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PROTOCOL

CLINICAL INVESTIGATION OF MEDICAL DEVICE

Title of the Study:	Multi-centre, randomized controlled, prospective study on the speed of healing, life quality and cost-effectiveness of the treatment with the blue light medical device EmoLED vs a standard treatment for leg ulcers (L.U.C.E. Study)
Reference number or identification code of the clinical investigation:	*EmoLED_003*
Protocol version number:	*3.0*
Issue date:	*24/02/2020*
Sponsor:	*EMOLED Srl* *Via della Cernaia, 5 – 50129 Firenze (FI)*
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APPROVAL OF THE PROTOCOL

The Investigators involved:

- approve this protocol
- declare that the study will be conducted according to GCP, UNI EN ISO 14155:2012 and in accordance to this protocol.

Dott. M. Romanelli — 18/06/2021 — Data

Dott. P. Baù — 04.06.2021 — Data

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Information about the medical device/s

The device used in this study is a CE marked phototherapy medical device (emitting blue light) for the treatment of skin lesions. It is a IIa class portable device, internally powered. The trade name is EmoLED (product code 9800010001), the version is V.1 and it includes a software (version 3.0) that operates the applications. The accessories provided are: safety glasses for the operator, charging adapter for the internal batteries, a shield for visual comfort and the device's case. The supplier is EMOLED S.r.l.

EmoLED is CE marked as a phototherapy device for the treatment of skin lesions. It is intended for use by medical personnel in a hospital or outpatient setting. The patients' demographic consists of individuals with skin lesions that are at least 16 old, regardless of their ethnicity. EmoLED is conceived and designed to treat chronic and acute lesions of the skin by healthcare professionals such as doctors and nurses operating in the vulnology; in particular the EmoLED treatment is part of the wound bed preparation.

The EmoLED treatment is additional to the conventional treatment, that consists in bandaging of lesions and application of conventional or advanced medications. The recommended posology is of one treatment for each dressing change, to be performed after the cleaning of the wound and before the following bandaging.

The device is constituted by a "body" to hold, containing the battery pack, the motherboard and a touch screen, and by a rotating arm containing the optical part and the proximity sensor. The optical head has a cylindrical shape with a 50mm diameter and a 180 degrees rotation. The full rotation is prevented by mechanical means.

The front of the device contains a touch screen display through which it is possible to select the language and register as a user, manage the treatments by entering the size of the lesion and see information related to the treatment such as time and number of applications remained. The screen also displays information about the status of the device, such as the remaining charge and any error messages or warnings.

The casing is equipped with two openings on the left side containing:
- A power jack for connection to the power supply dedicated to charging the batteries;

- A microUSB port to download data related to the use of the device collected by the way during its use. Access to these data is subject to the entry of a security password provided only to authorized personnel.

On the back of the device there is an On/Off button.

The user interface is essential and intuitive, in order to minimize the risks related to usage error. The commands are simple and like "confirm"/"cancel"/"next". The information shown on the screen is easy to understand and the meaning of all the symbols used is given in the appropriate paragraph of the User Manual.

EmoLED is a portable device, powered by rechargeable lithium-ion batteries, non-invasive and non-contact, fast in the treatment and that can be disinfected.
The interface through touch screen allows, asking 2 simple questions to the user, to set the treatment in terms of number of applications; the duration of the individual application and the delivered dose are set at the factory and are not modifiable by the user.

The device is equipped with a movable and rotating part containing the Optical Head, from which the incoherent light beam generated by 6 LEDS that emit in the blue range (between 410 and 435 nm, Optical Power Density 120 mW/cm^2) comes out, in order to facilitate the treatment of the lesions of interest. The

emission of light radiation is subject to the correct positioning of the device towards the lesion: target treatment distance 40 ± 10 mm. The positioning is controlled by an electronic card containing a distance sensor.
The use of the device is excluded when charging the batteries.

Accessories supplied with the device:
- Power supply;

- Screen for visual comfort;

- UV filter glasses.

The visual comfort screen is located just behind the light beam area and provides physical protection against emitted components that can prove troublesome when reflected. The charger is a 24 V power supply with an integrated cable to connect the device and a separable cable to connect to the network. On request, protective glasses with UV filtration and Optical Density (OD) of 4+ up to 400 nm and at least 2+ between 400 and 460 nm are provided. These PPE are recommended for both the doctor and for all people within a radius of 1 meter from the source of light. The glasses available are the 450#53 model of the NOIR Laser Company.
The device is supplied by manufacturer with certified quality management system, traceability is guaranteed by the manufacturer's system which, through the serial number, is able to know both where the device in question is located, and the details of the internal components of the device.
The EmoLED device is very simple and intuitive in its use, the user manual illustrates all the steps to be taken for proper use. Moreover, the health personnel that is going to use the device participates in a training session organised by the manufacturer in which the operation of the device is explained and a demonstration test is carried out. EMOLED staff is always available for any questions, requests and/ or assistance.
The blue light emitted by the device interacts with the endogenous chromophores of the skin, which trigger reactions that lead to the activation of certain cellular pathways. In particular, the wavelength emitted by the device is absorbed by Protoporphyrin IX present in Cytochrome C, an essential protein for cellular respiration at the mitochondrial level. The energy absorbed in this way is used by the cell to increase the production of ATP, a fundamental molecule for all the processes involved in tissue repair. In addition, blue light is able to stimulate the production of ROS (Reactive Oxygen Species) through the excitation of flavins and flavoproteines. Nowadays, multiple evidence has been produced about how ROS can be considered signal transducers of numerous cellular pathways. This consideration validates the evidence that ROS (at physiological concentrations) are crucial for multiple cellular functions such as differentiation, proliferation, migration and contraction.

