity

The majority of patients (90.1%) had some degree of oral mucositis. Mild to moderate severity (grades 1 and 2) was observed in 68.0%, and more severe OM (grades 3 and 4) was described in 12.0% of the patients (Supplementary Table 1). The frequency of severe (grades 3 and 4) OM in autologous and allogeneic HCT was 6.7% and 16.0% respectively. The median day of OM onset was at day +4, and the median time duration of the lesions was 6 days.

Secondary neoplasms after transplantation

Only 7/693 patients (1.0%) had a secondary neoplasm after transplantation, 2 in patients who received autologous and 5 in patients who received in allogeneic HCT (Supplementary Table 2). All cases occurred in adult patients. One case was a myelodysplastic syndrome, and the other 6 cases were solid tumors: breast cancer (2), pancreatic cancer (2), and head and neck cancer (2). Head and neck cancers affected the tongue and esophagus.

The tongue cancer was diagnosed in a 50-year-old male, who received an allogeneic transplantation with R-BEAM conditioning. The neutrophil engraftment occurred on day +15, and the maximum degree of OM was grade 1. Treatment included a partial glossectomy followed by radiotherapy (60 Gy). Until the end of the study, the patient was well and had no history of recurrence. Oral GVHD was not detected during this period.

Impact of oral mucositis in the overall survival

The median follow-up in the study was 84.5 months (3–405 range). Considering all enrolled patients, the 5-year overall survival was 62.6% (95%CI = 58.0–67.0%). When the patients were stratified in accordance with the oral mucositis severity, moderate (HR = 1.61, p = 0.025) and severe (HR = 1.96, p = 0.008) oral mucositis reduced significantly the overall survival (Fig. 3).

Discussion

In this retrospective study, we aimed to evaluate the short and long-term safety of the PBMT in prevention and treatment of OM in HCT patients. We evaluated the development of adverse reactions of PBMT in the oral cavity and the occurrence of secondary malignancies. To our
knowledge, this is the first single-center long-term study with a high number of patients (693) focused on the analysis of the safety of PBMT in HCT.

The analysis of early and late oral complications PBMT-induced did not reveal any harm, suggesting that PBMT is a safe therapy in this patient population. There were also no associated systemic side effects. The frequency of the secondary malignancies in the head and neck region, particularly in the oral cavity, was low not revealing a specific association with PBMT. Another important finding was that moderate and severe OM affected the overall survival of these patients.

The variation of PBMT parameters

This was a long-term study over a period of 15 years. During this period of time, several changes occurred in the type of laser wavelength and parameters used in the delivery of PBMT. Most of the variations in parameters involved the increase of power and energy density delivered to the oral tissues and OM lesions. A significant variation was the implementation of a laser device that could deliver light in the red and infrared wavelengths simultaneously.

The increase of energy and power densities were related to two specific facts: First, the majority of low intensity
laser machines in Brazil have the power fixed at 0.1 W, not allowing adjustments of this setting; second, the clinicians observed that the clinical outcomes of the PMBT protocol with higher energy and power density produced better effect in the treatment of OM. The laser machine that emits the red and infrared wavelengths simultaneously promotes healing of inflammation and pain control. This is desired when delivering care at bedside. In addition, the PBMT protocol was adjusted over time based on the evidence of increased OM risk.

**The prevention and treatment protocols for OM**

The HCT patients enrolled in the present study were treated with two different PBMT protocols, one for prevention and other for the treatment of oral mucositis. The two protocols involved energy densities considered high (from 8 to 22.2 J/cm²) when compared to the literature for oral mucositis control.

The prevention protocol involved shorter irradiation time per point, leading to a lower energy density (up to 11.1 J/cm²). The main objective of this protocol was to maintain the epithelial and connective tissue integrity by stimulating keratinocytes and fibroblasts renewal [23]. Moreover, a prevention protocol can reduce the risk of oral mucositis in the critical periods of the transplantation. Recent systematic reviews and meta-analyses [24–26] have demonstrated efficacy of PBMT in the prevention of oral mucositis severity, although more clinical studies focused on HCT patients are necessary for improving the scientific evidence of this therapy.

