Protocol

Antimicrobial photodynamic therapy in the treatment of foot ulcers in people with diabetes mellitus: a randomised controlled trial protocol

ABSTRACT

Significance Diabetic foot ulcers are a major complication of diabetes and a significant public health issue, greatly impacting healthcare costs. These ulcers are commonly treated with conventional methods such as saline cleaning, debridement, antibiotics, and topical dressings. However, the rise in non-traumatic lower limb amputations related to diabetes, along with growing antimicrobial resistance, highlights the insufficiency of these standard treatments in achieving timely healing.

Recent advances New light source adjuvant therapies, such as antimicrobial photodynamic therapy, are being tested to aid the healing of foot ulcers. This therapy involves using light (LASER or LED) to irradiate the lesion in combination with a photosensitising agent and tissue oxygen. This process promotes oxidative stress and reduces the microorganisms present in the ulcer.

Critical issues A significant challenge in applying photodynamic therapy is the lack of comprehensive clinical studies and complete treatment protocols. Although there is growing evidence supporting this therapy's effectiveness in various conditions, the scarcity of well-documented clinical trials, and reliable replication of these studies, represents a major obstacle for other researchers seeking to replicate the results.

Future directions This article provides a detailed and transparent protocol, which can be easily reproduced by other researchers, making significant step towards consolidating and expanding the use of photodynamic therapy in the treatment of diabetic ulcers. It is hoped that this study and the presented protocol will serve as a foundation for future research and innovations in photodynamic therapy, opening up new therapeutic possibilities and contributing to improving quality of clinical practice.

Keywords photodynamic therapy, oxidative stress, diabetic foot, wound healing, wound care

For referencing Brandão MGSA, et al. Antimicrobial photodynamic therapy in the treatment of foot ulcers in people with diabetes mellitus: a randomised controlled trial protocol. WCET® Journal. 2024;44(4):28-38.

DOI https://doi.org/10.33235/wcet.44.4.28-38

Submitted 13 September 2024, Accepted 20 November 2024

Maria Girlane S A Brandão*
RN PhD student
Department of General and Specialised Nursing, School of Nursing, University of São Paulo, Ribeirão Preto, Brazil
Email girlanealbuquerque@usp.br

Idevania G Costa
RN NSWOC PhD
School of Nursing, Lakehead University, Thunder Bay, Canada

Mayra Gonçalves Menegueti
RN PhD
Department of General and Specialised Nursing, School of Nursing, University of São Paulo, Ribeirão Preto, Brazil

Roberto Bueno Filho
Doctor PhD
Department of Clinical Medicine, Ribeirão Preto School of Medicine, University Hospital, Ribeirão Preto, Brazil

Soraia Assad Nasbine Rabeh
RN PhD
Department of General and Specialised Nursing, School of Nursing, University of São Paulo, Ribeirão Preto, Brazil

** Corresponding author*

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease often leading to complications, including diabetic foot ulcers (DFU). Annually, 18.6 million people develop DFUs, which precede 80% of lower limb amputations in DM patients. Infections affect 50–60% of DFUs, with around 20% of severe cases resulting in lower limb amputations (LLA).[1]

From 2010 to 2020, there were more than 240,000 hospitalisations in Brazil related to lower limb amputation due to DM.[2] Furthermore, even after surgical intervention in patients with minor amputations, there is a high probability of readmission due to infections. Patients with major amputations have a high probability of readmission for treatment of sepsis.[3]

The conventional DFU care includes cleaning, debridement, circulation, moisture and infection control. [4] However, this is often insufficient for timely tissue repair, as DM impairs all healing phases.[5] Delays occur in inflammation, cytokine persistence disrupts proliferation and myofibroblast changes hinder collagen deposition and remodeling. Most DFUs are also colonised by multiple bacteria.[6,7]

These microorganisms form colonies and group together, creating a self-protective film on the ulcer bed. This film promotes inflammation and stagnation of healing, blocking the action of conventional treatments and dressings and the host's defence responses, potentially leading to the progression and chronicity of the lesion.[8]

Therefore, providing adequate treatment to manage microbial communities of bacteria is vital to preventing serious outcomes in people with DFU, such as LLA or death.[8] An adjuvant therapy that can help treat foot ulcers in people with DM is photodynamic therapy (PDT).[9,10]

PDT involves using a light source (LASER or LED) to irradiate non-toxic photosensitive agents that interact with tissue oxygen, generating reactive oxygen species. These reactive oxygen species have a lethal effect on infectious agents, without causing tissue damage.[11,12] The most used photosensitive agent is methylene blue, as it has good market availability, low cost and a low risk of adverse reactions.[13] Cost-benefit research in Italy showed that using PDT in DFU had a positive budgetary impact, reducing the time to reach the outpatient healing goal by 50%.[14] A recent systematic review on the effectiveness of PDT revealed only a limited set of four clinical trials on the topic.[15] None of these studies made their protocols (with essential methodological details) available, representing a significant barrier to the accurate and reliable replication of their investigations. Additionally, these studies have limitations, such as using swabs for microbiological assessment and a wide variation in PDT application parameters and clinical outcomes.

For the diagnosis and evaluation of infection in foot ulcers, a sample must be obtained for culture by collecting a tissue fragment through curettage or biopsy, which is considered best practice.[16,17] Many studies on PDT for treating DFU use varied light parameters, doses and wavelengths. To establish best practices and standardise its application, it is essential to conduct studies that systematically test and apply these parameters.[18]

Therefore, this study aims to fill critical gaps in the literature by making the complete clinical trial protocol transparently available. This includes the publication of the Standard Operating Protocol (SOP) for PDT and biopsy of foot ulcers for a more precise microbial assessment. This protocol is constructed based on scientific evidence, promoting its reproducibility for the development of a clinical trial to evaluate the effectiveness of antimicrobial PDT in the treatment of DFU.