Literature review and rationale of the clinical investigation

Chronic diseases, such as the development of ulcerative lesions, related to the ageing population, now tend to increasingly burden the NHS and, indirectly, also the families of patients, which are faced with problems such as, in addition to the care of the patient, also the lack of productivity (and profit) due to the need to leave work to accompany the patient to the doctor or to do the therapy.
This and many others are the things to consider, besides the purely medical and biological aspect, when the full impact of a disease on the patient and his family is assessed, before than on the entire population.
Wound healing is a complex and dynamic biological process that includes a series of organized phases,

including coagulation, inflammation, matrix deposition, angiogenesis, proliferation, cell remodeling and wound contraction (1, 2). So that each step of the
process is completed, complex interactions between various biological factors need to occur, such as growth factors and proteinases, matrix components and various cell types, such as platelets, macrophages, fibroblasts and endothelial cells (3). The interaction of these processes as a whole determines the restoration of tissue integrity and functional healing (4). If on the one hand it is generally accepted that, in most cases, acute wounds can heal by following the orderly sequence of events just described within 2-4 weeks, on the other one sometimes, due to alterations in the course of the natural process of healing, you can create a pathological scar (hypertrophic scar or keloid), or you can have a stop in the healing process at various levels of the regenerative path, which determines a failure in the healing of the affected area and the consequent development of chronic skin wounds. Many can be the factors contributing to the occurrence of such eventualities: some of these are known, such as
diabetes, venous or arterial insufficiency, infections, metabolic deficiencies and the advanced age of the subject, others are still under study.
Therefore the proper management and dressing of the wound after a surgical act, a trauma or a pathological process is an important part of the healing process, not only to prevent the onset of infections or other complications, but also to speed up the healing of the wound itself with as less scarring as possible.
Although much has been done towards the complete understanding of the processes that regulate this important biological function, there are many aspects that still need to be clarified. At the same time, if it is true, on the one hand, that technological advances have led to the creation of increasingly sophisticated medications, it is equally true, on the other hand, that there is a need to find new methods to further improve the healing process, regardless of the event causing the skin injury, reducing the pain and discomfort associated with the medication itself, possibly shortening the time needed to recovery, with relative reduction of related costs.
The purpose of this study is to compare two homogeneous groups of patients following different therapies:
- Group 1: Standard treatment of two visits per week with cleansing and dressing of the wound;
- Group 2: Experimental treatment group represented by a weekly visit during which the EmoLED treatment is added to the standard treatment in order to assess the clinical efficacy of the two therapies and their impact on economy and quality of life (QoI).
The EmoLED device has received the CE mark (certificate no. G1 18 02 99242 002) and was used, in its prototype version, in three different clinical trials. The first two studies analyzed the effectiveness of EmoLED on surgical lesions (acute), the third study, currently underway, on the basis of some spontaneous observations of professionals in the field, assesses the effectiveness of continuous therapy on chronic injuries.

The clinical safety of the device has therefore been assessed in previous studies which have shown the not inferiority of treatment with EmoLED compared to other advanced therapies.
In the context of this study, venous or mixed skin ulcers will be considered. The application of the treatment in details will be explained later, however the treatment with EmoLED consists in radiating the lesion for 60 seconds with the light emitted by the device.
This treatment does not interfere with other systemic therapies that may be in place, nor does involve any additional risk compared to the standard treatment; the potential benefit is attributable to a reduced time of healing, which is followed by the consequent indirect benefits.

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Principle of operation of the EmoLED medical device to help wound healing

The EmoLED device is a medical device for the healing of wounds, CE marked, whose operation is based on LED sources operating in the blue range.
The choice of blue light was made on the basis of Anderson and Parrish's theory of selective photothermolysis which states that selective heating of a target chromophore is achieved when the wavelength of the source - our LEDS - is preferentially absorbed by the chromophore - the Protoporphyrin IX of the heme group and of the Cytochrome C.
As for the use of light in medicine, the scientific literature is rich in data and evidence on its use for the treatment of acne (5) and psoriasis (6) and its effectiveness in accelerating the process of healing of an induced wound on animal models (7), (12).
When considering the absorption coefficients of the skin chromophores in the visible light range, we note that hemoglobin has the peak of maximum absorption in the range of blue: around 410 nm for oxyhemoglobin (Hbo2) and around 430 nm for deoxyhemoglobin (Hb). This particular property is exploited to induce a localized temperature increase in order to stimulate a rapid coagulation effect. The use of the selected wavelength also ensures the circumscription of the thermal effect only to the areas where hemoglobin is present, limiting the effect on the healthy tissues surrounding the wound thanks to the reduced absorption of the other chromophores of the skin (11).
A way through which EmoLED can act in tissue repair is the chain of mitochondrial electronic transport. EmoLED in particular can act on the last two complexes that contain Cytochrome C, that is sensitive to visible light in the range of emission of the device.
The resulting effect is the strengthening of this process and the increase in ATP production, related to the development of a proton gradient dependent on the electron transport chain.

