Photomedicine and Laser Surgery
Volume 26, Number 4, 2008

Pp. 393–400
DOI: 10.1089/pho.2007.2144

# Low-Level Laser Therapy in the Prevention and Treatment of Chemotherapy-Induced Oral Mucositis in Young Patients

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## Abstract

***Objective:*** A pilot clinical study was conducted to evaluate the efficacy and feasibility of low-level laser therapy (LLLT) in the prevention and treatment of chemotherapy (CT)-induced oral mucositis (OM) in young patients.

***Background Data:*** Besides compromising the patient's nutrition and well-being, oral mucositis represents a portal of entry into the body for microorganisms present in the mouth, which may lead to sepsis if there is hematological involvement. Oncologic treatment tolerance decreases and systemic complications may arise that interfere with the success of cancer treatment. LLLT appears to be an interesting alternative to other approaches to treating OM, due to its trophic, anti-inflammatory, and analgesic properties.

***Materials and Methods:*** Patients undergoing chemotherapy (22 cycles) without mucositis were randomized into a group receiving prophylactic laser-irradiation (group 1), and a group receiving placebo light treatment (group 2). Patients who had already presented with mucositis were placed in a group receiving irradiation for therapeutic purposes (group 3, with 10 cycles of CT). Serum granulocyte levels were taken and compared to the progression of mucositis.

***Results:*** In group 1, most patients (73%) presented with mucositis of grade 0 ($p = 0.03$ when compared with the placebo group), and 18% presented with grade 1. In group 2, 27% had no OM and did not require therapy. In group 3, the patients had marked pain relief (as assessed by a visual analogue scale), and a decrease in the severity of OM, even when they had severe granulocytopenia.

***Conclusion:*** The ease of use of LLLT, high patient acceptance, and the positive results achieved, make this therapy feasible for the prevention and treatment of OM in young patients.

## Introduction

Patients who undergo chemotherapy (CT) and radiotherapy (RT) for the treatment of malignant neoplasia often present with oral mucositis (OM) as a side effect. This stomatotoxicity strongly impacts the quality of life of these individuals, with higher morbidity and mortality rates and length of hospital stay, that lead to higher costs of treatment.[1] Thus from the patient's point of view, OM is the most debilitating complication accompanying a bone marrow transplant.[2,3]

The estimated incidence of oral complications[4] varies with the type of therapy patients undergo, but occurs in approximately 40% of patients receiving chemotherapy, 80% those receiving bone marrow transplants, and 100% of patients receiving radiotherapy of the head and neck, if the oral cavity is in the irradiated field. Younger patients have a higher incidence of oral complications[5] than older patients receiving similar oncologic treatment.

CT's cytotoxic action on the epithelial basal cell layer leads to a decrease in the renewal rate of these cells, with atrophy and ulceration of the tissues. These drugs simultaneously at-

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tack the bone marrow, inducing granulocytopenia and thrombocytopenia, and predisposing the patient to infections and bleeding.[6] The ulcerated oral epithelium allows entry into the body of the oral microbiota, and may cause local and systemic infections. With the oral pain they suffer, patients tend to become dehydrated and malnourished.

The cellular and molecular changes seen as mucositis progresses[7] occur soon after the administration of RT or CT, and lead to DNA strand breakage and the liberation of reactive oxygen species (ROS). The ROS activate transcription factors such as P-53 and nuclear factor-$\kappa$B,[8] leading to cell death. These transcription factors also induce liberation of cytokines, such as tumor necrosis factor-$\alpha$, interleukin-11$\beta$, and interleukin-6, which are responsible for alterations in the connective tissue[9] and endothelium,[10] with consequent damage to the basal layer of the epithelium. The healing of non-infected mucositis occurs in a physiological way, and is associated with decreases in drug toxicity and re-establishment of the granulocyte count,[7,11] which favor the healing process. These biological events are influenced by various factors, such as drug toxicity, dose, the interval between cycles, associated radiotherapy, the general health of the patient, susceptibility to the CT agents, and patient dental condition and oral hygiene.