METHOD

Study design

This is a pragmatic, longitudinal Randomised Clinical Trial (RCT) protocol. In this study, we intend to analyse the effectiveness of PDT treatment for DFU, in two groups: the intervention group (IG) and control group (CG). The research protocol is outlined following the precepts of Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT).[19]

Search scenario

The research will be conducted in the outpatient service of two tertiary hospitals located in Ribeirão Preto, São Paulo, Brazil. The first is the Endocrinology and Metabolism Outpatient Clinic of the *Hospital das Clínicas* of the Faculty of Medicine of Ribeirão Preto of the University of São Paulo. The second site is the Vascular Surgery outpatient clinic at the *Beneficência Portuguesa* hospital in Ribeirão Preto.

Study participants and eligibility criteria

Participants will be selected through convenience sampling and randomly allocated to the intervention and control groups. The study will include: patients of both sexes; aged 18 years or over; who have DFU; who agree to undergo a biopsy of the lesion; and are available to attend the outpatient clinic weekly, for seven weeks (six weeks of treatment and a following week for assessment of outcomes and repeat biopsy).

The exclusion criteria are patients who have: a diagnosis or are undergoing treatment of neoplasms; chronic renal insufficiency or peripheral vascular insufficiency; suspected or confirmed osteomyelitis; a lesion with an area greater than 5cm by 5cm; or Ankle-Brachial Index (ABI) less than 0.7; absence of pulses with ischemia, as oxygen must reach the treatment site for PDT to work.[20] Patients using immunosuppressive medications, with a score greater than 12 in the Tardivo Algorithm[21] will also be excluded. The justification for excluding patients with these characteristics is the low probability that the treatment will be effective for them. Therefore, it is permissible to exclude them from the research.[22]

Participants who miss two consecutive appointments or experience skin irritation resulting from the use of methylene blue will be discontinued from the study and this data will be computed.

Recruitment

Hospital das Clínicas of the Faculty of Medicine of Ribeirão Preto: patients will be selected through the Endocrinology and Metabolism Outpatient Clinic and according to the eligibility criteria. During the nursing consultation and DFU assessment, the researcher, who is authorised to accompany the nursing consultations, will assess whether the patient meets eligibility requirements. If the person qualifies, the researcher will provide an invitation and explain the research process including risks and benefits, follow-up time and the need to voluntarily sign the Research Ethics Board approved informed consent form. Following this, the biopsy procedure will be carried out by a dermatologist and the patient will then be randomised.

Beneficência Portuguesa Hospital of Ribeirão Preto: patients will be selected through the Vascular Surgery Outpatient Clinic. During the consultation with the vascular surgeon, the researcher, who is authorised to attend medical consultations, will assess whether the patient meets the eligibility requirements. If the patient meets the inclusion criteria, the researcher will provide an invitation and explain all details about the study including the risks and benefits, follow-up time and the need to voluntarily voluntarily sign the Research Ethics Board approved informed consent form. Subsequently, the biopsy procedure will be scheduled by a vascular surgeon and the patient will then be randomised.

Randomisation

Patients will be randomised into two groups:

- **IG:** Cleaning with 0.9% saline solution, instrumental/sharp debridement (as needed), methylene blue plus activated PDT and application of secondary dressing (calcium alginate without silver).
- **CG:** Cleaning with 0.9% saline solution, instrumental/sharp debridement (as needed), methylene blue plus inactivated PDT and application of secondary dressing (calcium alginate without silver).

The random allocation will be carried out using individual, sealed, opaque, non-translucent and tamper-proof envelopes, provided to the research team by a person unrelated to the study (an individual with no involvement in the research and opening of the envelopes) and without external notes.[22,23] The envelopes will be opened sequentially as the trial processes, ensuring that each patient has an equal chance of being allocated to the IG or CG. For each eligible participant, an envelope will be opened containing the treatment group assignment and the procedure will be recorded in writing in a field diary.[24]

Blinding

When discussing blinding, the term double-blind is still widely used. However, this term is falling into disuse due to its ambiguity, in favour of clear specifications about who will be masked and who will know the allocation of participants.[24] Therefore, the blinding plan is explained in Table 1.

Therefore, only one researcher, responsible for applying the PDT, will have knowledge about the allocation of participants. In this case, it would not be technically possible for the researcher not to know what they are doing when using the light source and the photosensitiser. However, care has been taken to mask the other participants involved in the study.

Research team

To meet the specifications of the study design, a team of researchers was organised, consisting of four nurses (two are specialised in enterostomal therapy nursing), a dermatologist, a vascular doctor and three nursing students.

Data collection and measurement of baseline variables

Data collection and baseline variables will be conducted using a semi-structured instrument that includes sociodemographic and clinical data of the participants and assessment of DFU. The following sociodemographic and clinical data will be collected:

- General information: age, sex, occupation, place of birth, skin colour, marital status and education

Table 1. Distribution of those involved in the study and their blinding condition.

Study participants	Blinding condition
Patients	The entire procedure will be done blindly to patients, who will not be informed about which group they are in.
Doctor responsible for the biopsy	The professional responsible for collecting biopsies from patients' ulcers will not be informed which patients are in the IG or CG.
Intervention applicator	The PDT and pseudo-intervention will be carried out by a nurse qualified in LASER therapy in complex wounds, who will have knowledge about the allocation of participants, in order to carry out the treatment protocol indicated for each group.
Biopsy assessor	The microbiology laboratory professional, responsible for microbiological culture, will not be informed about the allocation of patients.
Statistical	The professional responsible for statistics and analysis of results will receive the data collected in Microsoft Excel® spreadsheets, in which each group will be coded with Greek symbols, (ω) omicron and (λ) lambda, without any identification of intervention or control group.

- Assessment of risk factors: associated systemic diseases, duration of DM, smoking, alcohol consumption, nutrition, hygiene, mobility and medications.

The assessment of ulcers will be conducted using the Bates-Jensen Wound Assessment Tool (BWAT) validated for Brazilian Portuguese, which provides a practical, objective and conclusive method for monitoring the healing evolution.[25] The scale contains 13 items that evaluate the lesion including size, depth, edges, detachment type and amount of necrotic tissue, characteristics and amount of exudate, edema and periwound induration, periwound skin colour, quality of granulation tissue and epithelialisation. Each item is classified into scores ranging from 1 to 5, where 1 indicates the best condition and 5 the worst condition of the wound.

