

Long-term survival of cancer patients after photobiomodulation therapy for prevention and treatment of oral mucositis

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ABSTRACT

Background: Photobiomodulation therapy (PBMT) has been employed for the prevention and treatment of oral mucositis in patients with head and neck cancer undergoing treatment. However, information about the survival of patients receiving PBMT is still lacking. Therefore, the aim of this study was to correlate the survival of patients who either received or did not receive PBMT, combined with cancer treatments, and to assess whether the number of PBMT sessions had a positive impact on survival.

Methods: The records of 751 patients seen at Hospital de Caridade São Vicente de Paulo diagnosed with head and neck cancer, treated by D.L.P., the sole operator of PBMT, between 2000 and 2016, were analyzed. The median survival rates were compared by analyzing diagnostic staging, the combination of cancer treatments, and the number of PBMT sessions. Correlation analyses were performed to assess the interactions between the number of PBMT sessions and survival.

Results: PBMT increased survival by 55.14 %, from 1.07 (Without PBMT) to 1.66 years (With PBMT) for the total population and more significantly in Group 41 (stages III and IV, radiation therapy/chemotherapy, with more than five PBMT sessions), the median survival value of 1.83 years, which represents a 66.36 % higher survival rate. In all the analyzed populations, the larger number of sessions was associated with better survival. In terms of survival, advanced stages III and IV almost equated with stages I and II in the absence of PBMT.

Conclusions: PBMT seems to interfere with the survival of patients with head and neck cancer, corroborating its recommendation for the treatment of mucositis, but further research is needed to investigate possible synergistic effects between PBMT and cancer treatments.

1. Introduction

Surgery, radiation therapy, and chemotherapy have been predominantly used for the treatment of head and neck cancer [1]. Other treatment approaches, such as photodynamic therapy [2,3,4,5,6], immunotherapy [6,7], targeted therapy [8] and gene therapy [9] have been employed to a lesser extent. Damage induced by head and neck radiation therapy and chemotherapy can have major side effects in the orofacial region, including mucositis, xerostomia, ageusia, candidosis, fibrosis, edema, osteonecrosis, osteoradionecrosis, radiodermatitis,

trismus, muscle contracture, and paresthesias [10].

Oral mucositis is one of the major complications caused by the main treatments for the head and neck cancer, which are radiation therapy and chemotherapy. It also occurs in chemotherapy used in conditioning process for hematopoietic stem cell transplantation. These complications significantly worsen patients' quality life. Clinically, the mucous membrane appears reddish at first and then single ulcers emerge as the condition progresses, and these ulcers may eventually coalesce. Patients report burning sensation, pain, and difficulty swallowing, feeding, and speaking. A weak immune system, poor oral hygiene, and biofilm

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accumulation lead to opportunistic infections, which can compromise the patient's systemic health, considering that oral ulcers serve as portals of entry for opportunistic microorganisms. In more severe cases, cancer treatment must be discontinued to treat these complications and allow the patient to recover, resulting in a worse prognosis. Therefore, oral mucositis can have an impact on the survival rates of patients receiving cancer treatment [11].

There is a lack of consensus in the literature on basic oral care strategies for the prevention and treatment of oral mucositis. The Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society for Oral Oncology (MASCC/ISOO) recommends the implementation of multi-agent combination oral care protocols. These protocols consist of a multifaceted approach to oral hygiene, such as toothbrushing with a soft-bristled toothbrush, regular replacement of toothbrush, flossing, and the use of bland rinses and moisturizers [12]. In addition, for the management of mucositis secondary to cancer therapy, the MASCC/ISOO recommends oral cryotherapy (for specific melphalan and 5-FU protocols), anti-inflammatory agents (benzydamine mouthwash), antimicrobials, coating agents, anesthetics, and analgesics (morphine 0.2 % mouthwash), growth factors, natural and miscellaneous agents (honey and oral glutamine), and photobiomodulation therapy (PBMT) [13].

Photobiomodulation therapy (PBMT) employs a light source (e.g., low-level laser) in the red or infrared wavelength. This technique has demonstrated remarkable efficacy in the prevention and treatment of oral mucositis [14,15,16]. The fundamental effects of PBMT on the irradiated tissue include analgesia, tissue repair, and modulation of the inflammatory process. Moreover, PBMT can also modulate the immune system [17,18].

Recently, some researchers have reported that the use of PBMT for the prevention and treatment of oral mucositis in patients undergoing head and neck radiation therapy has been associated with an increase in the survival of cancer patients [19]. Also, the concurrent use of PBMT and radiation therapy (RT) in animal models has potentiated the effects of this cancer treatment [20]. Therefore, PBMT can act as a radiosensitizing procedure for neoplastic cells and protection of normal cells from radiation [21]. Decreased tumor growth, improved clinical conditions, reduced metastatic spread and, consequently, enhanced survival rates have also been observed [22].

As evidenced, PBMT has been utilized for many decades for the prevention and treatment of oral mucositis in patients undergoing cancer treatments or conditioning therapy before bone marrow transplantation. Nevertheless, data on the survival rates of patients subjected to PBMT are still lacking in the literature. In the long term, the aim of this retrospective descriptive observational study was to correlate the survival rates of patients treated and not treated with PBMT combined with cancer treatments and to examine whether the number of PBMT sessions prolonged the lives of these patients.

2. Materials and methods

This study was approved by the Research Ethics Committees of the Faculty of Dentistry at the University of São Paulo (FOUSP) and of the Hospital de Caridade São Vicente de Paulo (HCSVP Jundiaí – São Paulo - Brazil). The methodology used in the study sought to verify whether PBMT had an impact on the survival rates of patients who either received or did not receive PBMT.

2.1. Data collection

This research is a descriptive observational study. It was based on the clinical activity over a long period that generated data in the hospital system, making its prior design and execution not possible as in a study of completely controlled conditions. In this type of study, the database was not originally developed for the specific purpose of the research.

The study material was obtained from the database of HCSVP

Jundiaí, comprising 1094 male and female patients diagnosed and treated between 2000 and 2019. The dataset included anatomical sites of the cancer (topography - codes C000 to C148) and cell type of each cancer, histological diagnosis (morphology - codes 80,001 to 99,893) based on the International Classification of Diseases (ICD) for Oncology [23].

The data were exported to an Excel database, tabulated, and consolidated with each patient's medical record number, name, date of diagnosis, morphology, diagnostic stage, type of cancer treatment administered, sex, age at diagnosis, patient's last record in the system (indicated as death), years of survival, and number of PBMT sessions performed. Duplicate data were removed. A filter was applied to select the records of patients diagnosed with head and neck cancer between 2000 and 2016 and whose treatment had been recorded in the system until 2019. The data were then sorted alphabetically by patient names and numbered from 1 to 751, generating a database referred to as the total study population. This database was used to analyze patients who received and did not receive PBMT.

Original names and medical record numbers were discarded. From 2000 to 2010, PBMT was not administered to treat oral mucositis. From 2010, the hospital's Department of Oral Medicine offered photobiomodulation therapy to patients with head and neck cancer, which was carried out by D.L.P., the only operator of the technique.

2.2. PBMT and oral care

The PBMT conducted from 2010 to 2016 utilized a low-level semiconductor diode laser (gallium aluminum arsenide; GaAlAs): Photon Laser III (DMC→), emitting a total of 30–50 mW, with a fiber contact area of 0.059 cm², resulting in an intensity ranging from 0.5 to 0.8 W/cm². The laser was administered at either 660 or 808 nanometers (nm), with doses ranging from 0.6 to 3 joules (10–50 J/cm [2]) and application for 20 to 60 s per point. The treatment was applied to all areas affected by mucositis, three times a week. The 660 nm wavelength was applied every one cm and the 808 nm wavelength every 1.5 cm. The most used wavelength was 660 nm due to the clinical observation of better results in continued PBMT applications. The 808 nm wavelength was little used, only in extreme pain conditions and when the ulcers were deeper. The PBMT was applied to HCSVP patients based on parameters obtained at conferences in the area, experience in other hospital care center that it can find in more update reference [24,25].