There is a wide range of procedures and products to alleviate the effects of oral mucositis, such as antimicrobial rinses, mucosa protectants, cryotherapy, topical analgesics, and more recently the use of keratinocyte growth factor-1[12] and phototherapy such as low-level laser therapy (LLLT).

The mechanism behind the laser's interactions with biological tissues is only partly understood, but it is described as a photobiological phenomenon in which primary photoacceptors (chromophores) such as cytochrome c oxidase, flavins, and porphyrins, absorb certain wavelengths, causing a cascade effect in the respiratory chain, which results in the production of energy to fuel cell metabolic processes.[13,14]

LLLT's photostimulation of biological tissues depends on the use of the proper parameters, and this is still under investigation, but since the 1960s[15,16] there has been strong evidence indicating the potential of LLLT to hasten healing. Laser therapy enhances microcirculation,[17–20] improves lymphatic drainage,[21–23] promotes pain relief,[24–29] and increases proliferation and mobility of epithelial cells.[16,30–32] There are also significant increases in fibroblast production and activity,[33–38] which accelerate collagen synthesis.[39–42]

Our improved understanding of the pathophysiology of OM has made possible the introduction of new approaches to treat the manifestations of stomatotoxicity. Recently published studies have demonstrated the wide range of benefits of LLLT for biological tissues. Several trials have been proposed to demonstrate these beneficial effects for the prevention and treatment of OM in oncologic patients,[43–54] but comparisons are difficult due to the lack of standardization of protocols.

OM is a result of various etiological agents and has characteristics that change as it progresses. LLLT acts in a beneficial and non-invasive way, producing no collateral effects.

Based on the accumulated evidence, the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology (MASCC/ISOO)[55,56] suggest the use of LLLT for OM prevention in hematopoietic stem cell transplant patients. Nevertheless, no guidelines have been established and further studies should be well designed. The aim of this clinical pilot study is to evaluate the efficacy and feasibility of LLLT in the prevention and treatment of OM in young patients who are undergoing high-dose chemotherapy.

## Materials and Methods

### *Patients*

This prospective, randomized, placebo-controlled study was approved by the Ethics Committee and developed for outpatients of the Pediatric Oncology Institute (GRAACC) of São Paulo Federal University between February and August 2003. All patients or their parents or guardians signed an informed consent.

A total of 13 patients were included, with a total of 32 CT cycles, of which 21 cycles were for osteosarcoma treatment, and 11 cycles were for high-risk acute lymphoid leukemia (ALL) treatment. There were 5 males and 8 females and their mean age was 14.6 y (range 7–23 y).

The CT regimens of the osteosarcoma patients are summarized in Table 1. The patients who underwent ALL treatment received CT in accordance with the criteria of the Brazilian Group for the Treatment of Leukemia in Childhood (GBTLI 99) (Fig. 1).

Patients with unstable clinical condition, severe oral infections, and those with head and neck malignancies were excluded.

### *Method*

Standardized methods of oral hygiene were established with the use of soft toothbrushes and mouth rinsing with bicarbonate solution four times a day. Adequate oral intake was achieved before CT to avoid trauma and to reduce the likelihood of infection.

Patients without OM when they begun CT were randomized into group 1 (prophylactic laser irradiation) or group 2 (placebo laser irradiation).