In each assessment, the lesion will be measured with a disposable measuring ruler, placed next to the edge of the DFU. In addition, at the initial stage, blood collection will be scheduled (within 7 days) to assess glycated hemoglobin at the beginning of treatment; and the assessment of the Ankle-Arm Index (ABI) will be conducted on the same day as the first treatment session.

The ABI will be performed on all patients by the same nurse, using Portable Vascular Doppler equipment DV610 MegaMED, to locate arterial pulses, using a transducer at a frequency of 10Mhz with a very high level of sensitivity; and a sphygmomanometer to measure systolic pressure. With the participant in the supine position, after 10 minutes of rest, systolic pressure measurements will be collected from the dorsal artery of the foot and brachial artery, bilaterally. The result will be obtained through the ratio between the highest pressure of the dorsal artery of the foot at the ankle and the highest pressure of the brachial artery of the upper limb, thus obtaining the ABI. Normal values for ABI between 0.9 and 1.3 will be considered.[26]

DEFINITION OF TREATMENT PROCEDURES

Protocol for applying photodynamic therapy treatment, intervention group (Appendix 1)

To perform PDT, a photosensitive compound and a light source are necessary to generate oxidative stress with tissue oxygen. Thus, the methylene blue solution (1%) will be used as the photosensitising agent. This solution will be formulated upon request by Imbralab – *Química e Farmacêutica Ltda* (CNPJ-05.123.544/0001-64), located in the city of Ribeirão Preto, São Paulo. Methylene blue was chosen because it has low toxicity, good market availability and is often used in combination with PDT in clinical research.[13,27]

This photosensitiser will be applied to the lesion (covering a 0.5cm edge and central portion) using a disposable 3ml pipette. The amount used will depend on the size of each lesion, for example, 0.5ml for lesions up to 4cm^2 and 1ml for lesions larger than 4cm^2. After application, a five-minute absorption into the tissue will be timed using a smartphone.

Following absorption, light irradiation will be performed using Therapy EC equipment from the company DMC (regulated by ANVISA Registration 80030819013). The irradiation will use a wavelength of 660nm, a dose of 9Joules and an irradiation time of 90 seconds per point.[28] The application will use a point contact technique, maintaining a standardised distance of 1cm between one point and another around the lesion and 0.5cm from the lesion edge, ensuring the entire ulcer area receives light irradiation. This procedure is safe and painless for the patient.[10]

To minimise the risk of contamination of the LASER tip with the ulcer, the tip will be covered with plastic film and re-covered for each patient, after cleaning the device with 70% alcohol liquid. All PDT applications will be carried out by a nurse qualified in LASER therapy. After applying PDT, with a secondary dressing of calcium alginate without silver will be used to cover the lesion. Each patient will receive the indicated coverage to be able to change the dressing at home. These changes will occur according to the assessment of the level of exudation and both patients and companions will be duly informed about this. Calcium alginate without silver was chosen because it has a moderate to high capacity of exudate absorption and the ability to maintain a physiologically moist environment, favouring autolytic debridement without having antimicrobial activity that could interfere with PDT results.

Treatment application protocol, control group (Appendix 2)

After cleansing of the ulcer as described previously and performing conventional treatment, methylene blue will be used as a pseudo-intervention under the same conditions and concentration as that used in the IG. Methylene blue was chosen because it leaves the ulcer bed dark blue even after removing the excess. This ensures that participants, who might exchange information in the waiting room and other study collaborators cannot determine where the active intervention is allocated.

After applying methylene blue and after the rest time, LASER light will be irradiated with the same device and the same application techniques as for the IG. However, the device's tip will be inactivated by blocking it with silicone rubber to prevent actual irradiation of PDT on the lesion, as shown in Figure 1.

Measuring outcomes

In this study, the reduction of bacteria through tissue biopsy was selected as the primary outcome, while the clinical evolution, described by signs of improvement of the lesion and healing rate are secondary outcomes.

- **Assessment of bacteria**

A recent study that compared the aspiration technique with biopsy concluded that biopsy is the most effective and sensitive method for identifying microorganisms in skin lesions.[29] Therefore, a biopsy procedure will be performed by a physician collaborating with the study, following the procedure protocol in Appendix 3.

To perform the procedure, the periphery of the ulcer will receive anaesthesia by injecting 2–2.5 mL of 2% lidocaine into the deep dermis using a sterile, disposable 3mL syringe and a 21g needle (0.8 x 25mm); 30G needle (0.3 x 13mm. A disposable punch, consisting of a handle and a circular cutting edge with a diameter of 3mm will be used to remove the material. The biopsied fragment will be lifted with forceps and its base will be sectioned in the deepest portion with a number 15 scalpel, followed by mechanical compression with sterile cotton.

The fragment will be stored in a sterile plastic collection bottle, labeled and promptly sent to the hospital's microbiology laboratory for identification of the microorganisms present in the tissue and antibiogram. The analysis will be conducted according to BrCAST standardisation rules in the outpatient clinics of both hospitals.

- **Clinical assessment of the injury**

All ulcers will be thoroughly evaluated to provide a clinical analysis of the lesions, including a qualitative description of data related to type of tissue, exudate, edges, odour and appearance of the periwound skin. For statistical analysis, assessments taken in the first and last treatment sessions in both groups will be considered.

- **Ulcer Healing Index**

An image database of research patients will be built. At each treatment session, the ulcer areas will be photographed with the iPhone 7 smartphone camera, with a 12MP wide-angle lens and ƒ/1.8 aperture, with the aid of a light ring (Mini Ring Light LED). The images will be recorded at a standardised distance of 10cm from the lesion area. Additionally, the Imito Measure® smartphone application will be used to calculate the measurements of length, width and wound area at each treatment session. The measurement taken in the first and last treatment sessions will be considered for statistical analysis. Subsequently, the Ulcer Healing Index (UHC) will be calculated[10] as shown in Figure 2.