The increase of ATP production determines an increment of the available energy for the cell that can intensify its metabolic activity, a necessary process during the repair of an injury that involves activation of different cell types and an additional energy effort for the organism.
The device has been designed and set to deliver a power density of about 7 J/cm2 in an acceptable treatment time for both doctor and patient: 60 seconds for each circular area of 5 cm diameter that needs to be treated.
The choice of the duration of the treatment was also made on the basis of what is stated in literature on thermal damage: the treatment induces a temperature between 45 and 50 º C in the treated area, ideal condition to stimulate the reversible and physiological phenomena that we want to induce.
On (13) page 78 there is an attached table showing the physiological phenomena induced by temperature:

Table 3.6. Thermal effects of laser radiation

Temperature	Biological effect
37°C	Normal
45°C	Hyperthermia
50°C	Reduction in enzyme activity, Cell immobility
60°C	Denaturation of proteins and collagen, Coagulation
80°C	Permeabilization of membranes
100°C	Vaporization, Thermal decomposition (ablation)
> 150°C	Carbonization
> 300°C	Melting

The same volume shows a curve that links the duration of the thermal stimulus with the reversibility of the process induced by this stimulus.

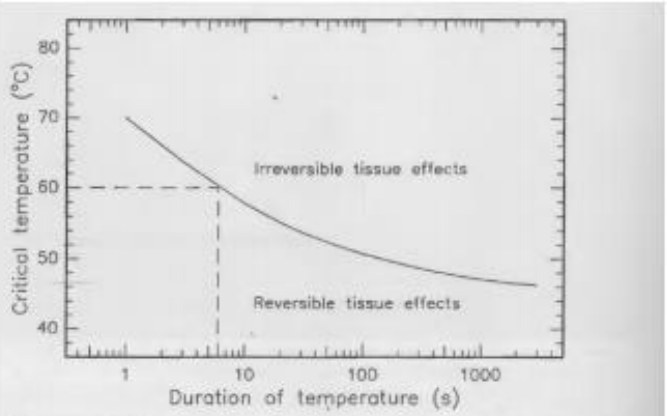

Fig. 3.21. Critical temperatures for the occurrence of cell necrosis. Data according to Henriques (1947) and Eichler and Seiler (1991)

This data on the physiological effects of temperature was the first constraint for the definition of treatment parameters.
In addition to studies related to the influence of temperature, the decision of treatment time is based on a finite element model (14) which correlates optical power, treatment time and final temperature induced by the treatment with the device in question. The initial model led to establish the conditions of optimal work, that were then tested in an animal model study.
The animal study, as reported in the article, allowed us to verify the effectiveness of the treatment sized this way relatively to the standard on an abrasion-induced wound with mechanical abrader on the back of the animal. The observations that were made showed the absence of thermal damage in the areas adjacent to the wound and confirmed that only one treatment is sufficient to trigger and accelerate the process of wound healing.
The mechanisms described above are in line with what is directly observed in a preclinical study on animal model, which is part of the Lightpatch project in the context of the BiophotonicPlus call 2012, where a fiber-optic version of the EmoLED device was used to treat injuries caused by abrasions on the back of CD1 albino male mice. Previously, two clinical studies on acute lesions - skin sampling areas - were carried out, along with one study
on chronic injuries, currently in progress.
The first study performed with EmoLED v.0 devices (completely analogous to the v.1 devices regarding the type of energy and power emitted) was performed on 20 patients afferent at the Great Burns Centre AOUP - Santa Chiara Hospital in Pisa. The collection areas were divided into two not bordering sub-areas of the same size (10x10 cm), one of which treated only with standard therapy – or application of dressing and bandage - while the second was treated with EmoLED for 30 seconds immediately after skin exportation. The results showed, in the 15 days of observation, a tendency to heal faster in the treated area compared to the control area.
The second study was carried out on 20 patients belonging to the Santa Maria Annunziata Hospital in Ponte a Niccheri - regional reference center for melanoma - in Florence. Again patients needed an autologous skin transplant, but they were not hospitalized, so they went to the clinic for a visit every 3 days for the 15 days of observation. The results are currently under development, but from a first analysis it emerges that the lesions treated with EmoLED are healed better and have had fewer complications.
A multicentric study on chronic leg ulcers is currently ongoing. The study involves the recruitment of 90 patients who, as in the previous cases, have a second control lesion, or

have only one particularly extensive lesion, which is only half treated. In this case data are still not available as recruitment is at 25% and just a small number of patients completed the 10-week study. However, no unexpected side effects or adverse events were found.
In conclusion, due to the fact that no adverse events or side effects have been recorded and injuries treated with EmoLED tended to heal faster and better than control injuries, the risk-benefit ratio can be assessed sharply shifted towards the EmoLED treatment.
The purpose of this study, in addition to the assessment of the effectiveness and safety of the device, is the evaluation of the economic impact due to the introduction of EmoLED treatments in the clinical practice, by also quantifying economically the reduction of healing time and the impact of the therapy on the quality of life perceived by patients.

Objectives of the study

The present clinical trial aims to compare, from the clinical, social and economic point of view, two groups of patients with lower extremity ulcers of venous or mixed aetiology, in the 16-weeks observation period.

Group 1 (or control group) will follow the standard treatment that requires two visits per week. More specifically, the treatment consists of: dressing change, cleansing and eventual debridement of the lesion, the topic treatment and compression bandage.

Group 2 (or experimental EmoLED protocol group) will be visited once a week. Therapy in this case includes, in addition to the standard therapy, a treatment with EmoLED device; it consists in irradiating each 5 cm diameter area of the lesion for 60 seconds, with the blue light emitted by the device. For lesions larger than 5 cm, several applications will be applied on adjacent areas, until the whole lesion is covered.