Table 1. Chemotherapy Protocol for the Patients with Osteosarcoma

| *Week* | *0* | *3* | *6* | *9* | *12* | *15* | *18* | *21* | *24* |
|---|---|---|---|---|---|---|---|---|---|
| CT | CDDP DOXO | HD IFO | CDDP DOXO | HD IFO | Surgery | CDDP DOXO | HD IFO | CDDP DOXO | HD IFO |

| | | |
|---|---|---|
| Cisplatin (CDDP): | 60 mg/m$^2$ D1, D2 = 120 mg/m$^2$ × 4; | 480 mg/m$^2$ total |
| Doxorubicin (DOXO): | 40 mg/m$^2$ D1, D2 = 80 mg/m$^2$ × 4; | 320 mg/m$^2$ total |
| High-dose ifosfamide (HD IFO): | 2.7 g/m$^2$ D1–D5 = 13.5 g/m$^2$ × 4; | 54 g/m$^2$ total |
| Mesna: | 600 mg/m$^2$ at hours 0, 3, 6, and 12; | 2.4 g/m$^2$/d total |

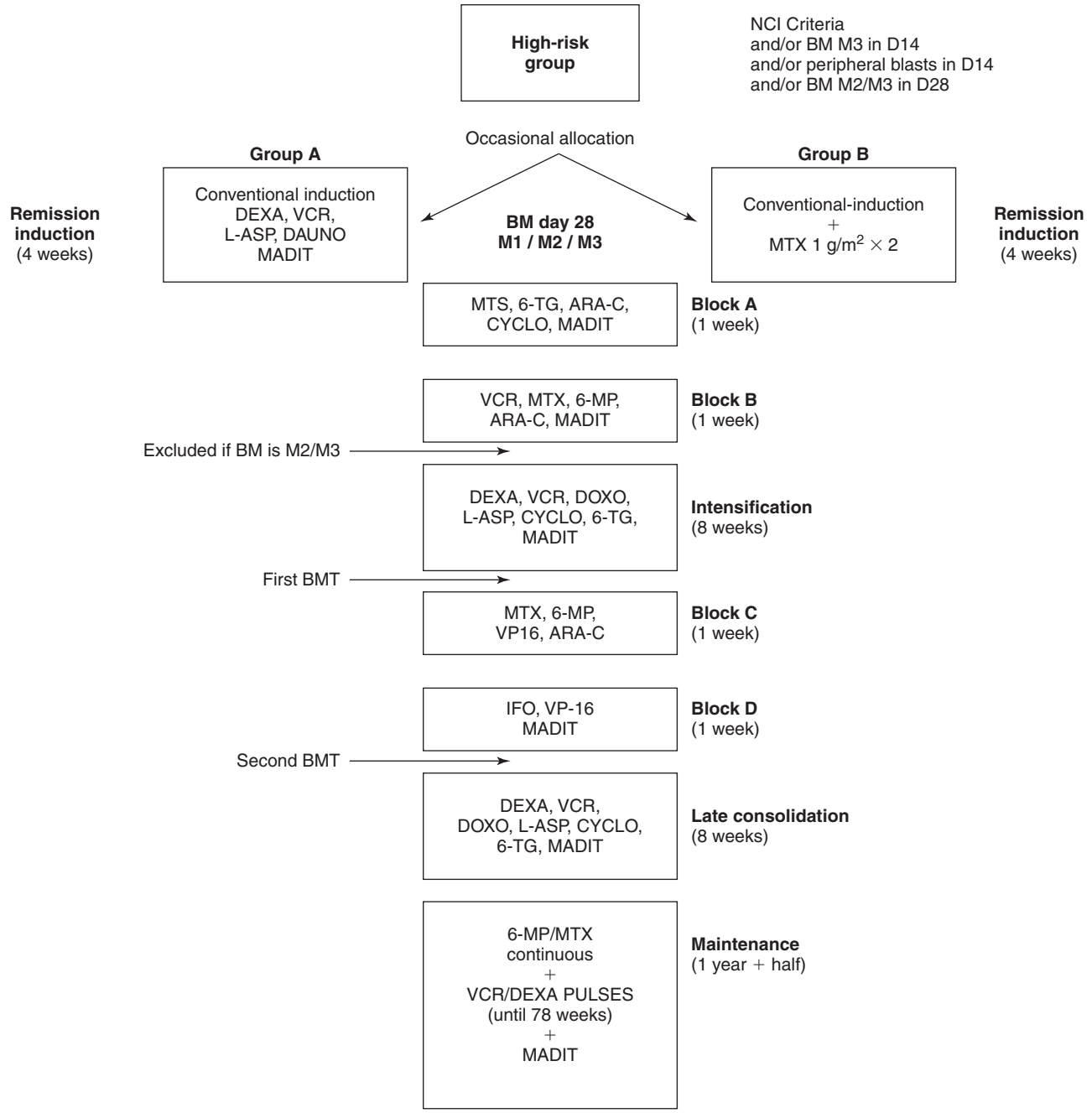


**FIG. 1.** The chemotherapy protocols of the patients with high-risk ALL. BM, bone marrow; BMT, bone marrow transplantation; MTX, methotrexate; DEXA, dexamethasone; VCR, vincristine; L-ASP, L-asparaginase; DAUNO, daunorubicin; 6-TG, 6-thioguanine; CYCLO, cyclophosphamide; 6-MP, 6-mercaptopurine; ARA-C, cytarabine; VP-16, etoposide; IFO, ifosfamide; MADIT, methotrexate + ARA-C + dexamethasone intrathecal.

Patients presenting with OM at their first examination were placed in group 3 (therapeutic laser irradiation).

The beginning of each CT cycle was considered a new case and the patients were subjected to new randomization.

### *The prophylactic laser group and the placebo laser group*

The first irradiation was performed within 24 hours of beginning CT, and the next two irradiations were performed every other day. The third one corresponded with the beginning of the period of highest risk of developing mucositis, between the sixth and ninth days after beginning CT. The patients in both groups were blinded as to whether they received real or sham laser therapy.

### *The therapeutic laser group*

The first laser irradiation session was done as soon as mucositis was diagnosed, and the two following sessions were performed every other day.

The clinical evaluations were conducted prior to irradiation. After a total of three laser therapy sessions, patients