The treatment protocol was indicated when clinical signs of oral mucosal injury were present. This protocol increased the irradiation time and used a higher energy density (up to 22.2 J/cm²). In addition to higher doses, the laser machine delivered 660 nm and 808 nm wavelengths simultaneously, aiming to improve the photon interaction with different chromophores and promoting the photon resorption at different depth levels [27]. The main objective of this protocol was to induce analgesia, for the reestablishment of oral intake and the improvement of patient’s quality of life.

Past studies have demonstrated efficacy in reducing pain caused by oral mucositis in HCT patients [6, 10], but their protocols used red lasers and lower energy densities. A previous study demonstrated a significant reduction of oral mucositis severity and analgesics prescription in cancer patients submitted to radiotherapy in the head and neck region when 660 nm and 808 nm were associated with a higher energy density (300 J/cm²) [28]. Furthermore, the association of red and infrared wavelengths can improve tissue repair by the increasing the collagen matrix and reducing inflammation [7]. Nevertheless, more comprehensive clinical studies involving the oral mucosa, variations on the dosimetry, and association of the two wavelengths are necessary to confirm this trend.

Scientific evidence has suggested that dosimetry up to 6 J/cm², 150 mW, and use of 633–685 nm and 780–830 nm wavelengths are safe [29]. Systematic reviews of PBMT used to prevent and control oral mucositis recommended higher values, including 12, 35, and 70 J/cm² [30, 31]. Others showed that PBMT applied in patients who underwent radiotherapy in the head and neck region was not associated to any adverse effects [15, 32]. Another study used 10 J/cm² daily, without adverse events and safety issues reported in H & N cancer patients [14]. However, the majority of the studies in the current literature had short follow-ups. Therefore, the question about the risk of secondary malignancies or tumor recurrence in the head and neck region needs further investigation [15].

In the current study, the highest doses were 11.1 J/cm² and 22.2 J/cm², which were compatible with the range of dosimetry values reported in other studies with HCT patients (Table 1). Based on the absence of adverse effects, no association with secondary malignancies in the head and neck region, and low frequency of severe of oral mucositis (grades 3 and 4), we can consider the parameters used in the present study to be safe.

**Secondary malignancies**

Secondary malignancies are one of the most important late complications in post-HCT period, affecting mainly patients receiving allogeneic transplantation [17]. Second primary oral cancers are one of the most frequent neoplasms in the HCT patients [18]. A study showed 2.7% incidence of oral squamous cell carcinoma as a second primary malignancy in allogeneic HCT. Risk factors associated with the malignancy development included myeloablative conditioning and presence of chronic GVHD in the oral cavity [18].

In the present study, only 1/693 (0.01%) patient developed a secondary malignancy in the oral cavity. We were not able to find any association of PBMT adverse reactions with the development of this neoplasm.

**Overall survival and oral mucositis**

The frequency of severe oral mucositis was low (12.0%). A comprehensive systematic review [2] showed frequencies of severe oral mucositis varying from 19.4 to 83.0% and from 23.5 to 90.6% in allogeneic HCT performed with myeloablative conditioning and reduced intensity conditioning, respectively. Although oral care protocols and oral cryotherapy were used, none of them implemented the use of PBMT. In the current study, a daily specialized oral care protocol was done for all HCT patients. In addition, oral cryotherapy was used in patients who underwent...
melphalan conditioning. Therefore, based on the very low frequency of grades 3 and 4 oral mucositis, the implementation of oral care, cryotherapy when indicated, and PBMT use can be recommended in the transplant setting [33]. Further clinical studies with HCT patients are necessary to confirm this hypothesis.

Although the frequency of severe oral mucositis was low, grades 3–4 oral mucositis reduced significantly the overall survival, suggesting that the prevention and control of OM is one of the most important steps in transplantation. New PBMT strategies focused on the patients at high risk for severe oral mucositis, such as those receiving allogeneic transplantation, myeloablative regimens, and with GVHD prophylaxis using methotrexate, must be investigated.