EmoLED is a medical device for light therapy (Class IIa), portable and battery-powered. Its intended use is the care of acute and chronic skin lesions by healthcare staff on patients over 16 years old, regardless of their ethnicity. Related adverse events, incidents or side effects haven't been observed during previous clinical trials and clinical experiences; therefore, no risks are reported in addition to the ones related to the use of light in medicine. However, the device is labelled and written instructions are provided for a safe use.

The objective of the study is to assess eventual differences in the outcome between the two groups and to determine if the therapy of Group 2 is a valid alternative to the standard therapy in terms of healing rate, quality of life and economic impact:

- The primary endpoint of the study is the percentage/rate of lesion area reduction
- The secondary endpoints are, together with other clinical parameters aimed at evaluating the general clinical situation, quality of life and costs related to the two treatments.

There is a follow-up after 4 weeks since the end of the period of observation (it may end at the 16th week or earlier).

Ethical considerations

The study will be submitted to the Ethical Committee of each investigator hospital for approval and in any case will be performed according to the GCP, the Helsinki Declaration and the Italian laws and requirements in the matter of human clinical trials.

Informed consent procedure

The Principal Investigator (PI) or their delegate selects the enrolled patients on the basis of the inclusion and exclusion criteria of the study. Once the patient's correspondence with the study's criteria has been verified, the PI or their delegate asks the patient for consent to use the EmoLED treatment device, and briefly explains the characteristics of the device, the expected effect, the possible trial-related risks, the guarantees to protect the confidentiality of the collected information. The PI asks the patient if they are interested in participating, leaving them twenty-four hours to decide. If the patient manifests their interest to partecipate in the study, during their next visit they will sign the Informed Consent and will decide whether to inform their family doctor of their participation in the study. The patient must give their availability to return to the structure every week for the trial observation time, including the follow up visit.

A copy of the Informed Consent, subscribed by the patient and by the Principal Investigator and a copy of the study information will be given to the patients.

Any news about the trial and/or the medical device under investigation and any eventual amendment to the protocol of clinical investigation will be notified to the patients during the first scheduled visit immediately after the Principal Investigator receives any of them.

Pre-clinical tests and previous clinical experience

Preclinical animal model study observations

Preclinical observations aimed at characterize safety and efficacy of the EmoLED medical device have been preventively performed on murine models. On each anesthetized animal, two wounds (1 cm diameter) were inflicted using an abrasive method. The 30-seconds treatment with EmoLED device has been performed on one of the two wounds, then both were/have been medicated to avoid the development of infections. After the treatment, animals have been placed in individual, thermostat cages, until they fully came out from anesthesia.

The observations reported below were extrapolated from tests performed on biopsies taken at specific time points: after the treatment, each animal was observed over a given follow-up period before being sacrificed, and the areas under study were collected and embedded in a compound suitable for cryosectioning (frozen section). They were later used both for histological and immunohistochemical analyses. A large number of observations were collected and they are reported below by making reference to the observed phenomenon.

Inflammatory infiltrate: based on the data obtained 0, 1, 3, 6, 9, 12, 18 and 24 hours after inflicting the wound, it is noted that within the first few hours the amount of inflammatory infiltrate in the areas treated with the EmoLED device is higher compared to the untreated wound. The difference decreases between 9 and 18 hours until, after 24 hours, the situation is reversed and the amount of inflammatory infiltrate in the untreated wound is higher than that in the treated wound.

Mast cells and Mast Cell Degranulation Index: although no particular differences in the number of mast cells were found between the treated and untreated samples, this was not the case when mast cell activation was studied: in fact, potent degranulation was observed in the treated sample at 3 hours, which was not seen in the wound not treated with the EmoLED device.

Macrophage populations: observations made 0, 3, 6, 9, 18 and 24 hours after inflicting the wound on the populations of M1 macrophages (proinflammatory) and M2 macrophages (pro-healing) show that the anti-inflammatory phase begins 6 hours after inflicting the wound in animals treated with the EmoLED device,

whereas it is necessary to wait 18 hours for the inflammatory phase to end with the standard treatment. Moreover, after 18 and 24 hours, both the M1 and M2 populations are comparable in the treated and untreated samples, demonstrating that treatment with the EmoLED device does not induce responses other than normal physiological reactions.

Based on these studies, it can be concluded that the EmoLED device exerts its effect within the first few hours after wound infliction (and therefore within the first few hours after treatment). Our hypothesis is that treatment with the device exerts its effect early in the inflammatory phase of healing and promotes healing both in terms of time (observable based on the data obtained from the inflammatory infiltrate study) and quality, evident from the significant mast cell activation, resulting in greater histamine release, causing increased activation of various mediators and cell types that may be involved in the healing process.

In-human acute wound study observations and ulcer case study

The safety and efficacy clinical validation to obtain the CE marking for the EmoLED device for the treatment of acute surgical wounds is ongoing. **In particular, the acute observations, whose detailed results are provided in the attached final Report on the clinical trial, have once again demonstrated the device's safety and efficacy in shortening the healing time of spontaneously healing wounds.**

Isolated observations in patients with chronic lesions, of various aetiologies, have shown that the device is capable of unblocking the healing process, thus allowing the wound to progress beyond the inflammatory phase by inducing re-epithelialisation and wound closure. Observations made using a thermal camera have also shown that, due to the compromised condition of the tissues surrounding the ulcers, the baseline temperature of the limb is lower than normal and that both during and after treatment with the EmoLED device, the temperature never exceeds 40°C.