Table 2. Changes in Mucositis Grade in Patients in the Prophylactic Laser Group

| *Mucositis grade* | *First evaluation* | | *Third evaluation* | | p *value* |
|---|---|---|---|---|---|
| | n | % | n | % | |
| 0 | 11 | 100% | 8 | 73% | 0.062 |
| I | 0 | 0% | 2 | 18% | 0.138 |
| II | 0 | 0% | 1 | 9% | 0.306 |

who did not present with mucositis were excluded from the protocol. If mucositis persisted, more laser sessions were scheduled.

### *Laser application*

An AsGaAl diode laser was used (THERA LASER; DMC Equipments Ltda. São Carlos, Brazil) operating at 685 nm, 35 mW output power, continuous wave, and a 600-$\mu$m spot. The energy delivered was 2 J per point of application, and the fluence was approximately 72 J/cm$^2$. The time spent at each point was 54 sec. Laser energy was application punctually, perpendicular to the tissue. The tip of the laser was disinfected with 70% alcohol solution and wrapped with a plastic film. Patients and operators wore glasses for eye protection.

The following areas were irradiated: the left and right jugal mucosa (two points on each side), the superior and inferior internal lip mucosa (one point in each quadrant), the floor of the mouth (one point on each side), the lateral edge of the tongue (two points on each side), the tip of the tongue (one point), the smooth palate (one point on each side), and the labial commissure.

### *Evaluation*

Patients were clinically evaluated with regard to OM severity, painful symptomatology, esophagitis, and they were asked if they used filgrastim (Granulokine®). The changes in granulocyte levels were evaluated through hematological assessment.

Oral mucosal toxicity and esophagitis were graded according to the National Cancer Institute's Common Toxicity Criteria, version 2.0.[57] Pain was evaluated before and after laser application via a visual analog scale scored from 0–10.[58] Pain scored from 1–4 was considered mild, that scored from 4.1–7 was moderate, and scores ≥7 were considered intense.