This research was based on data from a real, routine hospital service. Patients diagnosed with head and neck cancer, treated with PBMT or not, came from different professionals, sectors and origins like basic healthy unit, medical schools, hospital's own emergency room, private clinics from Jundiaí and other cities. There was no single standardized protocol for oral care.

2.3. Descriptive statistics

Measures of central tendency (years of survival), dispersion (survival in relation to patient age), and distribution (relative and absolute frequencies) were employed for histological diagnosis, sex, anatomical sites, staging at diagnosis, age range, number of individuals per survival internal, and number of PBMT sessions and cancer treatments.

2.4. Statistical studies

For statistical analyzes were used the programs Excel R programming language, RStudio console and Jamovi.

In the first phase focused on studying the influence of PBMT on the survival of patients in the total population, the second method investigated the impact of PBMT on the survival of specific groups within the studied population based on different combinations of other variables of interest in addition to PBMT using factorial median crossings between all possible combinations variables groups - cancer treatments,

diagnostic stage, PBMT therapy or not and PBMT sessions number. For this purpose, the R-Kolmogorov-Smirnov Normality Test was applied to characterize the distribution of the studied population in terms of normality [26], the RWilcoxon Rank Sum Test (Mann-Whitney test) to compare the measures of central tendency between two groups of paired data (single pair) and Kruskal-Wallis test (Dunn’s post-hoc test using Bonferroni-adjusted p value) to compare several paired groups.

In the second phase, the Mann Whitney test was performed to study survival in the comparison between early stages (I and II) and late stages (III and IV), with and without PBMT, < or >= 5 PBMT sessions. A survival study was carried out for the period from 2010 to 2016, using the Mann-Whitney test comparing measures of central tendency for survival between populations with PBMT and without PBMT following for logistic regression including anatomical sites, measuring the effect of each variable selected individually on survival, concluding with specific univariate and multivariate studies for survival like Kaplan-Meier and Cox regression.

2.4.1. Total population

Comparison of the medians for individuals treated and not treated with PBMT. Considering the non-normal distribution of the data within the total population of 751 individuals, the Wilcoxon rank sum test (Mann-Whitney test) non-parametric method was used to compare the medians and assess the range of values.

2.4.2. Analysis of specific groups according to combinations of variables

All possible combinations were performed among the variables: a) treated and not treated with PBMT; b) those who received fewer than five sessions or five sessions or more of PBMT; c) diagnostic stage (early – I and II, and advanced– III and IV); d) cancer treatments (surgery only; radiation therapy only; chemotherapy only; surgery (plus) + chemotherapy + radiation therapy; surgery + radiation therapy; surgery + chemotherapy; chemotherapy + radiation therapy), yielding 56 combinations denoted as Group 1 (G1) through Group 56 (G56), each representing a study group (Fig. 1).

Using the Kruskal-Wallis method with Dunn’s post-hoc test and Bonferroni-adjusted p value, the median of each group was compared with the medians of the remaining 55 groups. Pairs with numerical differences between their medians and an adjusted p value less than 0.05 were selected for separate comparative analysis.

A correlation analysis was performed between the number of PBMT sessions and survival of individuals treated with PBMT in both the total

population and in the selected groups of G1 to G56. This analysis aimed to assess the impact of the number of PBMT sessions (independent variable) on survival (dependent variable) using linear regression method of Spearman’s correlation and the correlation coefficient (Rho) with its corresponding scatter plots and regression lines.

2.4.3. Study of survival gain in interactions between PBMT and early stages (I and II) and advantages stages (III and IV)

Two groups of individuals were obtained from the total population: one labeled as “early-stage” (stages I and II) and one labeled as “advanced-stage” (stages III and IV). Within each of these groups, subgroups of individuals were formed based on the possible PBMT modalities: a) not treated; b) treated regardless of the number of sessions; and c) treated according to the number of sessions (fewer than five sessions or five or more sessions). A pairwise comparison of the possible combinations between these groups and subgroups was made, taking account the numerical and statistical differences in their median survival, focusing on the survival gain of one group in comparison to another. Those pairs with greater survival gains associated with significant clinical observations were selected for the analysis.

2.4.4. Study of survival for period with PBMT only

A survival study was carried out for the period from 2010 to 2016, using the Mann-Whitney test comparing measures of central tendency for survival between populations with PBMT and without PBMT.

2.4.5. Introduction of new methods to validate the results obtained

It was introduced the method multivariate logistic regression including histological diagnosis and anatomical sites, measuring the effect of each variable individually on survival, ending with specific univariate and multivariate studies for survival based on Kaplan-Meier Cox regression.

2.5. Characteristics of the total population

The following variables were assessed in this study: age, sex, histological diagnosis, anatomical sites, staging, cancer treatments, and PBMT.

Age at diagnosis: Age ranged predominantly from 41.3 to 76.7 years, accounting for 88.42 % of the total sample.

Sex: Approximately 12 % of the cancer patients were female and 88 % were male, indicating a sevenfold higher incidence of head and neck

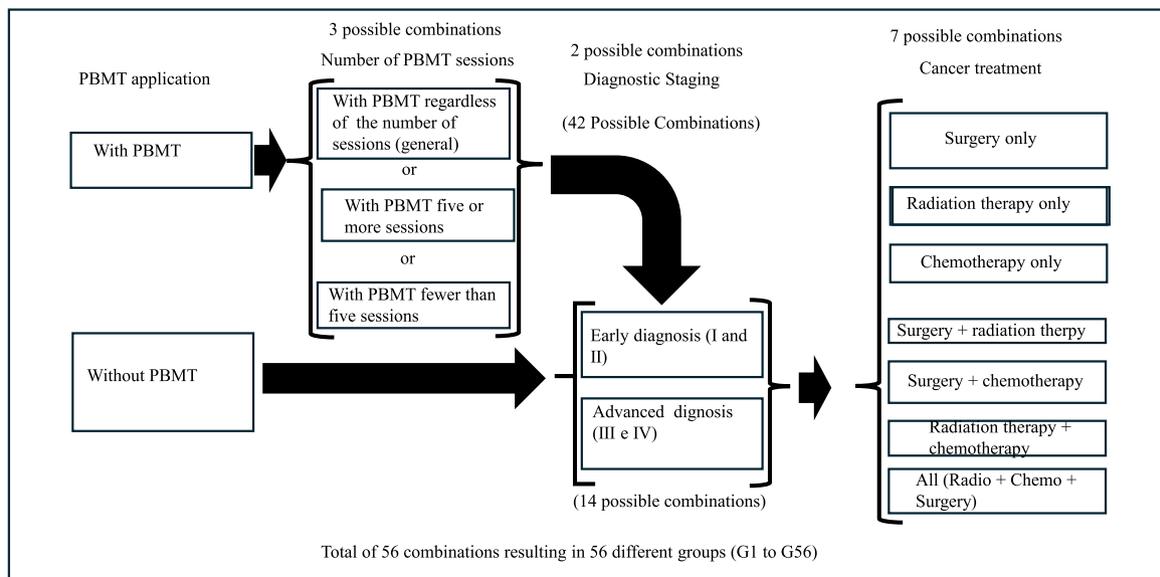


Fig. 1. Interaction of PBMT with groups of interest formed by different combinations of variables.

cancer among males. PBMT was applied to female patients, 4 % and male patients, 30 %.