A significant limitation of this study was the absence of a control group, not allowing a complete extrapolation regarding to the PBMT safety. Absence of oral GVHD data is also an important limitation, because the oral mucositis is considered a possible risk factor for this complication, and probably the PBMT may have a positive role on oral acute and chronic GVHD. The use of PBMT for other oral conditions, such as infectious, traumatic, and immune-mediated lesions, was not addressed, limiting also the knowledge about the PBMT effect and safety in these circumstances.

In conclusion, the PBMT protocols for oral mucositis prevention and treatment in the HCT patients were not associated with immediate and late adverse effects and were not related to the development of secondary malignancies in the oral cavity. The low frequency of severe OM detected in this study encourages the implementation of these protocols, with a special emphasis on the need for the correct use of dosimetry in PBMT.

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**Authors’ contributions** Letícia Mello Bezinelli contributed with study conception and design, data acquisition and interpretation, and critical review of the manuscript. Luciana Corrêa contributed with study design, data interpretation, and manuscript draft. Cristina Vogel contributed with study conception and design, data acquisition and interpretation, and review of the manuscript. Jose Mauro Kutner contributed with data interpretation and critical review of the manuscript. Andreza Feitosa Ribeiro contributed with data interpretation and critical review of the manuscript. Nelson Hamerschlak contributed with study design and critical review of the manuscript. Carlos de Paula Eduardo contributed with study conception and design, data acquisition and interpretation, and critical review of the manuscript. Fernanda de Paula Eduardo contributed with study conception and design, data acquisition and interpretation, and critical review of the manuscript.

**Data availability** All the data showed in the study support our claims and comply with field standards.

**Code availability** N/A.

**Declarations**

**Ethics approval** This retrospective chart review study involving human participants is in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. The Human Investigation Committee (IRB) of Hospital Israelita Albert Einstein approved this study (#3471–18 – HAE).

**Consent to participate** An informed consent to participate in this study was not signed by the patients because the data were collected from archived medical records, being impossible to contact the majority of the patients or their legal guardian. Principles of confidentiality, privacy, and data protection were entirely adopted in the study and in the manuscript.

**Consent for publication** Informed consent to publish this study was not signed by the patients because the data were collected from archived medical records, being impossible to contact the majority of the patients or their legal guardian. Principles of confidentiality, privacy, and data protection were entirely adopted in the study and in the manuscript.