Diabetic Foot Ulcer Classification System (SINBAD)

The Classification System and Score in Comparing Outcome of Foot Ulcer Management (SINBAD) will be used to classify the diabetic foot at the final moment and on the last day of treatment (week 7). The SINBAD system evaluates six categories including site, ischemia, neuropathy, bacterial infection, ulcer area and depth. The score varies between zero and one in each category. Therefore, the closer to six the total score is, the worse the healing and the lower the probability of cure.[30]

The Figure 3 presents a flowchart that summarises the procedures and interventions that will be performed in the study.

Organisation and analysis of data

The data will be stored in spreadsheets coded in Microsoft Excel, using the technique of double entry for the responses

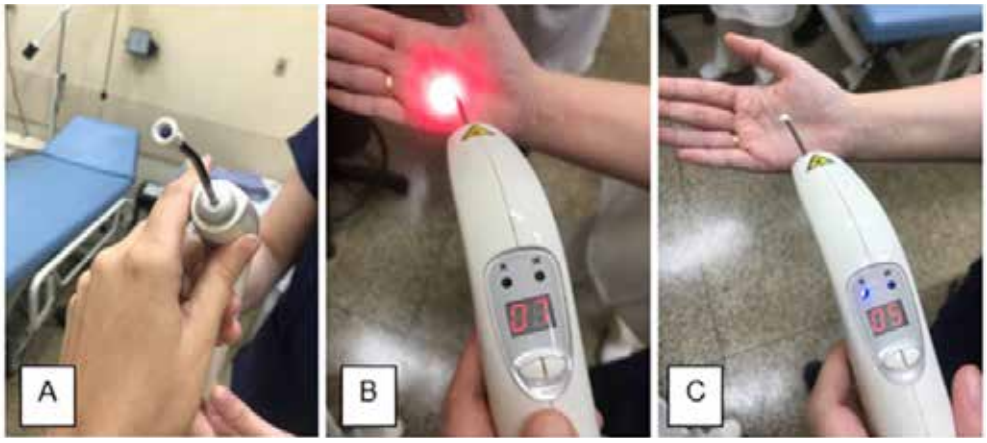

Figure 1. Illustration of the light-blocking technique, while maintaining the sounds of the LASER device.
A: LASER Tip with silicone rubber, blocking the light output and also the therapeutic action.
B: The LASER device is turned on, and the device tip has no blockages and emits light normally.
C: The LASER device is turned on, but the tip of the device has a silicone rubber to block the light.

$$UHC = \frac{[(\text{Starting Area}) - (\text{Finishing Area})]}{(\text{Starting Area})}$$

UHC = 1 -- Means total re-epithelialization
UHC = 0 -- Indicates signs of re-epithelialization
UHC > 0 -- It means reduction of the lesion area
UHC < 0 -- It means an increase in the area of the lesion

Figure 2. Formula for calculating the Ulcer Healing Index.

and subsequent validation of the data. After validation, the spreadsheets will be transferred to the SPSS statistical program.

A statistician will conduct simple frequency descriptive analyses for categorical variables and calculate measures of central tendency (mean or median) and dispersion (standard deviation or minimum and maximum) for quantitative variables, depending on the distribution of the variables. The Kolmogorov-Smirnov normality test will be performed to analyse the distribution of the data.

For the analysis of numerical variables, the Student's t-test will be used for independent samples if data with normal distribution, or the Mann-Whitney test if the distribution is not normal. For the analysis of categorical variables, the Chi-Square test will be used. The significance level of 5% will be adopted in the analytical procedures.

Protection of research participants

The research will adhere to the principles of the Declaration of Helsinki and the ethical guidelines for research involving human beings as outlined in Resolution 466/2012 of the National Health Council, Brazil.

This trial has been authorised by the manager of the Hospital's endocrinology outpatient clinic and approved by the Research Ethics Board (REB) of both School of Nursing of Ribeirão Preto (5.802.182/2022) and Hospital das Clínicas of the Faculty of Medicine of Ribeirão Preto (6.071.033/2023). Additionally, it obtained the Universal Test Number (UTN) from the World Health Organisation (U1111-1286-7818) and the Brazilian Clinical Trials Registry (REBEC) under number RBR-2dm7t97.

Patients' participation will be voluntary and upon agreement with the terms expressed in the informed consent form, which allows them to withdraw from the research any time without affecting their treatment or suffering judgments or penalties.

Expected results

It is anticipated that the defined PDT protocol will promote clinical improvement of the DFU in participants in the intervention group compared to the control group. Additionally, the study aims to to raise awareness about two important topics:

1) Implementation of biopsy in health services: Emphasising the importance of using biopsy to follow up on DFU cases based on the microorganisms present in the wound bed, considering its polymicrobial etiology.

2) Investment in nurse training and qualification: Highlighting the need to invest in the training and qualification of nurses to develop new care protocols and therapies focused on innovative and more cost-effective adjuvant technologies.

Moreover, the PDT protocol presented here, built considering scientific evidence, could serve as a guideline for researchers and clinicians to replicate it in different geographic locations and wound care settings.