Information related to the clinical investigation

Study design

This is a multi-center prospective, randomized, controlled study aimed to verify the clinical efficacy of a portable battery-powered device blue LEDs based. This study aims to compare the existing SOC (consisting in two visits per week) to a protocol that requires only one visit per week, during which the EmoLED treatment is administered in addition to the current therapy.

Primary and secondary objectives

As further detailed in the section on statistical analysis, it is expected to register, during the time of observation, a difference in efficacy between EmoLED protocol group (“Group 2”) and SOC protocol group (“Group 1”), in terms of “healing rate”, intended as a reduction of the wound area, but also as a progress, in a broad sense, of the overall clinical situation of the lesion, in terms of pain and quality of life.

Endpoint

Primary endpoint of the study

The primary endpoint will be the outcomes comparison in terms of healing rate of lesions treated with SOC only versus lesions treated with EmoLED and SOC, on week 16th.

Secondary endpoint

1) Safety of treatment
2) Evaluation of the healing time of the lesions treated with the standard method (Group 1) versus the lesions treated with the EmoLED Protocol (group 2);
3) Evaluation of the treatment of chronic ulcers in patients undergoing standard treatment versus EmoLED treatment about life quality (QoL) in 16 weeks, through generic questionnaires as the EuroQiL-5D2 and specific questionnaires as the Wound-Qol3;
4) Evaluation of costs related to the treatment and control group, in both the NHS and society perspective
5) Evaluation of VAS scale in both groups;
6) Evaluation of satisfaction, complexity and helpfulness of EmoLED device, considering both the perspective of patients, and the one of doctors and health care workers involved;
7) Evaluation of cost-effectiveness using EmoLED for the treatment of ulcers.

Measurable parameters

The key data and lesion's pictures of each patient will be collected in the Data Collection Form, after the enrollment and the signature of the Informed Consent:

- Group 1: V1 (Visit 1 and first treatment), V2, V3, V4… until V32 (32 treatments are planned during 16 weeks, 2 per week)
- Group 2: V1 (Visit 1 and first treatment), V2, V3, V4… until V16 (16 treatments are planned during 16 weeks, 1 per week)

The parameters used to define the study result will be found through:

- Photographic images of lesions treated with EmoLED and control lesions
- Data Collection Form filled by the medical staff during the visit
- Economic/organizational impact survey
- Medical/clinical/social impact survey
- Survey on the usability of the device
- Detection of pain through VAS scale

Registration of clinical data

The data related to the evaluation of the study endpoints are acquired, for both groups, during the visits.

The parameters of the Data Collection Form are acquired and kept on paper for the clinical trial lenght, and conserved in both paper and digital form; the pictures of the lesion are acquired and kept in digital form, during the length of the clinical trial and afterwards.

During each visit, a picture of the lesion will be taken so that the process of healing can be valued also in a visual way. Each picture will be named with the patient code and the date of the visit. In case of multiple lesions matching with both inclusion and exclusion criteria, they will be all treated following the same protocol, depending on the group of treatment, but only the lesion with the wider area will be registered; to distinguish the area of interest, the perilesional tissue will be labelled with single-use permanent markers.

Data Collection Form

The Data Collection Form is unique for each patient and includes: Clinical Section, Quality Life Section and Economic Section. The Clinical Section contains identification code of the patient, date of birth, sex, etiology of the ulcer, any eventual concurrent disease, any drug therapy (in terms of active substances), ulcer's date of onset, date of entry to the facility.

The second part of the form is organized as a matrix where the row represents the measurable parameters for each time of observation (reported in column).

The investigator marks with an "X" the chosen value for each parameter and the most suitable description. The Principal Investigator will also fill a check list, for each visit, to ensure that also the pain level has been measured and that the picture of the lesion has been taken.

There is also a "Notes" section to register information not planned at first, that might be helpful during the data analysis.

The last part of the Data Collection Form is about follow-up visit (after 4 weeks).

The Data Collection Form is already completed with the patient's recruitment code and the group they belong to, so that the allocation to the EmoLED group and the control group is random. The allocation sequence of the group is generated by Matlab.

On each visit, the patient will be asked to rate their current pain level (VAS scale 0-10).

The quality section survey will be administered on the first and last visits, and also every 4 weeks. In detail, standard questionnaires as the EQ-5D2 and the Wound-Qol3 will be administered, both available and validated in Italian language.

Specific questionnaires for recognition of costs (Economic section) will be administered to patients and their primary caregiver (if present) during the first visit, with the aim of identifying the resources absorbed by the patients (and their caregivers) treated in the two different approaches. The consumption of resources will be measured by gathering information about the quantification of direct health costs (visits, hospitalizations, emergency healthcare, medications etc…), direct non-health-related costs (informal medical assistance, transportation and accommodation costs for each visit etc..) and indirect costs (productivity loss for both the patient and the caregiver/s) and also by taking into account both the costs associated with the adherence to the treatment, taking the first visit as a reference parameter, and the costs related to complications and events associated with the ulcer that determined the need for treatments/ exams and/or medical evaluations. The Principal Investigator will be given also a questionnaire to determine the costs incurred by their facility for handling patients with leg ulcers.

The questionnaires meant to value customer satisfaction, complexity and helpfulness of EmoLED device will be administered to doctors and to healthcare personnel involved in the treatment at the end of the trial.