Histological Diagnosis: Approximately 86 % of individuals from the total population developed squamous cell carcinoma (Not Otherwise Specified - NOS). Among these, approximately 33 % received PBMT. The remaining histological diagnosis account for almost 14 % of the individuals (Fig. 2).

Anatomical sites: The oral cavity and the oropharynx account together for approximately 45 % of all diagnosed cases. The remaining topographies include the larynx, hypopharynx, nasopharynx, and parotid gland, representing approximately 55 %. PBMT was applied to the oral cavity (mouth, floor of the mouth, tongue) in 6.5 % of the patients, and to the oropharynx (posterior tongue, tonsils, soft palate, hypopharynx, and larynx) in 11.8 %. Patients with cancer of the oral cavity and oropharynx were those who most frequently received PBMT, representing nearly 20 % of the total.

Staging: Advanced stages of cancer (III and IV) were observed in approximately 74 % of individuals from the total population, while early stages of cancer (I and II) affected around 16 % of individuals. Considering the last more advanced stage of cancer and earliest stage, nearly 60 % of participants were diagnosed at stage IV and 6 % at stage I. There was a 1:2 ratio for patients who received PBMT and those who did not receive it, regardless of staging.

Cancer treatments and PBMT: The relative frequencies of each cancer treatment were compared with the total number of procedures performed. The frequencies were approximately 42 %, 37 %, 16 %, and 5 % for radiation therapy, chemotherapy, surgery, and no treatment, respectively. Additionally, the relative frequencies of cancer treatments conducted exclusively or in combination were analyzed in relation to the total number of treated individuals, taking into account whether PBMT was performed.

The cancer treatments used and the percentage of treated participants in relation to the total population were ranked in decreasing order as follows: Radiation therapy + chemotherapy (RT + CT) without PBMT (25.43 %) and with PBMT (17.98 %), amounting to 43.41 %; Surgery + Radiation therapy + Chemotherapy (S + RT + CT) without PBMT (9.45 %) and with PBMT (6.13 %), amounting to 15.58 %; Radiation therapy without PBMT (7.59 %) and with PBMT (3.06 %), amounting to 10.65 %; Chemotherapy without PBMT (6.79 %) and with PBMT (1.07 %), amounting to 7.86 %; Surgery + Radiation therapy (S + RT) without PBMT (3.73 %) and with PBMT (2.93 %), amounting to 6.66 %; Surgery (S) without PBMT (3.86 %) and with PBMT (1.46 %), amounting to 5.32 %; and Surgery + Chemotherapy (S + CT) without PBMT (1.73 %) and with PBMT (0.13 %), amounting to 1.86 %. The remaining 8.66 % of the patients did not receive any cancer treatment because of death or

withdrawal prior to the sessions and procedures.

2.6. Survival study

Individuals as per age and number of PBMT sessions:

Approximately 90 % of the patients survived up to 5.5 years, while the remaining 10 % survived from 5.5 to 15 years. Most of these individuals were aged 40 to 80 years (Table 1A and Fig. 3). As for the number of PBMT sessions, 73 % underwent up to 12 sessions, while the remainder of the patients underwent 13 to 46 sessions (Table 1B).

Given the non-normal data distribution according to the R Lilliefors test (Kolmogorov-Smirnov normality test) applied to the total population, with p value = 2.20E-14 < 0.05, and the following hypotheses: H0 – normal distribution; H1 – non-normal distribution, H0 rejected and H1 accepted, nonparametric methods were used, and the medians of the populations treated and not treated with PBMT were compared. The data distribution pattern was quite similar for the total population with and without PBMT. Their respective histograms and distribution curves show the same pattern, number of classes and shape, ruling out the possibility that the differences between their medians could be due to other factors than those associated with the investigated treatments, e.g., in case of comparison of populations with different origins, procedures, and sampling characteristics (Figs. 4 and 5).

Based on the R Wilcoxon rank sum test (Mann-Whitney test) applied to populations treated and not treated with PBMT, we observed that the median for the population treated with PBMT significantly differed from the population without PBMT (p value = 1.45E-09 < 0.05, assuming hypothesis H1 – different medians). The median values for survival were 1.66 years for populations who received PBMT and 1.07 years for those who did not receive it. Numerically, in absolute terms, survival gain amounted to 0.59 years, while in relative terms, the survival gain was 55 % for the population treated with PBMT as compared to the population not treated with PBMT. In the histograms where the areas are delineated by their respective data distribution curves, we graphically perceive these differences - how they are distributed and where they are concentrated, in the comparison between the populations treated and not treated with PBMT (Fig. 5).

Study of groups formed by variables: diagnostic stage, cancer treatments, application or not of PBMT and number of PBMT sessions.

Groups G 27 and G 41 were the only ones among the 56 formed groups that yielded statistically significant differences in the comparison between the medians according to the Kruskal-Wallis test with Dunn's post-hoc test and a more stringent Bonferroni-adjusted p value. The adjusted p value was less than 0.05, which indicates an adequate n (number of individuals) for detection by the test and a statistically

	PBMT Total Population		
	With	Without	Sum
1 Squamous cell carcinoma (WOS)*	29,70%	56,72%	86,42%
2 Undifferentiated carcinoma (WOE)	0,67%	1,20%	1,87%
3 Carcinoma (WOS)	0,00%	1,20%	1,20%
4 Carcinoma in situ (WOS)	0,27%	1,07%	1,34%
5 Adenocarcinoma (WOS)	0,00%	0,93%	0,93%
6 Adenoid cystic carcinoma	0,27%	0,80%	1,07%
7 Squamous cell carcinoma in situ	0,53%	0,53%	1,06%
8 Lymphoepithelial carcinoma	0,53%	0,00%	0,53%
9 Acinar cell carcinoma	0,00%	0,40%	0,40%
10 Mucoepidermoid carcinoma	0,27%	0,40%	0,67%
11 Inmunoblastic lymphoma malignant (WOS)	0,40%	0,00%	0,40%
12 Papillary adenocarcinoma Intraductal with invasion	0,13%	0,00%	0,13%
Sum 12 with higher incidence	32,77%	63,25%	96,02%
Remaining of the 22 other histological diagnoses			3,98%
Total			100,00%
* Without other specification			

Fig. 2. Histological diagnosis of total population with neck and head cancer.

Table 1
Relative frequency of individuals from the total population as per.

A) Survival interval (in years)												
Intervalo	(0.1 - 1.39]	(1.39 - 2.77]	(2.77 - 4.16]	(4.16 - 5.55]	(5.55 - 6.93]	(6.93 - 8.32]	(8.32 - 9.7]	(9.7 - 11.1]	(11.1 - 12.5]	(12.5 - 13.9]	(13.9 - 15.2]	Total
%	56,46%	19,84%	7,72%	5,06%	5,06%	2,26%	0,93%	0,93%	0,67%	0,53%	0,53%	100,00%
soma %	89,08%				10,92%							

B) Interval for number of sessions												
Intervalo	(1 - 4]	(5 - 8]	(9 - 12]	(13 - 17]	(18 - 21]	(22 - 25]	(26 - 30]	(31 - 33]	(34 - 38]	(39 - 42]	(43 - 46]	Total
%	30,95%	24,21%	18,25%	11,90%	7,54%	3,97%	1,98%	0,40%	0,40%	0,00%	0,40%	100,00%