DISCUSSION

The present protocol involves a randomised study that will analyse the effectiveness of PDT in treating DFU. The strengths of this research include:

- A study method with a team of physicians and nurses, using double-blind method with controlled randomisation,
- The use of tissue biopsy technique with sterile management before and after six weeks of treatment,

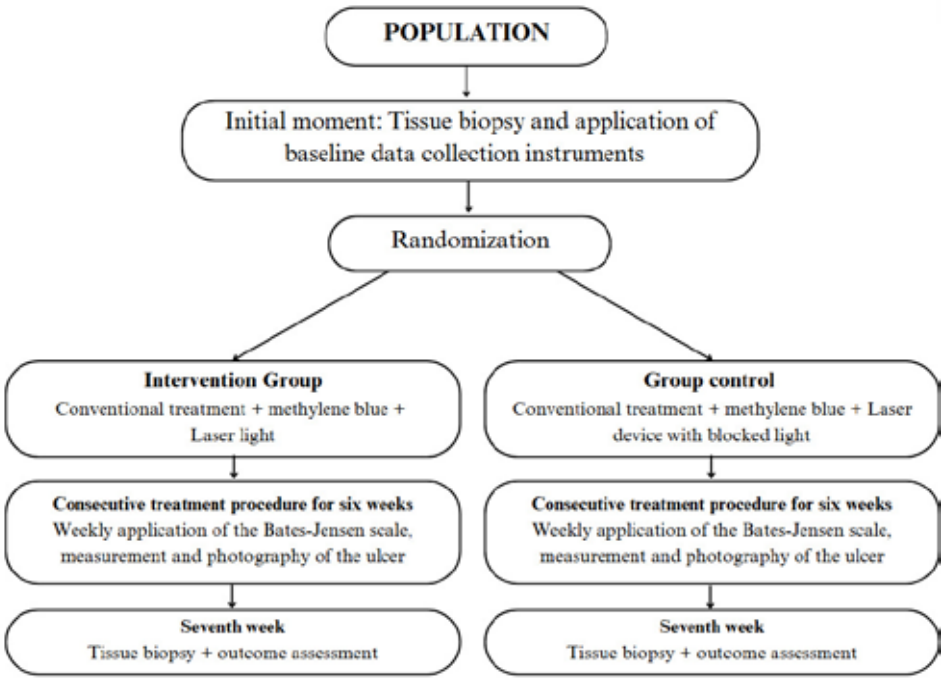

Figure 3. Flowchart illustrating the study steps and procedures.

totalling seven weeks, with follow-up. This approach will allow for reliable assessment of the types of bacteria present in the ulcer, and;

- A PDT treatment protocol developed based on scientific evidence, such as systematic review with meta-analysis, to understand the main parameters used in PDT.[15]

The choice to conduct a clinical trial is due to the lack of clinical studies comparing PDT with a control group, despite the presence of case series and review studies with converging results for PDT.[9,15,31] Furthermore, there is considerable variation in the ideal light parameters, such as dose in joules and irradiation time, which are crucial for the effectiveness of therapy.[32]

In the present study, the treatment protocol was developed based on a previously published systematic review and meta-analysis, which sought to establish the best parameters for conducting PDT.[15] Subsequently, we performed a scoping review (currently in the post-processing review phase, approved in the journal Photobiomodulation, Photomedicine, and Laser Surgery), which corroborated the selected parameters: 660 nm, 9 J, in point and contact mode.

Another determining factor in choosing these parameters was the recommendation of the manufacturer of the equipment used. The Therapy EC device, from DMC, has automation for the application of red light, which operates at 660 nm (± 10 nm).[33] When selecting the 9 J energy, the device automatically adjusts the application time to 90 seconds, emitting an audible signal at the end of each cycle to indicate the need to change the point on the wound. This protocol is supported by DMC's research center, NUPEN, which recommends the use of methylene blue as a photosensitizer and the weekly application of red light at the power and parameters mentioned above.[34]

It is worth noting that, in the context of PDT, the energy in Joules (J) is the product of the power (W) by the irradiation time (s), according to the formula: E(J)=P(W)×t(s). In the case of the protocol used, a dose of 9 J distributed in 90 seconds implies that the device operates with an average power of 0.1 W (100 mW). The factory default of Therapy EC states that the useful power of the emitter is 100 mW, corresponding to the energy-time relationship automatically adjusted by the device.[33]

Additionally, the chosen parameters were also based on the best interaction with the photosensitizer used, methylene blue. This is a second-generation phenothiazine photosensitizer widely used in PDT, due to its high efficiency in generating singlet oxygen ($^1O_2$). A study conducted indicates that methylene blue presents the best quantum yield between the wavelengths of 600–900 nm.[35] However, wavelengths above 760 nm fall into the infrared spectrum, which reaches deeper tissues, while application to wounds requires light in the red range near 600 nm for greatest effectiveness.[36]

Through literature surveys, it was also identified that methylene blue is the most widely used photosensitizing agent due to its good availability on the market and low record of adverse events reported after application to the wound bed. Although a higher concentration of methylene blue (1%) was chosen compared to the literature, previous studies of patients with DFU in Brazil used a concentration of 2% and recorded no adverse events.[33]

Therefore, the adopted parameters not only follow scientific evidence, but also consider the technical characteristics of the equipment and the ideal interaction with the photosensitive agent, ensuring greater safety and efficacy in the application of PDT in the treatment of foot ulcers in people with DM.

For the clinical applications of PDT to evolve, studies comparing PDT directly with standard techniques and a placebo/pseudo-intervention PDT are needed, along with more objective clinical assessment methods, to provide useful data for clinically relevant PDT protocols.[34]

A more objective method for clinical evaluation of therapy is the biopsy of the lesion for microbiological analysis. A recent study that compared the aspiration technique with biopsy concluded that biopsy is the most effective and sensitive method for identifying the causative microorganisms in skin lesions.[29] Therefore, in this study a biopsy procedure will be performed by a physician to identify microorganisms before and after treatment with PDT.

In addition to the microbiological analysis, weekly monitoring of the clinical evolution of the lesion is relevant. Recent research on DFU cases that used the Bates-Jensen Wound Assessment Tool which demonstrated that PDT promoted improvements in the size of the lesion.[35] This can be related to the fact that the reduction of microorganisms present in the lesion provides a moisture balance and less friable and hemorrhagic environment, favorable for granulation tissue and healing progress.

In addition to these clinical benefits, the mechanism of action of PDT extends beyond antimicrobial action. The interaction of red light with the photosensitizer methylene blue generates reactive oxygen species, such as singlet oxygen, which contribute to microbial destruction and modulation of the inflammatory response. However, red light at 660 nm can additionally influence cellular components, such as mitochondria, stimulating ATP production and improving cellular repair processes. A systematic review study conducted by Nesi-Reis et al. (2018)[40] suggests that red light can also impact leukocytes and macrophages, which are crucial for controlling inflammation and promoting tissue repair. These combined effects—microbial reduction and biostimulation—create a favorable environment for the formation of granulation tissue and the progression of healing.