Image Acquisition

For the 16 weeks of the trial, during each visit a picture of the lesion will be taken, so that the process of healing can be valued also in a visual way. Each picture will be named with the patient code and the date of the visit. The quantitative analysis about the size of the lesions through the collected pictures will be performed at certain time points: at the beginning, in the middle and at the end of the treatment.

The image analysis is made through an appropriate analysis algorithm that measures the area of the lesion (mm^2). The lesion must be fully and frontally framed to obtain an optimal analysis. The ruler with patient's ID code and date must appear in every picture. It is important that the ruler is placed straight and in-focus to have the scale reference. If there is a wide lesion that needs more than one picture it's advisable to

locate several markers around the lesion and take photos of adjacent areas, including at least two visible markers of the previous picture, to have the complete image of the lesion through a sequential photo array.

Bias

The staff participating in the study is trained on the correct use of the device and accessories, as well as on the correct execution of the clinical protocol and on the compilation of the related forms. In any case, the EMOLED staff is always available to the team for any clarification or doubt during the study.
In order to promote a more homogeneous operation between the various centres, joint training sessions are organized and documents which serve as guidelines for the most critical passages or the ones which require particular attention, such as recording, saving and transmitting data, are prepared.
In order to avoid bias during the procedure of allocation in groups of patients enrolled in the study, it should be noted that the randomization grid is not distributed to the investigators: instead, paper folders are distributed containing the data collection forms. The progressive patient ID is on the outside of the folders, while on the inside a label is placed with the assignment of the patient to one of the two groups (Control or EmoLED treatment), which reflects the randomization grid. These folders are closed through an adhesive label with the signature of the Head of Clinical Affairs of the sponsoring company.

Patients selection

All patients that satisfy all the inclusion criteria, both hospitalised and outpatient, will be considered for inclusion in this study.

It will be used a standard procedure to value patients that includes the anamnesis evaluation and a physical examination.

Inclusion criteria

- Subjects suffering from venous and mixed skin ulcers;
- Presence of a lesion < 100 cm^2 of area and < 1 cm in depth;
- Men and women ≥ 18 years old;
- The patient must be able to understand the aims of the clinical trial and provide informed consent in writing;
- Chronicity of the lesion: at least 8 weeks.

Exclusion criteria

- Patients who participated in clinical trials about skin ulcers healing during the previous month;
- Patients who are not able to understand the aims of the trial;
- Patients with pressure ulcers;
- Patients with diabetic foot ulcers;
- Patients with circumferential leg ulcer (due to the difficulties in analysing the pictures);
- Patients with clearly infected ulcers or with systemic infection;
- Patients with ulcers caused by critical ischemia;
- Patients with a self-harm past that can purposely alter the process of healing;
- Patients with psychiatric disorders;

- Pregnancy or breast feeding;
- Patients with neoplasms or other diseases involving the use of cytostatic or immunosuppressive drugs;
- Patients with limited lifespan;
- Patients with photosensitizing illnesses or that take photosensitizing drugs.

All inclusion and exclusion criteria must be satisfied before recruitment. Any concomitant pharmacological therapy must be maintained.

Number of patients expected to enroll
A total of 80 subjects will be recruited, 40 per arm at the facilities involved in the clinical study. Each one of the four sites involved will enroll 20 patients. The expected time for enrollment is 7 months. Since this is a multicentric investigation with competitive recruitment the number of patients per site can vary, depending on the recruitment capability of each site.

Enrollment point
Once the patient has signed the informed consent, the Principal Investigator enters the data in the Enrollment Registry, carefully checking all the inclusion and exclusion criteria; if all criteria are met, the patient is considered to be enrolled in the clinical study.

Number of experimental devices expected to be used
Each experimental site will be equipped with an EmoLED medical device. In the study a total of 4 EmoLED medical devices will be made available.

Time-frame of the study

The study lasts 12 moths, with a recruitment time of 7 months.

Medical and surgical procedures

The lesions will be cleansed with saline solution and, if necessary, surgical debridement will be performed with the most suitable method. At this point, if patient is included in Group 2, the EmoLED treatment starts. After EmoLED application (Group 2) or after cleansing (Group 1), a polyurethane foam dressing will be applied on the lesion. In case of clinical signs of infection, a silver dressing will be applied.

Then an elasto-compressive double layer dressing of the limb with cohesive fixation bielastic latex-free bandage will be carried out.

The treatment with EmoLED, in addition to the SOC, will be performed during each visit for 60 seconds on each 5 cm diameter sub-area of the selected lesion.

In case of multiple lesions matching with both inclusion and exclusion criteria, they will be all treated following the same protocol, depending on the group to which the patient belongs; in this case the Principal Investigator will fill out a data-collection form and take pictures just of the lesion with the wider area.

Known or predictable factors that may impair outcomes and interpretation of outcomes

A different therapy standard between participating centers and/or within the same center can lead to very variable outcomes between one patient and another, all other conditions being equal. This problem is tackled by indicating in detail the therapy to be followed depending on the type of lesion.

An incorrect recording of photographic images does not make it possible to determine the area of the lesion via software. This problem is tackled by providing precise instructions on the correct use of the tools available for the retrieval of images.
Discontinuity of patients can affect recovery times. Before recruiting a patient, the doctor ensures that the patient is available to go, with the required timing, to the clinic, for the scheduled visits. This fact is underlined several times when providing study information to the patient.