In the presence of infections caused by fungi, bacteria, or viruses, a therapeutic regimen was established, and the evaluation of laser effectiveness was restricted to signs and symptoms of OM lesions, without consideration as to whether the laser therapy could cure the infection.

Granulocyte levels were evaluated because they behave similarly to the epithelial cells of the oral mucosa when exposed to CT agents. The granulocyte levels were classified one of three ways: ≥2000/mm$^3$, from 1000–2000/mm$^3$, and <1000/mm$^3$. Filgrastim use was also considered because it raises serum granulocyte levels.

### *Statistical analysis*

The mucositis grades and granulocyte levels were compared at the first and third evaluation. Then the mucositis grades of the prophylactic laser group were compared with those of the placebo laser group.

The results were analyzed using the comparison test for two proportions, which is a non- parametric qualitative test. The value considered statistically significant was $p < 0.05$.

## Results

### *Prophylactic LLLT*

There were seven patients in this group, with a total of 11 CT cycles. All patients were undergoing osteosarcoma treatment.

In the third evaluation, among the 8 patients without mucositis (Table 2), there were 5 cases with granulocyte levels <2000/mm$^3$ (Table 3). It must be emphasized that two patients with granulocyte levels >2000/mm$^3$ had taken filgrastim.

In the second evaluation, candidiasis was diagnosed in the patient who presented with grade II mucositis. Once the infection treatment regimen was established, laser application was performed and this patient reported pain reduction from 5 (moderate) to 3 (mild).

In the second evaluation, esophagitis was observed in just one patient. This patient reported intense pain in the pharynx (grade 9), but for the oral-cavity mucositis the grade was 0, and granulocyte levels were <1000/mm$^3$. In the third evaluation, three patients presented with esophagitis, but did not present with mucositis in the oral cavity.

All patients progressed toward the resolution of OM with no other type of therapy.

Table 3. Changes in Granulocyte Levels in Patients in the Prophylactic Laser Group

| *Granulocytes/mm$^3$* | *First evaluation* | | *Third evaluation* | | p *value* |
|---|---|---|---|---|---|
| | n | % | n | % | |
| >2000 | 7 | 64% | 4 | 36% | 0.201 |
| 1000–2000 | 3 | 27% | 1 | 9% | 0.269 |
| <1000 | 0 | 0% | 4 | 36% | 0.027 |
| Not recorded | 1 | 9% | 2 | 18% | 0.534 |

Table 4. Changes in Mucositis Grade in Patients Receiving Placebo Irradiation

| *Mucositis grade* | *First evaluation* n | *First evaluation* % | *Third evaluation* n | *Third evaluation* % | p *value* |
|---|---|---|---|---|---|
| 0 | 8 | 73% | 3 | 27% | 0.033 |
| I | 3 | 27% | 5 | 45% | 0.375 |
| II | 0 | 0% | 2 | 18% | 0.138 |
| III | 0 | 0% | 1 | 9% | 0.306 |
| IV | 0 | 0% | 0 | 0% | — |

### *Placebo LLLT irradiation*

Seven patients were evaluated, having a total of 11 CT cycles. Among them, 5 cycles were for osteosarcoma treatment, and 6 cycles were for ALL treatment.

Some patients (3) presented with grade I mucositis at the first examination, but they were randomized into this group because they had mild edema in the jugal mucosa. All these patients maintained grade I mucositis during all three evaluations.

In this group mucositis episodes were frequent (Table 4), but they were not as severe, because when grade II mucositis was diagnosed, therapeutic measures had already been established. Also, these patients were under supervision, which may have promoted better oral hygiene, which is known to decrease the severity of OM.

The levels of granulocytes in the placebo group did not show great variation (Table 5).

At the first evaluation, no patients reported pain, while at the third evaluation three patients mentioned pain in the oral cavity. Two patients had viral infections at the third evaluation, and therapeutic intervention was necessary in three patients.