However, it is important to consider that PDT should not be performed in isolation. It is essential to implement basic measures to progress healing, such as wearing appropriate

footwear to relieve plantar pressure, glycemic control, adherence to hygiene measures and strategies to strengthen self-care and self-management of the underlying disease.

The limitation of the study is the lack of assessment of the microbial load of DFU. Therefore, future studies should consider the possibility of applying PDT, with biopsy analysis, in partnership with a microbiological analysis laboratory that can support not only the identification of microorganisms but also counting of the microbial load.

ACKNOWLEDGMENTS

I, the first author, would like to express my gratitude to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the funding granted to postgraduate programs in Brazil, which are free of charge and allow the development of academic research in the country. I would like to thank the National Council for Scientific and Technological Development (CNPq) for supporting the productivity grant (process no. 140675/2021-3).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Research Ethics Committee of the Ribeirão Preto School of Nursing and Hospital das Clínicas of the Ribeirão Preto School of Medicine, São Paulo, Brazil.

FUNDING

The authors received no funding for this research.

REFERENCES

1. Voelker R. What are diabetic foot ulcers? JAMA. 2023;330(23):2314. doi:10.1001/jama.2023.17291
2. Silva AAS da, Castro AA, Bomfim LG de, et al. Amputações de membros inferiores por Diabetes Mellitus nos estados e nas regiões do Brasil. Research, Soc and Devel. 2021;10(4):e11910413837. doi: 10.33448/rsd-v10i4.13837
3. Walicka M, Raczyńska M, Marcinkowska K, Lisicka I, Czaicki A, Wierzba W, Franek E. Amputations of Lower Limb in Subjects with Diabetes Mellitus: Reasons and 30-Day Mortality. J Diabetes Res. 2021 Jul 24;2021:8866126. doi: 10.1155/2021/8866126. PMID: 34350296; PMCID: PMC8328738.
4. Andrade LL de, Carvalho G de CP, Valentim FAA de A, et al. Characteristics and treatment of diabetic foot ulcers in an ambulatory care. R de Pesq: cuidado é fundamental. 2019;11(1):124–8. doi: 10.9789/2175-5361.2019.v11i1.124-128
5. Ananian CE, Davis RD, Johnson EL, Regulski MJ, Reyzelman AM, Saunders MC, Danilkovitch A. Wound closure outcomes suggest clinical equivalency between lyopreserved and cryopreserved placental membranes containing viable cells. Adv Wound Care (New Rochelle). 2019 Nov 1;8(11):546-554. doi 10.1089/wound.2019.1028
6. Fuchs C, Stalnaker KJ, Pham L, et al. Unique Physiology And Intrinsically Delayed Wound Healing In Plantar Skin. WHS Annual Meeting. Wound Healing Society. 2022. Available from: https://woundheal.org/Meeting/Program/2022/O2.01.cgi
7. ShankarRao AG, Behera PK, Tripathy KP, et al. Clinico-microbiological profile and culture sensitivity pattern of micro-organisms isolated from diabetic foot ulcers: study from a tertiary care centre. J Assoc Physicians India. 2022;70(4):11–12.
8. Afonso AC, Oliveira D, Saavedra MJ, et al. Biofilms in diabetic foot ulcers: impact, risk factors and control strategies. Int J Mol Sci. 2021;22(15):8278. doi: 10.3390/ijms22158278
9. Li X, Kou H, Zhao C, et al. Efficacy and safety of ALA-PDT in treatment of diabetic foot ulcer with infection. Photodiagnosis Photodyn Ther. 2022;38:102822. doi: 10.1016/j.pdpdt.2022.102822
10. Aureliano PMC. Efeitos biológicos produzidos pela terapia fotodinâmica na reparação de feridas diabéticas em humanos. 127 f. Tese (Doutorado) – Curso de Programa de Pósgraduação em Engenharia Biomédica, Universidade Brasil, São Paulo, 2017. Available from: https://www.universidadebrasil.edu.br/portal/_biblioteca/uploads/20200316195511.pdf
11. Ning X, He G, Zeng W, et al. The photosensitizer-based therapies enhance the repairing of skin wounds. Front Med (Lausanne). 2022;9:915548. doi: 10.3389/fmed.2022.915548
12. Warrier A, Mazumder N, Prabhu S, et al. Photodynamic therapy to control microbial biofilms. Photodiagnosis Photodyn Ther. 2021;33:102090. doi: 10.1016/j.pdpdt.2020.102090.
13. Brandão MGSA. Terapia fotodinâmica no tratamento de úlceras infectadas nos pés de pessoas com diabetes mellitus: revisão sistemática e metanálise. 77 f. Dissertação (Mestrado) – Curso de Programa de Pós-Graduação em Enfermagem, Instituto de Ciências da Saúde - ICS, Universidade da Integração Internacional da Lusofonia Afro-Brasileira – Unilab, Redenção, 2020. Available from: https://repositorio.unilab.edu.br/jspui/bitstream/123456789/1995/1/MARIA%20GIRLANE%20SOUSA%20ALBUQUERQUE%20BRAND%c3%83O%20Disserta%c3%a7%c3%a3o.pdf
14. Lorenzoni V, Chiavetta A, Curci V, et al. New perspective to improve care of patients with infected diabetic foot ulcer: early economic impact of the use of photodynamic therapy with rlp068 (based) system. Clinicoecon Outcomes Res. 2021;13:135-144. doi: 10.2147/CEOR.S274897
15. Brandão MGSA, Ximenes MAM, Sousa DF, et al. Photodynamic therapy for infected foot ulcers in people with diabetes mellitus: a systematic review. Sao Paulo Med J. 2023;141(6):e2022476. doi: 10.1590/1516-3180.2022.0476.27022023
16. International Working Group on the Diabetic Foot (IWGDF). Guidelines on the prevention and management of diabetic foot disease. IWGDF Guidelines. 2019. Available from: https://iwgdfguidelines.org/guidelines/guidelines/
17. Lipsky BA, Senneville É, Abbas ZG, et al. International Working Group on the Diabetic Foot (IWGDF). Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). Diabetes Metab Res Rev. 2020;36(sup1):e3280. doi: 10.1002/dmrr.3280.
18. Hou C, Zhang L, Wang L, Zhao S, Nie J, Lv M, Zhang W, Su X, Tian S, Li Y. A meta-analysis and systematic review of photodynamic therapy for diabetic foot ulcers. Photodiagnosis Photodyn Ther. 2024;48:104228. doi: 10.1016/j.pdpdt.2024.104228.
19. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200–207. doi: 10.7326/0003-4819-158-3-201302050-00583
20. Tardivo JP, Adami F, Correa JA, Pinhal MA, Baptista MS. A clinical trial testing the efficacy of PDT in preventing amputation in diabetic patients. Photodiagnosis Photodyn Ther. 2014;11(3):342–350. doi: 10.1016/j.pdpdt.2014.04.007
21. Tardivo JP, Baptista MS, Correa JA, et al. development of the Tardivo Algorithm to predict amputation risk of diabetic foot. PLoS One. 2015;10(8):e0135707. doi: 10.1371/journal.pone.0135707