Interruption and withdrawal of subjects from the clinical trial

Patients who miss the visits for two consecutive weeks, before/until the 14th week, and the patients who miss the visits for one week, after the 14th week, will be reached by phone by the Principal Investigator to assess their availability to go on with the study; in the event that the patient does not intend to continue with the visits at the centre, they will be excluded from the study. Also, the patient who will turn out to be absent for more than half of the sessions will be excluded as well.

Following the resignation or withdrawal from the study by a patient, their data collection form will be identified, scanned and archived.

If a patient withdraws from the study, the Principal Investigator or his delegate records the event in the Patient Identification Register and on the Data Collection Form and stores all documentation.

Monitoring plan of the clinical investigation

To guarantee the conformity with ICH/GCP guidelines, the Clinical Research Coordinator (CRC) will be responsible for the study to be carried out in full observance of the Standard Operating Procedures, of the Protocol and other written instructions.

The main responsibilities of the CRC are to ensure adherence to the Protocol, to make sure that the data are accurately and fully registered and reported and to verify that the Informed Consent was obtained and registered for each subject before the beginning of the study.

The CRC will keep in touch and meet the Principal Investigator on a regular basis throughout the whole duration of the study. The CRC will be allowed to check the various documents (Data Collection Forms, CRF and other related documents containing original data) related to the study in order to verify the conformity with the Protocol and to ensure the accuracy of recorded data.

Quality assurance, control procedures, data management and record-keeping

All the information gathered during the Study will be considered strictly confidential. The patients' consent to the registration of personal data will be requested at the time of recruitment; nevertheless the data collection forms will contain the Identification Code of the patient and their date of birth only.
In order to guarantee the correct traceability of all parties involved, the number of the Study and the naming of the Center will be included as well.

At the end of the Study, all data will be archived using the appropriate safety measures at the Coordinating Site for a minimum of ten years.

If a patient withdraws their consent to data processing, they will be immediately destroyed to ensure total confidentiality, in addition each investigator will keep a copy of the study's documentation for at least two years after its conclusion.

The Principal Investigator builds and is responsible for the updating and custody of the Patient Identification Register or Enrollment Register.

The Patient Identification Register includes personal data of patients who agreed to take part in the study, the identification number assigned to the patient, their illness, indication of the eventual exclusion from the recruitment with its justification and date of recruitment. The patient code is constituted by a three-digit number: the first one indicates the center's number, as stated in this protocol, and the other two are sequential numbers, starting from 01. Only the Principal Investigator and, at their discretion, the members of their team have knowledge of the identities of patients enrolled in the study.

Deviations from the clinical protocol

In the case of isolated deviations from the protocol, these are registered in the data collection form, which is provided with a space to record any variation or comment the doctor deems appropriate. In case, during the study, a modification to the protocol is deemed necessary, the investigator communicates this need to the sponsor, who convenes the investigator of the coordinator centre, the study coordinator and any other experts in the field, and discusses the proposed variations and, where appropriate, submits a request for amendment to the Ethics Committees to approve the changes deemed necessary.

Adverse Events

Despite all the information in our possession and physical characteristics and performances of the device that do not suggest the possible occurrence of and adverse event, this section of the Protocol takes into consideration the possibility that said event could take place.

An Adverse Event (AE) is any untoward change in the state of health, or side effect, or unexpected benefit that could occur in a patient involved in in a clinical study while they receive the treatment or within a given timeframe following the conclusion of the therapy, not necessarily related to said treatment or clinical experimentation. An AE can consist in any unfavourable and/or unexpected sign or symptoms (including abnormal results from laboratory testing) or a pathology temporarily associated with the use of a medicinal product (subject of the experimentation) or experimental device, that whether or not is related to that medicinal product or device.

The morbid condition of each patient will be monitored the whole time of the study. During each visit any sign manifested by the patient will be carefully observed, in fact, the subject will be encouraged to report any symptom with direct questions, such as: "How have you been feeling since our last visit?". They will also be prompted to spontaneously report any adverse events that occurred during the study.

Any significant clinical anomaly encountered over the course of the study will be monitored and observed until its normalisation, or until it will be clinically explainable.

Serious Adverse Events

A Serious Adverse Event (SAE) is defined as any unfavourable medical occurrence that, at any dosage, will result in any of these consequences:

- Death of the patient
- Any inauspicious outcome in the short term
- Hospitalization or prolonged hospitalization of the subject
- Disability or significant/persistent incapacity
- Other major medical event

Other medical events that do not involve the death of the patient, or do not endanger their life, or do not require the hospitalization of the subject can be also classified as SAE when, according to proper medical assessment, they may constitute a risk for the subject or they may require medical intervention or surgery to prevent one of the outcomes previously described.

Hospitalization is defined as the eventuality in which the subject is withheld (usually for at least a night) in hospital or in the emergency room for observation and/or treatment. Whenever is questioned if the hospitalization took place or if it was necessary, the adverse event must be considered serious.
It is not necessary to consider as an Adverse Event a hospitalization due to elective surgery or due to the implementation of routine clinical procedures that are not consequences of an Adverse Event. If during the procedure an unfavourable eventuality is reported, it shall be considered as an Adverse Event, if Serious or not it will be decided according to customary criteria.