### *Placebo versus prophylactic laser group*

At the third evaluation, 73% of the patients in the prophylactic laser group did not have mucositis, and in the placebo group 27% had no mucositis, a difference that reached statistical significance ($p = 0.03$).

### *Therapeutic LLLT*

Six patients (10 CT cycles in all) were followed-up. Five cycles were given for osteosarcoma treatment, and five cycles were for ALL. These patients had already shown some degree of mucositis at the first evaluation. This group had CT beginning on the date of the first examination, and continued it for 1–7 d, with an average of 3 d.

At the first evaluation of the protocol, seven episodes of pain were noted, two of them (29%) mild pain, and five (71%) moderate pain. At the second evaluation, after one laser session, five reported (71%) episodes of mild pain, and two (29%) episodes of moderate pain. At the third evaluation, of the 10 CT cycles evaluated, only three (30%) cases of mild pain were noted; however, these patients were able to speak and eat in spite of the pain. At the third evaluation only one occurrence of moderate pain was noted, and after another laser session it became mild.

At the third evaluation, most patients regardless of their low granulocyte levels (80% of patients), did not have severe mucositis (Table 6), and they could eat and continue their cancer treatment regimens.

Also at the third evaluation, among the six patients with granulocyte levels $<1000/mm^3$ (Table 7), two of them presented with grade 0 mucositis, one patient had OM grade I, and three patients had OM grade II. The patients with grade II OM had grade III OM at the previous evaluations, and they improved after LLLT in spite of granulocytopenia. OM healing occurred in all of them, with no need for further LLLT treatment sessions.

## Discussion

LLLT was well tolerated, even when patients presented with severe lesions, which makes this therapy tolerable to children. There were no patients who discontinued LLLT because they found it to be unacceptable. With the improvement in signs and symptoms, some patients did not attend the following sessions, which made follow-up of these patients impossible.

Several patients repeated the CT cycles with similar drugs and dosages, and were randomized once again within the LLLT protocol. This procedure allowed the comparison of different approaches to treating OM in the same patient, or in those with similar CT protocols.

For instance, two patients with the same disease and similar CT regimens (high-dose methotrexate) were included in the prophylactic laser group, and later, because at the first examination they had already presented with signs of OM, they were included in the therapeutic laser group. When they were prophylactically irradiated they had mucositis grade 0 or I at the three evaluations. On the other hand, when the therapeutic laser protocol was used, they developed mu-

Table 5. Changes in Granulocyte Count in Patients Receiving Placebo Irradiation

| *Granulocytes/mm³* | *First evaluation* n | *First evaluation* % | *Third evaluation* n | *Third evaluation* % | p *value* |
|---|---|---|---|---|---|
| >2000 | 5 | 45% | 6 | 55% | 0.670 |
| 1000–2000 | 3 | 27% | 3 | 27% | 1.000 |
| <1000 | 1 | 9% | 2 | 18% | 0.534 |
| Not recorded | 2 | 18% | 0 | 0% | 0.138 |

Table 6. Changes in Mucositis Grade in Patients in the Therapeutic Laser Group

| *Mucositis grade* | *First evaluation* n | *First evaluation* % | *Third evaluation* n | *Third evaluation* % | p *value* |
|---|---|---|---|---|---|
| 0 | 0 | 0% | 4 | 40% | 0.025 |
| I | 3 | 30% | 2 | 20% | 0.606 |
| II | 6 | 60% | 4 | 40% | 0.371 |
| III | 1 | 10% | 0 | 0% | 0.305 |
| IV | 0 | 0% | 0 | 0% | — |

cositis grade III at the second evaluation, and mucositis grade II at the third evaluation.

It was also possible to compare three CT cycles with high-dose methotrexate in a patient. We noted remarkable results: in the first two cycles with prophylactic laser irradiations, the patient presented mucositis grades 0 and I. In the third cycle, this patient was randomized to the placebo group and mucositis grade III was diagnosed, and required additional therapeutic measures.