22. Gil AC. Como elaborar projetos de pesquisa. 7th ed. Barueri, Altas, 2022.
23. Hulley SB, Cummings SR, Browner WS, et al. Delineando a pesquisa clínica. Editora Grupo A. 2015.
24. Polit DF, Beck CT. Fundamentos de Pesquisa em Enfermagem. 9th ed. Porto Alegre, Artmed, 2019.
25. Alves DF dos S, Almeida AO de, Silva JLG, et al. Translation and adaptation of the Bates-Jensen Wound Assessment Tool for the brazilian culture. Text Context Nursing. 2015;24(3):826–833. doi: 10.1590/0104-07072015001990014
26. McClary KN, Massey P. Ankle Brachial Index. [Updated 2023 Jan 16]. In: StatPearls,Treasure Island (FL), 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544226/
27. Eduardo CP, Bello-Silva MS, Ramalho KM, et al. Terapia fotodinâmica como benefício complementar na clínica odontológica. Rev Assoc Paul Cir Dent. 2015; 69(3): 226–235.
28. Moura JPG, Brandão LB, Barcessat ARP. Estudo da terapia fotodinâmica (PDT) no reparo de lesões teciduais: estudo de casos clínicos. Est. Científica (Unifap), 2018; 8(1):103–110.
29. Tokarski J, Alves DC, Gioppo N, et al. Aspirado versus biópsia para o diagnóstico de feridas infectadas. Rev Enferm Atual In Derme. 2022; 96(38):e–021227.
30. Carvalho R, Paulino A, Alves A, et al. Tradução das recomendações do "International Working Goup on the Diabetic Foot" (IWGDF) pelo Grupo de Estudos de Pé Diabético (GEPED) da Sociedade Portuguesa de Diabetologia. Rev Port Diabetes. 2020;15(2): 58–69.
31. Pantò F, Adamo L, Giordano C, Licciardello C. Efficacy and safety of photodynamic therapy with RLP068 for diabetic foot ulcers: a review of the literature and clinical experience. Drugs Context. 2020;9:2019-10–13. doi: 10.7573/dic.2019-10-3
32. Cardoso VS, de Souza Lima da Silveira PR, Dos Santos CM, da Rocha RB, Hazime FA. Dose-response and efficacy of low-level laser therapy on diabetic foot ulcers healing: Protocol of a randomized controlled trial. Contemp Clin Trials. 2021;110:106561. doi: 10.1016/j.cct.2021.106561
33. Tardivo JP, Correa JA, Pinhal MAS, et al. A cohort study to evaluate the predictions of the Tardivo Algorithm and the efficacy of antibacterial photodynamic therapy in the management of the diabetic foot. Eur J Med Res Clin Trials. 2022;4:1–11. doi: 10.17303/ejmrc.2022.4.102
34. Boltes Cecatto R, Siqueira de Magalhães L, Fernanda Setúbal Destro Rodrigues M, Pavani C, Lino-Dos-Santos-Franco A, Teixeira Gomes M, Fátima Teixeira Silva D. Methylene blue mediated antimicrobial photodynamic therapy in clinical human studies: The state of the art. Photodiagnosis Photodyn Ther. 2020;31:101828. doi: 10.1016/j.pdpdt.2020.101828
35. Ferreira, R.C. Terapia fotodinâmica como coadjuvante no processo de cicatrização do pé diabético: série de casos. 2022. 82 f. Tese (Doutorado). Programa de Pós-Graduação em Biofotônica Aplicada às Ciências da Saúde. Universidade Nove de Julho, São Paulo. Available from: https://bibliotecatede.uninove.br/bitstream/tede/3057/2/Rita%20de%20C%C3%A1ssia%20Ferreira.pdf
36. Tsai SR, Hamblin MR. Biological effects and medical applications of infrared radiation. J Photochem Photobiol B. 2017;170:197-207. DOI:10.1016/j.jphotobiol.2017.04.014
37. Tardivo JP, Correa JA, Pinhal MAS, et al. A Cohort Study to Evaluate the Predictions of the Tardivo Algorithm and The Efficacy of Antibacterial Photodynamic Therapy in The Management of the Diabetic Foot. Eur J Med Res Clin Trials 4: 1-11. Available from: https://repositorio.usp.br/directbitstream/99fc9a31-1d92-4411-80d1-873b2b640988/3112259.pdf
38. Boltes Cecatto R, Siqueira de Magalhães L, Rodrigues MFSD, et al. Methylene blue mediated antimicrobial photodynamic therapy in clinical human studies:The state of the art. Photodiagnosis Photodyn Ther. 2020 Sep;31:101828. DOI: 10.1016/j.pdpdt.2020.101828
39. Ferreira, R.C. Terapia fotodinâmica como coadjuvante no processo de cicatrização do pé diabético: série de casos. 2022. 82 f. Tese (Doutorado). Programa de Pós-Graduação em Biofotônica Aplicada às Ciências da Saúde.Universidade Nove de Julho, São Paulo. Available from: https://bibliotecatede.uninove.br/bitstream/tede/3057/2/Rita%20de%20C%C3%A1ssia%20Ferreira.pdf
40. Nesi-Reis V, Lera-Nonose DSSL, Oyama J, et al. Contribution of photodynamic therapy in wound healing: A systematic review. Photodiagnosis Photodyn Ther. 2018;21:294-305. DOI:10.1016/j.pdpdt.2017.12.015