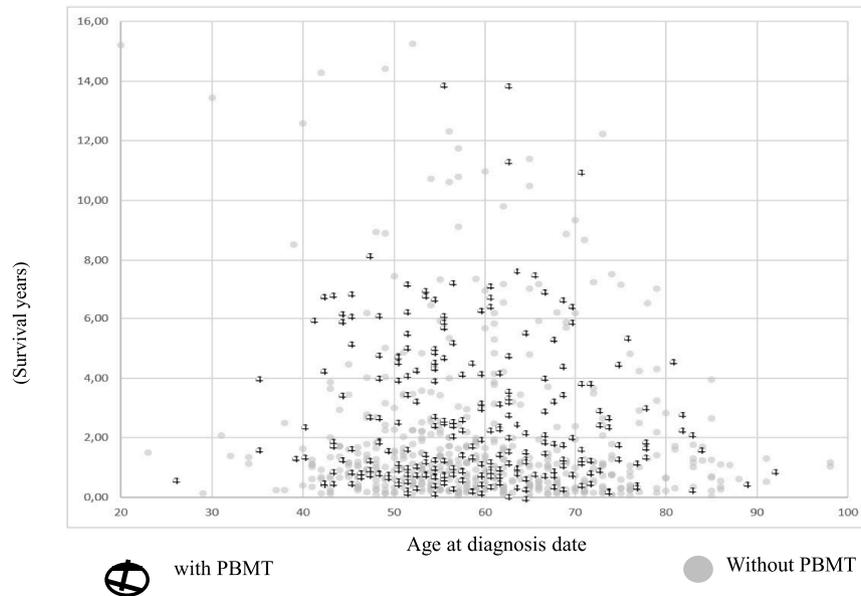


Fig. 3. Dispersion of the total population - age at diagnosis date x survival in years.

significant difference between the medians. The comparison between the respective histograms and data distribution graphs shows similar patterns, corroborating that the differences in the medians are due exclusively to the effect of combination of the variables analyzed in each group. The median survival for G41 was 1.83 years, compared to 1.1 for G27, representing 0.73 years (66.36 %), the largest median survival. The variable that differed in these two groups was the application of PBMT with five or more sessions in G41, confirming the tendency observed for the total population of individuals treated with PBMT (Fig. 6).

Correlation analysis between number of PBMT sessions and survival of the total population who received PBMT:

A positive correlation was observed in the linear correlation analysis of populations treated with PBMT, according to Spearman's Rho test and its respective lines superimposed on the scatter plots ($p < 0.001/\rho = 0.307$ for the total population and $p < 0.001/\rho = 0.319$ for G 41. The number of sessions (independent variable) had a positive impact on the survival of individuals treated with PBMT (dependent variable) (Fig. 7).

In G 41 (Fig. 8), the survival of patients treated with PBMT, expressed by medians, increased as the patients underwent a larger number of sessions, and not the other way around, as the interval between the number of sessions contained individuals from all survival ranges, including those who survived less and those who survived much longer. If the patients who underwent more sessions had received PBMT for having survived longer, and not the other way around, there would be a larger concentration of patients with shorter survival on the intervals with a smaller number of sessions, and those with better survival rates would be found on the intervals with a larger number of sessions.

Study of survival gain in interactions between PBMT and early stages (I and II) and advanced stages (III and IV).

Fig. 9 shows that the positive effect of PBMT mitigated the negative effect of the worst prognosis (stages III and IV), equating it with the best prognosis (stages I and II) in the presence of five or more sessions of PBMT. In other words, whereas the population without PBMT (stages I and II) had a survival of 0.65 years (59.09 %) more than those not treated with PBMT (stages III and IV), which was statistically significant, as expected, the population without PBMT (stages I and II) had a similar survival to that of the group that received PBMT (stages III and IV) ≥ 5 sessions.

Study of some variables for stages I, II, III and IV using multivariate binomial logistic regression (Fig. 10)

Survival study for the total population of individuals with oral and oropharyngeal cancer

In this study, showed in the Fig. 11, we evaluated survival in relation to PBMT using the univariate Kaplan Meier method, a renowned method for studying survival that assigns different weights to individuals who do not have confirmation of the 'death' event in the period studied (those censored). This study was carried out to confirm or not the first phase of the research in which we carried out the survival study for the population with PBMT and without PBMT, applying the Mann Whitney test (univariate method). The positive influence of PBMT on survival is maintained in the results in all methodologies, regardless of whether we consider the total population in which all cancer locations are included or when we study the influence of PBMT on survival only in relation to the locations where the light aimed at to treat mucositis, it was applied

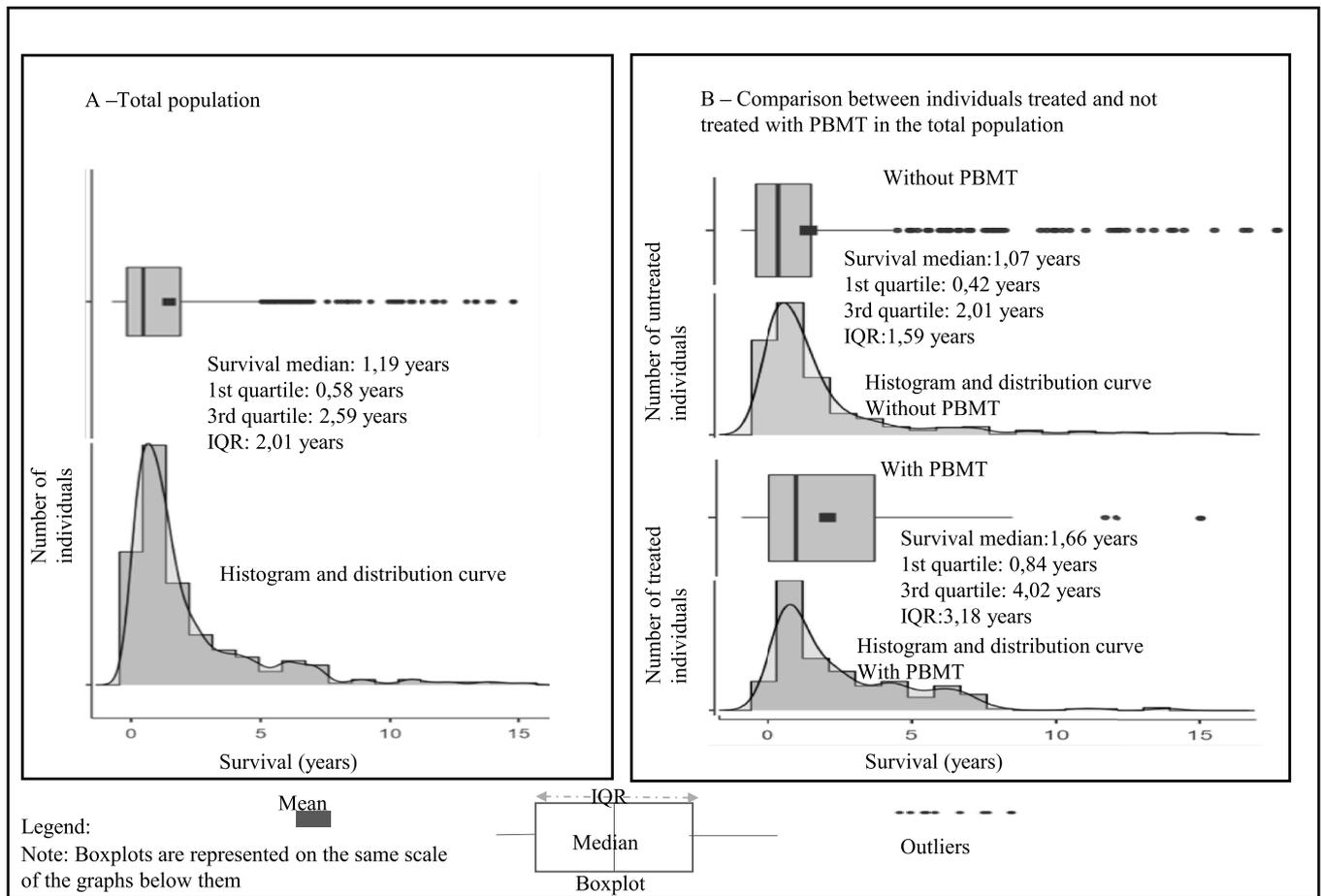


Figure 4. Comparative survival study between individuals treated and not treated with PBMT in the Total population, with comparison of their medians.