**Conflict of interest** The authors declare competing interests.

**References**

1. Granot N, Storb R (2020) History of hematopoietic cell transplantation: challenges and progress. Haematologica. 105(12):2716–2729. https://doi.org/10.3324/haematol.2019.245688
2. Chaudhry HM, Bruce AJ, Wolf RC, Litzow MR, Hogan WJ, Patnaik MS, Kremers WK, Phillips GL, Hashmi SK (2016) The incidence and severity of oral mucositis among allogeneic hematopoietic stem cell transplantation patients: a systematic review. Biol Blood Marrow Transplant 22(4):605–616. https://doi.org/10.1016/j.bbmt.2015.09.014
3. Eduardo FP, Bezinelli LM, Gobbi MF, Pereira AZ, Vogel C, Hamerschlak N, Correia L (2018) Impact of oral and gastrointestinal mucositis on body weight alterations during hematopoietic stem cell transplantation. Nutr Cancer 70(2):241–248. https://doi.org/10.1080/01635380.2018.1412476
4. Elad S, Raber-Durlacher JE, Brennan MT, Saunders DP, Mann AP, Zadik Y, Quinn B, Epstein JB, Blijlevens NM, Walmuto T, Passweg JR, Correa ME, Dahllof G, Garming-Leget KU, Logan RM, Potting CM, Shapira MY, Soga Y, Stringer J, Stokman MA, Vokurka S, Wallhult E, Yarom N, Jensen SB (2015) Basic oral care for hematology-oncology patients and hematopoietic stem cell transplantation recipients: a position paper from the joint task force of the multinational Association of Supportive Care in cancer/International Society of Oral Oncology (MASCC/ISOO) and the European Society for Blood and Marrow Transplantation (EBMT). Support Care Cancer 23(1):223–236. https://doi.org/10.1007/s00520-014-2378-x
5. Jaguar GC, Prado JD, Nishimoto IN, Pinheiro MC, de Castro DO Jr, da Cruz Perez DE, Alves FA (2007) Low-energy laser therapy for prevention of oral mucositis in hematopoietic stem
cell transplantation. Oral Dis 13(6):538–543. https://doi.org/10.1111/j.1601-0825.2006.01330.x
6. Schubert MM, Eduardo FP, Guthrie KA, Franquin JC, Bensadoun RJ, Migliorati CA, Lloidi CM, Eduardo CP, Walter NF, Marques MM, Hamdi M (2007) A phase III randomized double-blind placebo-controlled clinical trial to determine the efficacy of low level laser therapy for the prevention of oral mucositis in patients undergoing hematopoietic cell transplantation. Support Care Cancer 15(10):1145–1154. https://doi.org/10.1007/s00520-007-0238-7
7. Santos NR, de M Sobrino JB, Almeida PF, Ribeiro AA, Cangussu MC, dos Santos JN, Pinheiro AL (2011) Influence of the combination of infrared and red laser light on the healing of cutaneous wounds infected by Staphylococcus aureus. Photomed Laser Surg 29(3):177–182. https://doi.org/10.1089/pho.2009.2749
8. Hodgson BD, Margolis DM, Salzman DE, Eastwood D, Tarima S, Williams LD, Sande JE, Vaughan WP, Whelan HT (2012) Amelioration of oral mucositis pain by NASA near-infrared light-emitting diodes in bone marrow transplant patients. Support Care Cancer 20(7):1405–1415. https://doi.org/10.1007/s00520-011-1223-8
9. Silva GB, Mendonça EF, Bariani C, Antunes HS, Silva MA (2011) The prevention of induced oral mucositis with low-level laser therapy in bone marrow transplantation patients: a randomized clinical trial. Photomed Laser Surg 29(1):27–31. https://doi.org/10.1089/pho.2009.2699
10. Ferreira B, da Motta Silveira FM, de Orange FA (2016) Low-level laser therapy prevents severe oral mucositis in patients submitted to hematopoietic stem cell transplantation: a randomized clinical trial. Support Care Cancer 24(3):1035–1042. https://doi.org/10.1007/s00520-015-2881-8
11. Salvador DRN, Soave DF, Sacofo NT, de Castro EF, Silva GBL, E Silva LP, Silva TA, Valadares MC, Mendonça EF, Batista AC (2017) Effect of photobiomodulation therapy on reducing the chemotherapeutic induced oral mucositis severity and on salivary levels of CXCL8/interleukin 8, nitrite, and myeloperoxidase in patients undergoing hematopoietic stem cell transplantation: a randomized clinical trial. Lasers Med Sci 32(8):1801–1810. https://doi.org/10.1007/s10103-017-2263-1
12. Sonis ST, Hashemi S, Epstein JB, Nair RG, Raber-Durlacher JE (2016) Could the biological robustness of low level laser therapy (Photobiomodulation) impact its use in the management of mucositis in head and neck cancer patients. Oral Oncol 54:7–14. https://doi.org/10.1016/j.oraloncology.2016.01.005
13. Zecha JA, Raber-Durlacher JE, Nair RG, Epstein JB, Sonis ST, Elad S, Hamblin MR, Barasch A, Migliorati CA, Milstien DM, Genot MT, Lansaat L, van de Brink R, Arnabat-Dominguez J, van der Molen L, Jacobi I, van Diessen J, de Lange J, Smeele LE, Schubert MM, Bensadoun RJ (2016a) Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations. Support Care Cancer 24(6):2781–2792. https://doi.org/10.1007/s00520-016-3152-z