APPENDIX 1

Standard operational procedure

Treatment of foot ulcers in the intervention group

Material for the procedure
Personal protective equipment (PPE) - disposable procedure gloves; sterile gloves; coat, mask; beanie; protective glasses for LASER light. Cleaning and dressing procedure - sterile dressing package; sterile gauze; 0.9% saline solution; plastic bag to discard the dressing; blades for debridement (number15 or 22), adhesive tape/micropore/bandage; absorbent cover. Injury assessment - disposable paper ruler and smartphone for photographic recording. Photodynamic therapy procedure - LASER device, disposable 3ml pipette, transparent film and methylene blue (1%).
Procedure
1. Perform hand hygiene with liquid soap and water and put on the necessary PPE
2. Put on procedure gloves
3. Remove the dirty dressing and discard it
5. Clean the wound with 0.9% saline solution
6. Dry with sterile gauze pads if necessary
7. Perform conservative instrumental debridement with blades for debridement (number15 or 22), if necessary

8. Measure, assess and photograph the lesion (all treatment sessions)
9. Perform punch biopsy procedure (1st session and one week after the 6th session)
10. Drip 1% methylene blue solution over the entire length of the lesion and wait for five minutes
11. Remove excess methylene blue solution with sterile gauze if necessary
12. Protect the LASER tip with plastic wrap
13. Put on LASER light protection glasses
14. Irradiate the lesion with the LASER, according to the following parameters Wavelength: 660nm; Irradiation mode: point and contact; joules per point: 9J/cm^2; time per point: 90 seconds
15. Cover with absorbent cover (calcium alginate)
16. Add sterile gauze, padding the wound
17. Fix the dressing with a crepe bandage and tape
18. Discard the material
19. Remove gloves and perform hand hygiene
20. Deliver the dressing to the participant and provide instructions for changing the dressing at home

APPENDIX 2

Standard operational procedure

Treatment of foot ulcers in the control group

Material for the procedure
Personal protective equipment (PPE) - disposable procedure gloves; sterile gloves; coat, mask; beanie; protective glasses for LASER light. Cleaning and dressing procedure - sterile dressing package; sterile gauze; 0.9% saline solution; plastic bag to discard the dressing; blades for debridement (number15 or 22), adhesive tape/micropore/bandage; absorbent cover. Injury assessment - disposable paper ruler and smartphone for photographic recording. Treatment - LASER device, silicone occluder, disposable 3ml pipette, transparent film and methylene blue (1%).
Procedure
1. Perform hand hygiene with liquid soap and water and put on the necessary PPE
2. Put on procedure gloves
3. Remove the dirty dressing and discard it
5. Clean the wound with 0.9% saline solution
6. Dry with sterile gauze pads if necessary
7. Perform conservative instrumental debridement with blades for debridement (number15 or 22)
8. Measure, assess and photograph the lesion (all treatment sessions)
9. Perform punch biopsy procedure (1st session and one week after the 6th session)
10. Drip 1% methylene blue solution with a pipette over the entire length of the lesion and wait for five minutes
11. Remove excess methylene blue solution with sterile gauze if necessary
12. Protect the LASER tip with plastic wrap and place the silicone occluder on the tip of the device to prevent light from escaping
13. Put on LASER light protection glasses
14. Calibrate the LASER with the following parameters, so that the device's sound pattern is the same in both groups, despite the tip being occluded. Wavelength: 660 nm; irradiation mode: point and contact; joules per point: 9J cm^2; time per point: 90 seconds
15. Cover with absorbent cover (calcium alginate)
16. Add sterile gauze, padding the wound
17. Fix the dressing with a crepe bandage and tape
18. Discard the material
19. Remove gloves and perform hand hygiene
20. Deliver the dressing to the participant and provide instructions for changing the dressing at home

APPENDIX 3

Standard operating protocol

Collection of material for biopsy

Material for the procedure
Biopsy procedure - lidocaine 2% with adrenaline (epinephrine) 1:200.000 solution for injection; punch 3 mm; 3ml syringe; 21g needle (0.8 x 25mm); 30G needle (0.3 x 13mm); blade no. 15; sterile gloves; sterile surgical gown, pack of sterile gauze and cotton, 50 ml sterile collection pot and 10 ml 0.9% saline solution.
Procedure
1. Hand hygiene with soap and water
2. Put on a sterile gown
3. Put on sterile gloves
4. Organise and prepare the material inside the sterile field
5. Place the fenestrated field over the foot, helping to isolate the area to be biopsied
6. Anesthetise the lesion with 2–2.5 ml of lidocaine 2% with adrenaline (epinephrine)
7. Introduce the punch through rotary movements, carried out in both directions
8. Hold and gently lift the biopsied fragment with tweezers or a needle and section its base at the deepest portion with a number 15 scalpel blade
9. Place the tissue fragment in a plastic collection bottle with 10 ml of physiological solution (0.9%)
10. Perform hemostasis with mechanical compression of the biopsied site with sterile cotton
11. Remove the fenestrated field, discard the material and wash your hands
12. Request and place the hospital's standard identification label on the sample vial
13. Send the sample to the microbiology laboratory