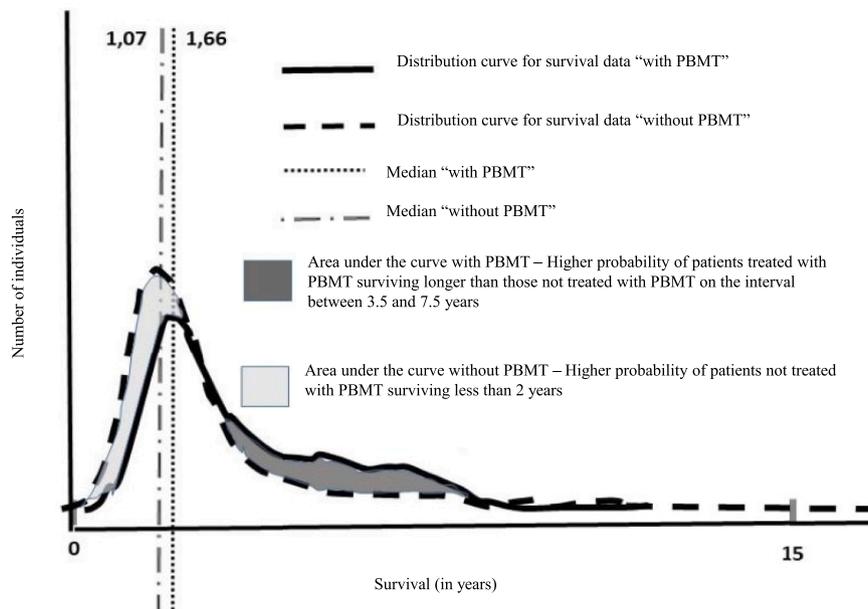
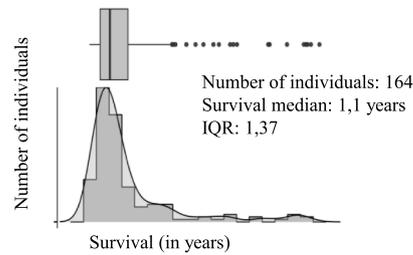


Fig. 5. Superimposed areas under the distribution curves for survival data on the populations treated and not treated with PBMT.

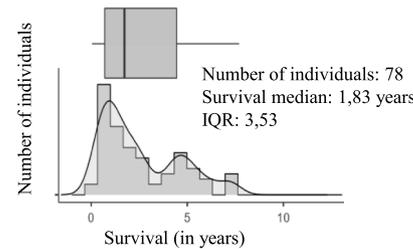
close to a neoplastic lesion in the oral cavity and/or oropharynx.
 Survival study in relation to some variables with the multivariate Cox Regression method for the population treated with PBMT The Fig. 12:

1) Compare survival within the population treated with PBMT, groups of individuals treated with more than 5 sessions (1) and less than 5 sessions (0), using the Kaplan Meier (Univariate) and Cox Regression (multivariate) method. Survival was higher for those who

Group G 27 – Advanced stage (III and IV), Radiation therapy + Chemotherapy, without PBMT



Group G 41 – Advanced stage (III and IV), Radiation therapy + Chemotherapy, with PBMT five or more sessions



Kruskal-Wallis test with Dunn's post-hoc test and p value adjusted by Bonferroni correction; adjusted p value = 0,04012368; significant difference between the medians at a 5% probability.

Fig. 6. Pair of groups formed by variables of interest with statistical difference in their medians.

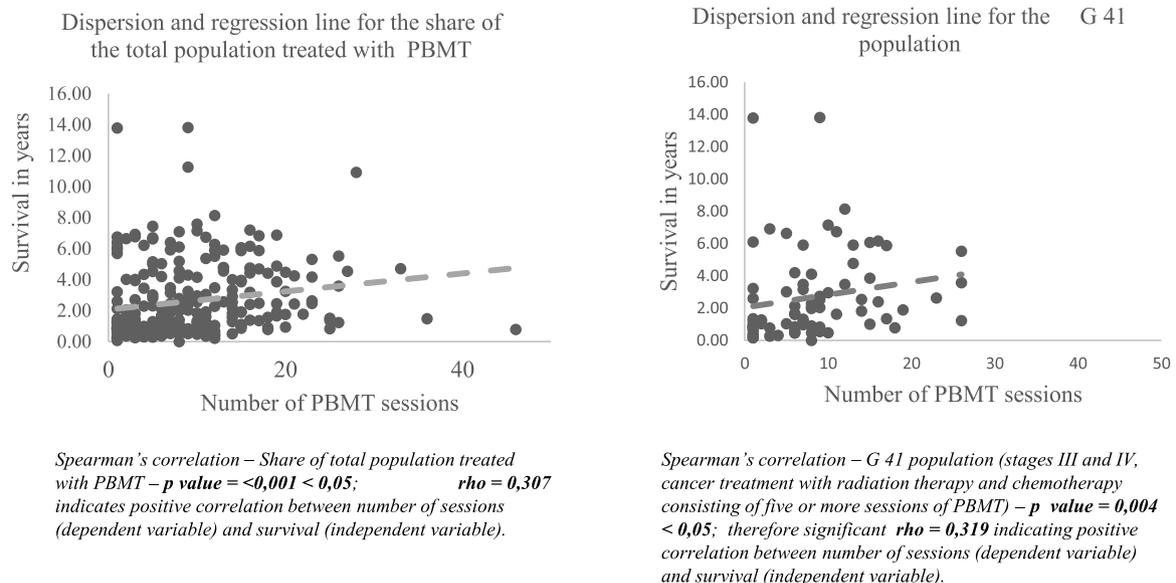


Fig. 7. Linear correlation analysis.

received more than 5 PBMT sessions. This result was repeated when we ran the multivariate factorial method to compare groups (56 × 56) and linear regression, Spearman's correlation.

- 2) It presents the variables PBMT and stage diagnosis, which were among the variables studied (PBMT, stage diagnosis, age and oncological treatments) the only variables that showed significance in relation to survival. These methods used show that PBMT and diagnostic staging had greater strength. They are the variables that can alter survival.

3. Discussion

Approximately 700,000 new cases of head and neck cancer occur worldwide every year, which corresponds to around 4.5 % of the number of cancer cases in relation to all cancers. In Brazil, there is an

estimate around 40,000 new cases of head and neck cancer per year and 904,980 for all types of cancer, which corresponds to 4.41 % of the number of cancer cases in relation to all cancers [27,28,29]. For some authors, [30,31] low socioeconomic development, limited access to education, alcoholism and tobacco are some of the reasons that may justify the higher incidence of head and neck cancer in some countries. The patients with head and neck cancer treated at HCSVP in Jundiá (Brazil) share common characteristics with those treated at other centers. Most patients are male, possibly for some authors due to greater consumption of tobacco and alcohol [30] aged 40 to 80 years; most frequently in the oral cavity and oropharynx with predominance of diagnosis squamous cell carcinoma, stages III and IV; and surgery, radiation therapy, and chemotherapy, either as single treatment or combined [31].