14. Brandão TB, Morais-Faria K, Ribeiro ACP, Rivera C, Salvajoli JV, Lopes MA, Epstein JB, Arany PR, de Castro G Jr, Migliorati CA, Santos-Silva AR (2018)Locally advanced oral squamous cell carcinoma patients treated with photobiomodulation for prevention of oral mucositis: retrospective outcomes and safety analyses. Support Care Cancer 26(7):2417–2423. https://doi.org/10.1007/s00520-018-4046-z
15. de Pauli PM, Araújo ALD, Arboleda LPA, Palmier NR, Fonseca JM, Gomes-Silva W, Madrid-Torocen CC, Silveira FM, Martins MD, Faria KM, Ribeiro ACP, Brandão TB, Lopes MA, Leme AFP, Migliorati CA, Santos-Silva AR (2019) Tumor safety and side effects of photobiomodulation therapy used for prevention and management of cancer treatment toxicities. A systematic review. Oral Oncol 93:21–28. https://doi.org/10.1016/j.oraloncology.2019.04.004
16. Bensadoun RJ, Epstein JB, Nair RG, Barasch A, Raber-Durlacher JE, Migliorati C, Genot-Klastersky MT, Treister N, Arany P, Lodewijckx J (2020) Robijns J; world Association for Laser Therapy (WALT). Safety and efficacy of photobiomodulation therapy in oncology: a systematic review. Cancer Med 9(22):8279–8300. https://doi.org/10.1002/cam4.3582
17. Heydari K, Shamsirian A, Lotfi-Foroushani P, Aref F, Hedayatizadeh-Omaran A, Ahmadi M, Janbabaei G, Keyhaniyan S, Zaboli E, Ghasemzadeh SM, Alizadeh-Navaei R (2020) The risk of malignancies in patients receiving hematopoietic stem cell transplantation: a systematic review and meta-analysis. Clin Transl Oncol 22(10):1825–1837. https://doi.org/10.1007/s12094-020-02322-w
18. Santarone S, Natale A, Angelini S, Papalinietti G, Vaddinelli D, Di Bartolomeo A, Di Bartolomeo P (2020) Secondary oral cancer following hematopoietic cell transplantation. Bone Marrow Transplant. https://doi.org/10.1038/s41409-020-01147-z
19. Khan FM, Sy S, Louise P, Ugarte-Torres A, Berka N, Sinclair GD, Stewart DA, Russell JA, Storek J (2010) Genomic instability after alloge neic hematopoietic cell transplantation is frequent in oral mucosa, particularly in patients with a history of chronic graft-versus-host disease, and rare in nasal mucosa. Blood. 116(10):1803–1806. https://doi.org/10.1182/blood-2009-10-249201
20. WHO. Handbook for reporting results of cancer treatment. Geneva: World Health Organization. 1979. [Offset publication n° 48:16–7]
21. Eduardo FP, Bezini L, da Carvalho DL, Lopes RM, Fernandes JP, Brumatti M, Vince CS, de Azambuja A, Vogel C, Hamerschlak N, Correa L (2015) Oral mucositis in pediatric patients undergoing hematopoietic stem cell transplantation: clinical outcomes in a context of specialized oral care using low-level laser therapy. Pediatr Transplant 19(3):316–325. https://doi.org/10.1111/petr.12440
22. Eduardo FP, Bezini L, da Graça Lopes RM, Nascimento Sobrino JJ, Hamerschlak N, Correa L (2015) Efficacy of cryotherapy associated with laser therapy for decreasing severity of melphalan-induced oral mucositis during hematological stem-cell transplantation: a prospective clinical study. Hematol Oncol 33(3):152–158. https://doi.org/10.1002/hon.2133
23. George S, Hamblin MR, Abrahame H (2018) Effect of red light and near infrared laser on the generation of reactive oxygen species in primary dermal fibroblasts. J Photochem Photobiol B 188:60–68. https://doi.org/10.1016/j.jphotobiol.2018.09.004
24. He M, Zhang B, Shen N, Wu N, Sun J (2018) A systematic review and meta-analysis of the effect of low-level laser therapy (PBMT) on chemotherapy-induced oral mucositis in pediatric and young patients. Eur J Pediatr 177(1):7–17. https://doi.org/10.1007/s00431-017-3043-4
25. de Lima VHS, de Oliveira-Neto OB, de Hora Sales PH, da Silva TT, de Lima FJC (2020) Effectiveness of low-level laser therapy for oral mucositis prevention in patients undergoing chemoradiotherapy for the treatment of head and neck cancer: a systematic review and meta-analysis. Oral Oncol 102:104524. https://doi.org/10.1016/j.oraloncology.2019.104524
26. Peng J, Shi Y, Wang J, Wang F, Dan H, Xu H, Zeng X (2020) Low-level laser therapy in the prevention and treatment of oral mucositis: a systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol 130(4):387–397.e9. https://doi.org/10.1016/j.orms.2020.05.014
27. Hamblin MR (2017) Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. AIMS Biophys 4(3):337–361. https://doi.org/10.3934/biophy.2017.3.337
28. Soares RG, Farias LC, da Silva Menezes AS, de Oliveira E Silva CS, Tabosa ATL, Chagas PVF, Santiago L, Santos SHS, de Paula AMB, Guimarães ALS (2018) Treatment of mucositis with combined 660- and 808-nm-wavelength low-level laser
therapy reduced mucositis grade, pain, and use of analgesics: a parallel, single-blind, two-arm controlled study. Lasers Med Sci 33(8):1813–1819. https://doi.org/10.1007/s10103-018-2549-y