Reporting of Adverse Events or Serious Adverse Events

All the Adverse Events reported by the subject or observed by the Ricercatore or by members of their team will be mentioned on the SAE form. For each Adverse Event must be gathered the following information:

- Date and Time of insurgence and resolution (duration)
- Extent (further defined in the text)
- Treatment eventually requested or measures taken
- Outcome
- Relation with the product used in the experimentation (further defined in the text)
- If the Adverse Event caused the subject to withdraw from the study

All the Adverse Events must be registered using the accepted diagnosis, whenever possible. Any alteration of laboratory results, vital sign, or reported by the physical examination must not be registered as an Adverse Event if it is known that they constitute a symptom or a sign of a diagnosis already reported as it is. For example: an ST segment elevation detected by an EKG and an increase of creatine phosphokinase are both well known signs of myocardial infarction and it will not be necessary to report them if myocardial infarction is already registered ad an Adverse Event. However, if both of them happen separately and a myocardial infarction has not been diagnosed, this eventuality must be registered as an Adverse Event.

Severity Classes and causal relationship of Adverse Events must be defined using the following parameters:

Gravity:

- *Mild* easily tolerated, does not interfere with daily activities
- *Moderate* causes some interference with daily activities
- *Severe* all daily activities are completely compromised

Relationship with procedures required by the study:

- *Not correlated* temporal association among the Adverse Event and the use of the product results in an extremely unlikely association between the two.
- *Possibly correlated* the adverse event occurs with a timing compatible with the beginning of the use of the product employed in the study, but it is also attributable to the clinical condition of the patient or the procedures/condition of the study.
- *Undoubtedly corelated* the adverse event occurs with a timing compatible with the beginning of use of the product employed in the study, recedes with its discontinuation and re-emerges when its reintroduced.

In addition to registration in the Data Collection Form (AE), the Adverse Events considered serious (SAE) that comprehend Suspicious and Unexpected Adverse Reactions (SUSAR) must be reported by the ricercatore in the Serious Adverse Events Report Form. This last document must be compiled and sent immediately to the Clinical Research Coordinator of the study, namely withing twenty-four from the moment the research staff diagnosed an SAE.

Safety follow-up for subjects withdrawn from the study

The subjects that withdrew from the experimentation after the treatment with the device under study for any reason, will undergo a continued monitoring of the Adverse Events in place until the results from the tests related to the Adverse Event, and required by the SOC, will recover to baseline or until the investigator will determine that those events are not clinically relevant anymore. The subjects who present Adverse Events up until thirty days from the last visit or the last follow-up check (whichever of the two eventualities is the latter) will be monitored until their test results are not recovered to baseline, or until the investigator does not determine that the results are not clinically relevant.

Safety follow-up for subjects who have completed the study

All the subject who still present Adverse Events upon completion of the study will be monitored until the test results are not back to baseline, or is no longer expected from them to change, or until the investigator does not determine that those results are no longer clinically relevant.

Amendments to the clinical evaluation plan

If, during the study, a modification to the protocol is deemed necessary the investigator communicates this need to the sponsor, who convenes the investigator of the coordinator centre, the study director and any other experts in the field, and discusses the proposed variations and, where appropriate, submits a request for amendment to the Ethics Committees to approve the changes deemed necessary.

If a substantive amendment request is necessary, the clinical trial will be suspended until approval has been received by the relevant ethics committees.

Early termination and suspension of clinical evaluation

In the event of suspension or early termination of the study by the sponsor, taken as a safety measure due to events related to the conduct of the study that may affect the health of the patients recruited, the promoter is required to notify the Ministry of Health and the Ethics Committees involved within 90 days from the recruitment of the last patient or the early closure. In case of suspension or early termination of the study not for safety reasons, the sponsor shall justify and explain their decision. The suspended clinical trial cannot be reactivated unless a substantive amendment and the positive opinion of the Ministry of Health and the Ethics Committees have been submitted.

Statistics

Calculation of the sample size

80 patients will be recruited during the study; they will be randomized 1:1 to the standard treatment group and the EmoLED Protocol group. In the control group, which follows the standard treatment, patients will be visited twice a week. The EmoLED experimental Protocol group will undergo medical examination once a week; in this case, therapy will consist in the standard treatment plus EmoLED treatment.

This sample size is sufficient to compare the average rate of the injured area reduction, considering Student-T test for the comparison between independent samples, by establishing $\alpha = 0.05$, power level $(1-\beta) = 0,8$, under the assumption to have an area reduction rate after 16 weeks amounting to 50% in Group 1 (control group), a difference of 20% between Group 2 (experimental group) and Group 1, a standard deviation of averages equal to 50% of the mean values and a bilateral hypothesis.

Statistical analysis

The average rate of reduction of the injured area between the two groups will be compared using the Student-T-test, the quality of life in both groups at the baseline, after 16 weeks and the difference with the baseline will be compared using Student-T-test for independent samples.

Linear regression models will be used to value the differences between the groups to exclude any confounding factors. Due to the usually asymmetrical nature of cost distribution, the comparison of the costs related to the treatment and control group, in both the NHS and society perspective, will be made using the non-parametric Mann-Whitney U test.

Generalized linear regression models will be used to value the differences between the groups to eliminate any confounding factors.

The cost-effectiveness of both treatments in the time frame of the study (16 weeks) will be valued by calculating the incremental cost-effectiveness ratio (ICER) and expressed in terms of incremental costs for "quality adjusted years (QALY).

Extrapolations of results about the cost-effectiveness that simulate longer time-frames will be made considering decision-making models (e.g. Markov models) and eventually including the data gathered during the study with literary data and/or evidence from other studies.

Data publication policy

The study results will be available for publication on national and international journal or for presentations made by investigators during scientific conventions and on medical journals, at the end of the clinical trial, when its results have been already analysed.

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