Cancer patients have numerous orofacial complications because of

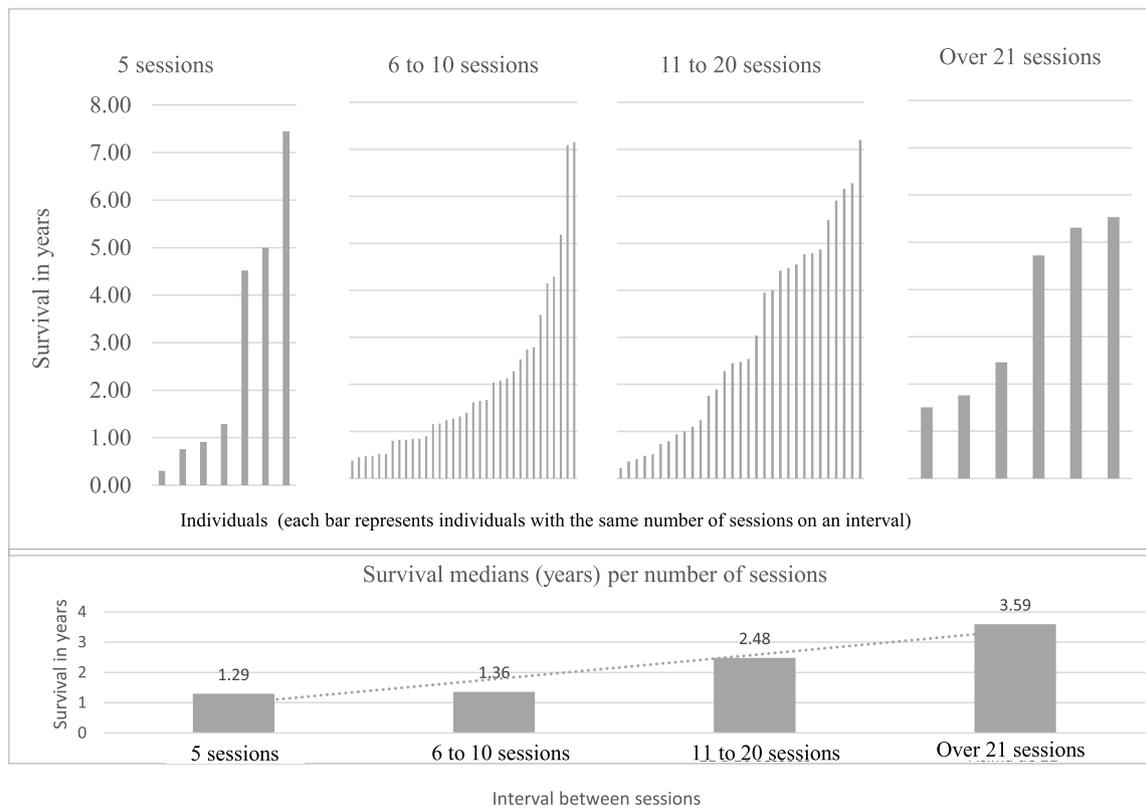


Fig. 8. Survival distribution of individuals from the G 41 population relative to the number of PBMT sessions.

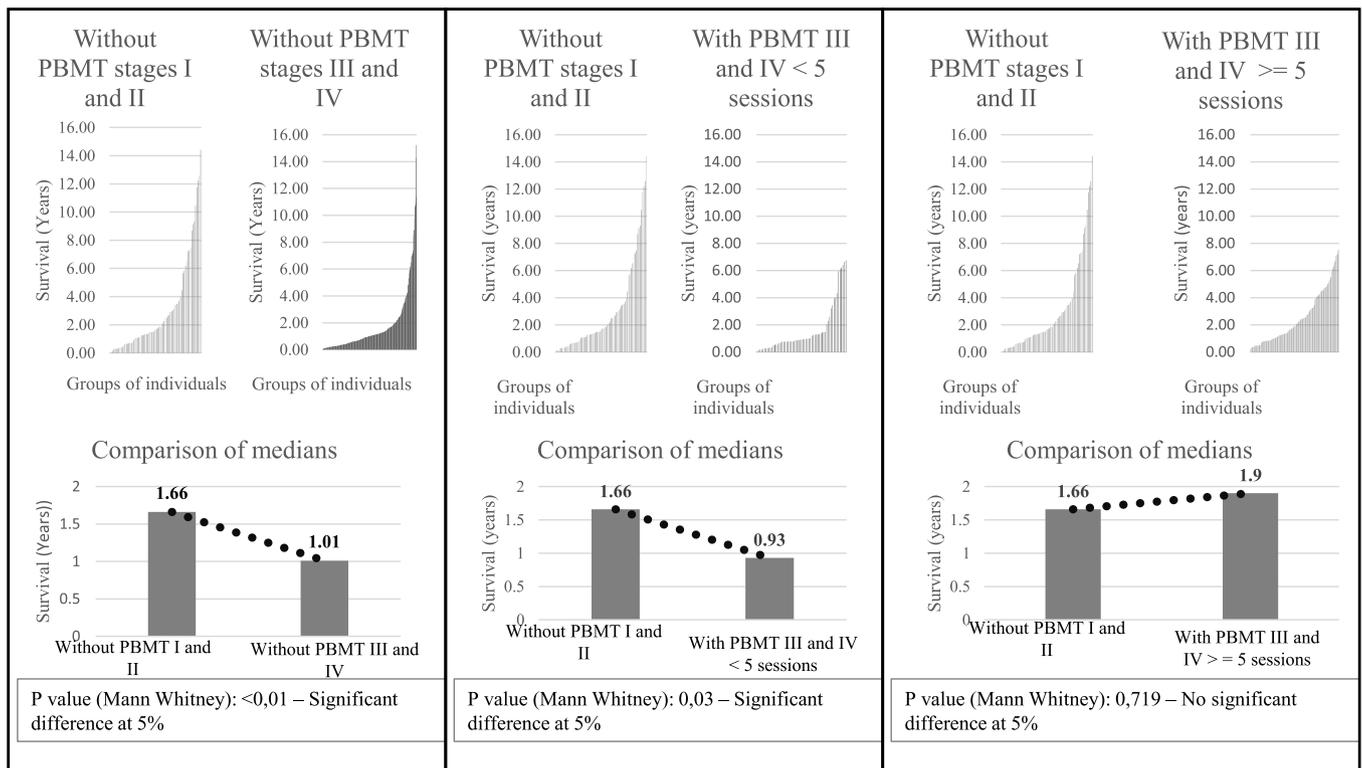
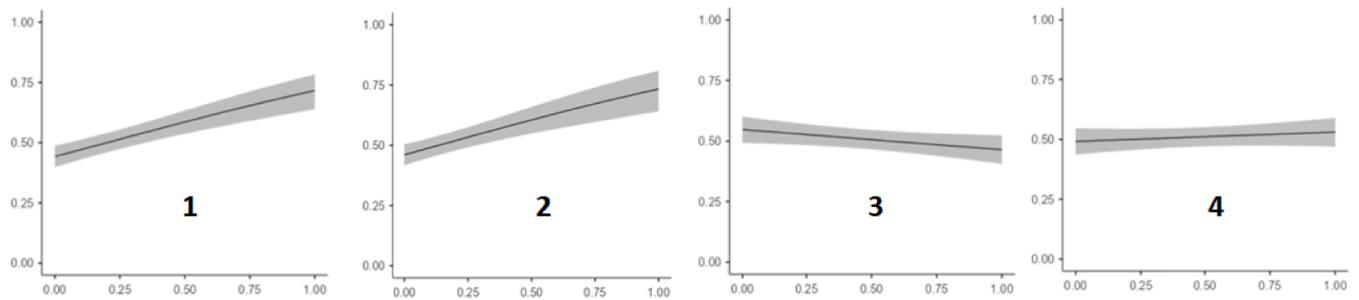