29. Zecha JA, Raber-Durlacher JE, Nair RG, Epstein JB, Elad S, Hamblin MR, Barasch A, Migliorati CA, Milstein DM, Genot MT, Lansaat L, van der Brink R, Arnabat-Dominguez J, van der Molen L, Jacobi I, van Diessen J, de Lange J, Smeee LE, Schubert MM, Bensadoun RJ (2016b) Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 2: proposed applications and treatment protocols. Support Care Cancer 24(6):2793–2805. https://doi.org/10.1007/s00520-016-3153-y

30. Migliorati C, Hewson I, Lalla RV, Antunes HS, Estilo CL, Hodgson B, Lopes NN, Schubert MM, Bowen J, Elad S, Mucositis study group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO) (2013) Systematic review of laser and other light therapy for the management of oral mucositis in cancer patients. Support Care Cancer 21(1):333–341. https://doi.org/10.1007/s00520-016-1605-6

31. Zadik Y, Arany PR, Fregnani ER, Bossi P, Antunes HS, Bensadoun RJ, Gueiros LA, Majorana A, Nair RG, Ranna V, Tissing WJE, Vaddi A, Lubart R, Migliorati CA, Lalla RV, Cheng KKF, Elad S, Mucositis study group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO) (2019) Systematic review of photobiomodulation for the management of oral mucositis in cancer patients and clinical practice guidelines. Support Care Cancer 27(10):3969–3983. https://doi.org/10.1007/s10103-019-04890-2

32. Antunes HS, Herchenhorn D, Small IA, Araújo CMM, Viégas CMP, de Assis RG, Dias FL, Ferreira CG (2017) Long-term survival of a randomized phase III trial of head and neck cancer patients receiving concurrent chemoradiation therapy with or without low-level laser therapy (LLLT) to prevent oral mucositis. Oral Oncol 71:11–15. https://doi.org/10.1016/j.oraloncology.2017.05.018

33. Bezinelli LM, de Paula EF, da Graça Lopes RM, Biazevic MG, de Paula EC, Correa L, Hamerschlak N, Michel-Crosato E (2014) Cost-effectiveness of the introduction of specialized oral care with laser therapy in hematopoietic stem cell transplantation. Hematol Oncol 32(1):31–39. https://doi.org/10.1002/hon.2050

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