Methotrexate (MTX) and cytarabine (ARA-C) are antimetabolite cell-cycle-phase specific drugs with significant cytotoxic potential for the oral and gastric mucosa. In the protocol for ALL treatment these agents were administrated simultaneously or over a short time interval. For osteosarcoma treatment, the recommended dose of MTX was 12 $g/m^2$, as an alternative therapy regimen for patients with refractory disease.

The use of LLLT for prophylactic purposes showed the most satisfactory outcomes, a fact in accordance with data found in the literature.[43–46,48–50,52–54] For therapeutic applications, the patients noted pain relief, and there were no reports of OM worsening after LLLT, even when the granulocyte levels were $<2000/mm^2$.

Some authors also demonstrated that light-emitting diodes are helpful in treating OM.[59,60] Their effects similar to those of LLLT are explained by the fact the coherence of laser light is lost in the top layers of biological tissues, and thus the photo-stimulatory effects are more related to the light's parameters than to the light source employed.[61]

In the present study, the comparison of the evolution of mucositis with the granulocyte count allowed differentiation of beneficial laser effects from those of the physiologic evolution of OM. Also, when drug toxicity is reduced, the re-establishment of conditions that favor healing occurs. We observed a decrease in the intensity of OM, even in those with severe granulocytopenia.

The difficulty of demonstrating the laser's efficacy in oncologic patients with OM results from the variety of disease types and chemotherapy and radiotherapy protocols. There is also a lack of a consistent classification system to evaluate the severity of OM, and the possibility of spontaneous healing in many cases without complications. As CT agents have different toxicity levels, the short interval between CT cycles makes it difficult to determine which drugs may intensify OM.

Also, it was difficult to determine the appropriate laser parameters to use in pediatric patients. The energy levels used here were based on several small pilot studies performed by our group. From an initial energy level of approximately 1 J, we evaluated clinical responses with levels up to 3 J. We found 2 J to be a good compromise between the results obtained and the time spent on laser application.

The reduction in OM-induced pain was the most remarkable effect reported by our patients, a fact in agreement with data found in the literature.[43–45,47,51,53,54] This prompt alleviation allowed patients to improve their nourishment, which improved their overall state of health.

The ease of use of LLLT, the ability to treat OM in a few irradiation sessions, the low cost of the equipment, the high patient satisfaction with the protocol, and the successful results obtained in this study, make this therapy feasible for the prevention and treatment of OM in pediatric patients.

## Conclusion

In spite of our small patient sample, it is worth noting that most did not develop OM when prophylactically irradiated, and there was quick recovery and pain relief for those in the therapeutic laser group, even in those with significant granulocytopenia, thus enabling them to better tolerate their chemotherapy regimens. This demonstrates that LLLT has both a preventive and a therapeutic role in those prone to develop OM. Further clinical studies are needed with greater numbers of patients to optimize the laser parameters, to test LLLT's effectiveness with various CT regimens, and to further explore this treatment option for OM, which could greatly benefit these patients.

## Acknowledgments

We want to thank all of the patients and their families. Thanks also to Armando Reis Tavares, Jimmy Adans, Maria

Table 7. Changes in Granulocyte Levels in Patients in the Therapeutic Laser Group

| *Granulocytes/mm³* | *First evaluation* n | *First evaluation* % | *Third evaluation* n | *Third evaluation* % | p *value* |
|---|---|---|---|---|---|
| >2000 | 4 | 40% | 1 | 10% | 0.121 |
| 1000–2000 | 3 | 30% | 2 | 20% | 0.606 |
| <1000 | 3 | 30% | 6 | 60% | 0.178 |
| Not recorded | 0 | 0% | 1 | 10% | 0.305 |

Cristina Iglesias, and Ricardo B. Iannuzzi for assistance in manuscript preparation. We also thank Mr. Renaldo Mansini, Jr. of DMC Equipmentos Ltda., São Carlos, Brazil, for the aid with the laser equipment.

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