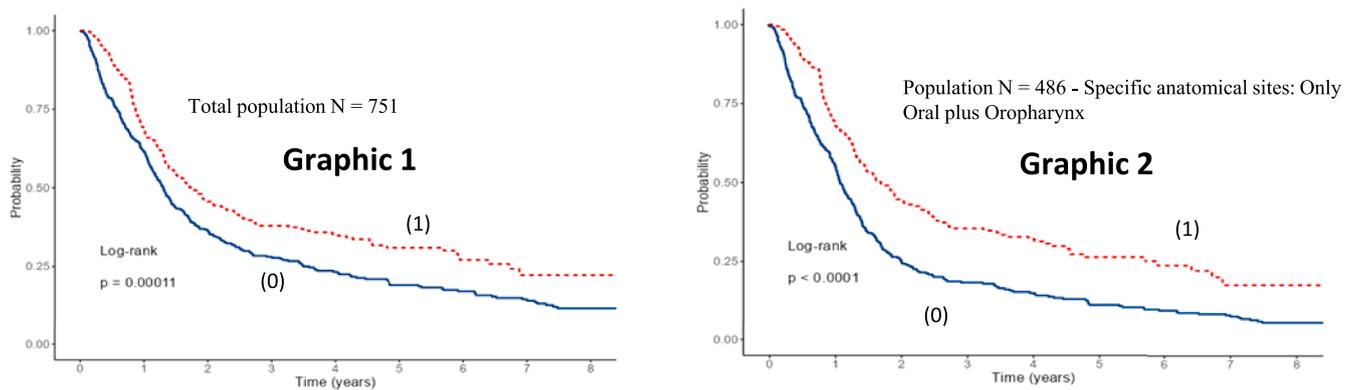
Fig. 9. Survival gain in interactions between PBMT and early (I and II) and advanced (III and IV) stages.



Graphic	Legend	p	Significance (95%)	Odds Ratio	Interpretation
1	PBMT sessions - Less 5 (0) x 5 or more (1)	<0.001	High significance	3.179	The predictor 5 or more PBMT sessions (1) has a survival probability 3.179 times higher than less 5 PBMT sessions (0)
2	Advanced stage (III and IV) (0) x Early stage (I and II) (1)	<0.001	High significance	3.215	The predictor Early stage (I and II) (1) has a probability survival probability 3.215 times higher than advanced stage (III e IV) (0)
3	Staged age years less 60 (0) x 60 or more (1)	0.042	Low significance (close p < 0.05)	0.716	The predictor staged with less 60 years (0) has a survival pobability 0.284 times higher than staged with 60 or more years (1)
4	RT (Radiotherapy) + QT (Chemotherapy) (1) x Cancer therapeutic scheme remaining (S - Surgery, RT, QT, S+R, S+RT+QT) (0)	0.363	Not Significant	1.16	The predictor RT+QT (1) has not a survival probability higher than remaining cancer therapeutic scheme (S, RT, QT, S+R, S+RT+QT) (0)

Fig. 10. Multivariate binomial logistic regression in the population composed of I, II, III and IV diagnostic stages.

Survival Curves for With (1) x Without (0) Based on Kaplan – Meier estimates



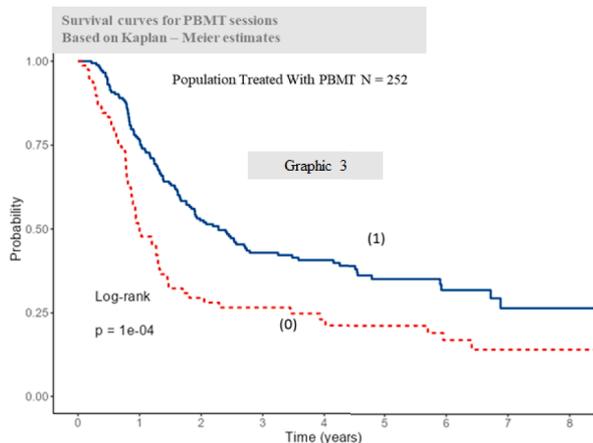
Graphic	Legend	Median		Median Kaplan - Meier		p Kaplan - Meier	Significance (95%)	Hazard Ratio	Survival Ratio		Interpretation
		Without PBMT (0)	With PBMT (1)	Without PBMT (0)	With PBMT (1)				Without PBMT (0)	With PBMT (1)	
1	Without PBMT (0) x With PBMT (1)	1.07	1.65	1.29	1.76	<0.0001	High Significance	0.7	5.7%	22.2%	Based on Hazard Ratio: with PBMT (1) has a survival probability 30% higher than without PBMT (0)
2	Whithout PBMT (0) x With PBMT (1)			1.08	1.7	<0.0001	High Significance	0.58			High significance 0.58 With PBMT (1) has a survival probability 42% higher than without PBMT (0)

Fig. 11. Survival study using specific methods - survival function estimator based on kaplan - meier (univariate) and Cox Regression (multivariate).

head and neck radiation therapy and chemotherapy. These complications compromise the quality of life of the patients, in addition to increasing their mortality. The most common complications include oral

mucositis, opportunistic infections, hyposalivation and xerostomia, and osteonecrosis, among others. In the past few decades, PBMT and photodynamic therapy have been used as important tools in the

Graphic	Legend	Median		Median Kaplan - Meier univariable		p Kaplan - Meier	Significance (95%)	Hazard Ratio	Survival Ratio		Interpretation
		less 5 PBMT sessions (0)	5 or more PBMT sessions (1)	Without PBMT (0)	With PBMT (1)				less 5 PBMT sessions (0)	5 or more PBMT sessions (1)	
3	5 or more PBMT sessions (1) X less 5 PBMT sessions (0)	0.965	2.23	1	2.28	1,00E-04	High Significance	1.85	14.2%	26.5%	Based on Hazard Ratio: PBMT with 5 or more sessions (1) has a survival probability 85% higher than less 5 PBMT sessions (0)
		N = 78	N = 174	N = 78	N = 174						



Effect of variables groups on a dependent variable survival	p	Significance (95%)	Hazard Ratio Multivariable	Interpretation
5 or more PBMT sessions (1) X less 5 PBMT sessions (0)	p<0.001	High Significance	1.96	For a variable number of PBMT sessions, patients undergoing 5 or more PBMT sessions (1) had a 96% higher survival probability than those undergoing less than 5 PBMT sessions (0)
Remaining stage (III, IV, X, Y, O) (0) X Early stage (I and II) (1)	p=0.001	High Significance	0.41	For the staging variable, the early-stage group (I and II) (1) has a 59% higher probability of survival than the remaining group of stage III, IV, X, Y, O) (0)

Conclusions: The effects of the variables number of sessions and diagnostic stage on survival are significant and equivalent. Note: X, Y and O refer to patients not staged, diagnosed and treated for cancer, and should therefore not be confused with patients that have "censored" data according to the Kaplan - Meier Cox methodology. The other available variables analyzed together (Multivariable HR and Univariable HR) not showed significant differences in terms of survival probabilities.

Fig. 12. Survival study with specific methods using Multivariable Cox Regression - Population treated with PBMT (N = 252).

management of these oral complications in patients subjected to cancer treatment and these therapies have been gaining ground multidisciplinary teams dealing with high complexity patients [32,33,34,35].

Given that laser irradiation is contraindicated in areas with neoplastic lesions, professionals are concerned about irradiating tumor cells that are not clinically visible. [36] In situations, for example, when we have a neoplastic lesion on the edge of the tongue and we are applying PBMT to the mouth floor, due to the scattering of light, it is possible to irradiate neoplastic cells. There is the same risk when we apply the laser to other areas with potentially cancerous lesions not clinically visible. There are few and controversial studies on the subject in the literature, so much so that some experimental studies have suggested that the application of laser could enhance the effect of cancer treatment, thus increasing the survival of irradiated patients. [19]

The present study demonstrated a positive relationship between PBMT and survival for both the total population and the specific group (G41), which included patients with stage III and IV cancer subjected to radiation therapy + chemotherapy who received five or more sessions of PBMT. Moreover, this study showed that the increase in survival was closely related to the larger number of PBMT sessions. The patients with more advanced cancer (stages III and IV) basically had the same survival of patients with early-stage cancer (stages I and II) when they underwent five or more PBMT sessions. It is possible that the number of sessions plays an important role. In our study, only patients who underwent more than five PBMT sessions had their survival increased. It is important to highlight that even though patients who underwent fewer than five PBMT sessions did not show an increase in survival, being that a single laser application can stimulate tissue repair and promote analgesia [39]. More sessions can provide enhanced control of pain, better tissue repair and, consequently, less severe mucositis [1].

Most of the patients in this study underwent radiation therapy + chemotherapy and received five to twenty PBMT sessions. However, further studies are needed to elucidate the role of the frequency and number of PBMT sessions, whether the application of PBMT before or after cancer treatment (radiation therapy and chemotherapy) has some influence, and what would be the best PBMT protocols in association

with cancer treatment protocols in order to increase survival.

The positive relationship between PBMT and survival might have occurred due to direct or indirect effects. The direct effects of PBMT might have occurred because of its capacity to make neoplastic cells remain in the same phase of the cell cycle, on which radiation therapy [37] and chemotherapy [38] would have a stronger effect. For example [20], in an in vitro study evaluating neoplastic cells subjected to PBMT and Radiation therapy, there was a greater number of neoplastic cells in G2/M of the cell cycle and the best result to kill these cells was with 2.4 Gy of ionizing radiation. In another in vitro study [21], ionizing radiation was applied for 60 min after the cells were pre-exposed to PBMT at energy densities [1 and 5 J/cm²] with 685 nm and 830 nm. The authors concluded that there was a possible radiosensitization effect at 685 nm in HeLa cancer cells with an energy density of 5 J/cm² and a radio-protection effect at 830 nm with [1 J/cm²] for normal NIH3T3 cells. The parameters of ionizing X-ray radiation from a linear accelerator were energy of 6 photons (Mev), total dose rate of 200cGy/min and dose of 2.4.6 Gy. The authors had more HeLa cell death when they used 6 Gy for 800 Hela cells. Regarding another in vivo study [37], the authors developed a radiotherapy method using a low-power laser device as a radiomodifier in 78 patients with local cancer (cutaneous, oral and oropharyngeal) comparing 83 patients as controls. The method adopted dynamic fractionation and multifractionation of the total focal dose of 60–60.8 Gy. Patients received fractions of radiation at the time when the tumor reached the maximum level of oxygenation after laser application. They showed an increase in mitotic cells but not an increase in tumor. In another in vitro study [38], the authors reported that the failure of cisplatin to respond to cancer could be related to ATP consumption and photobiomodulation could help. In this in vitro study that associated cisplatin with PBMT, there was an increase in the cytotoxicity of cisplatin, an increase in the death of neoplastic cells by apoptosis with a large consumption of ATP.

In literature regarding possible synergistic effects between PBMT and cancer treatments, authors [21,42] start by considering that PBMT has a biphasic dose-response effect and lower energy densities of PBMT can act to stimulate and repair cells and tissues, but higher energy densities

have inhibitory effects, such as inducing apoptosis. PBMT produces oxidative stress and reactive oxygen species (ROS) in the body that are eliminated by antioxidant systems. ROS homeostasis is very important in cell signaling following an adaptive response to ionizing radiation. ROS generated by PBMT produce imbalance and activate nuclear factor KB (NF-KB) which at lower energy densities of PBMT inhibits apoptosis through Akt/Gsk3beta and when present at higher energy densities, there is oxidase and ROS stress which increase cytotoxic levels and induce apoptosis through the same Akt/Gsk3beta pathway. The higher energy densities of PBMT can lead to cancer cell death and this can be explained by ROS homeostasis. Perhaps the radiomodulatory effect of PBMT is related to the exponential growth of cancer cells, normal cells, and higher energy densities to DNA damage. Other authors [20] showed that lighting increased cellular differentiation and angiogenesis, which may be related to the synergistic effect of photobiomodulation plus radiation and that there was no induction of cell proliferation *in vitro* but increased the G2/M fraction after 24 h of lighting and increased the effect of radiation by 30 %.

Therefore, in addition to the direct effect of laser irradiation on cells, there is also an indirect effect on survival, as with PBMT and the consequent improvement in clinical conditions (analgesia, repair, modulation of the inflammatory process), patients end up having a better diet, gain weight, reduce their chance of interrupting cancer treatment because of orofacial complications, improve their immune response, avoid or reduce the length of hospital stay, reduce their risk of local and systemic infections, all of which may contribute to improving quality of life and prognosis [38] what was observed clinically in our study.

There are limitations to a retrospective observational descriptive study. To overcome them, we rely on the large total number of individuals in the population (751) and some methodological strategies. It is important to observe that is very difficult to obtain this representation of cancer patients in a controlled study.

The present author was the PBMT operator, the size of the tumor in relation to PBMT was not measured during the period studied and laser light was not applied directly to the tumor. In the regions of the mouth and oropharynx, mucositis tends to occur close to the tumor, while in other anatomical locations, such as, the nasopharynx, mucositis tends to occur further away from the tumor. When we apply PBMT to areas close to the tumor, it may be possible for it to grow. In the literature [10,17], studies are described that PBMT may be related to reducing of the tumor and its aggressiveness. There are studies that demonstrate that PBMT may have effects contrary to those [10].

We carried out a survival study for patients who did or did not receive PBMT for the total population in which all anatomical sites were included and in patients treated with PBMT presented a 30 % higher probability of survival than those without PBMT. When it was studied the survival for patients who did and did not receive PBMT in relation to the anatomical sites, oral and oropharynx (areas most affect by mucositis) the group treated with PBMT presented a 42 % higher probability of survival than without PBMT. These results suggest that in cases where mucositis is close to the tumor and receive PBMT there is a greater chance of increased survival, but currently, PBMT directly on the tumor is contraindicated because we do not have enough *in vivo* scientific studies that can guarantee the safety of applying PBMT in cancer and little is known about the possible mechanisms involved yet.

We have not found until now studies that have demonstrated metastases resulting from PBMT when patients underwent oncological treatments. There is a study in which the authors conclude that patients submitted to PBMT, and oncological treatments had no impact over primary cancer treatment, recurrence of new primary cancers or survival based on the comparison of its results to the indices found in the literature.

The PBMT had established to treatment of mucositis, but there are still few clinical studies regarding its influence of PBMT +RT+QT on the tumor [40,41,43].

The evolution of oncological treatments with the possible inclusion of PBMT is of great importance in the survival of patients with head and neck cancer, especially in countries like Brazil, as it has a low cost. However, to improve our survival rates for head and neck cancer, at the same time as we advance in the efficiency of treatments, it is necessary that we move extremely quickly in expanding educational campaigns for prevention, determining early diagnosis, and reducing the time for initiating and carrying out oncological treatments [44,45].

In conclusion, our data suggest that patients undergoing cancer treatment can increase their survival if subjected to PBMT, underscoring the importance of laser application not only for the prevention and treatment of oral mucositis, but also for new investigations into the possible synergistic effects between PBMT and cancer treatments. The survival outcome was favorable. Thus, the results obtained could motivate several subsequent studies.

CRediT authorship contribution statement

Deise Luciane Paiva: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valdecir Rodrigues Oliveira:** Writing – review & editing, Writing – original draft. **Vanderlei Salvador Bagnato:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Alyne Simões:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

Declaration of Competing Interest None.

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