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ORIGINAL ARTICLE

Effectiveness of Blue light photobiomodulation therapy in the treatment of chronic wounds. Results of the Blue Light for Ulcer Reduction (B.L.U.R.) Study

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ABSTRACT

BACKGROUND: Lower limb ulcers not responding to standard treatments after 8 weeks are defined as chronic wounds, and they are a significant medical problem. Blue light (410-430 nm) proved to be effective in treating wounds, but there is a lack of data on chronic wounds in clinical practice. The aim of the study was to determine if blue light photobiomodulation with EmoLED (Emoled Srl, Sesto Fiorentino, Florence, Italy) medical device in addition to standard of care is more effective compared to standard of care alone in promoting re-epithelialization of chronic wounds of lower limbs in 10 weeks.

METHODS: Ninety patients affected by multiple or large area ulcers were enrolled. To minimize all variabilities, each patient has been used as control of himself. Primary endpoint was the comparison of the re-epithelialization rate expressed as a percentage of the difference between the initial and final area. Secondary endpoints were: treatment safety, pain reduction, wound area reduction trend over time, healing rate.

RESULTS: At week 10, the wounds treated with EmoLED in addition to standard care showed a smaller residual wound area compared to the wounds treated with standard of care alone: 42.1% *vs.* 63.4% (P=0.029). The difference is particularly evident in venous leg ulcers, 33.3% *vs.* 60.1% (P=0.007). 17 treated wounds and 12 controls showed complete healing at week 10. Patients showed a significant reduction in pain ($P=2\times10^{-7}$).

CONCLUSIONS: Blue light treatment in addition to standard of care accelerates consistently the re-epithelialization rate of chronic wounds, especially venous leg ulcers and increases the chances of total wound healing in 10 weeks.

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KEY WORDS: Wound healing; Wounds and injuries; Ulcer; Low-level light therapy.

Chronic wounds have a substantial impact on the patients' quality of life and are a therapeutic challenge as they often do not respond to standard treatments.[1, 2] The definition of chronic wounds varies in literature, it usually includes a prolonged healing time considered as lack of clinical signs of re-epithelialization for a period that goes from a minimum of four weeks up to three months and the alteration of the sequence of events leading to healing.[3, 4] The objective of the therapies is to manage the healing process reactivating it and sustaining it to achieve non-disturbing cicatrization. Unfortunately, chronic wounds respond poorly even to treatments with advanced dress-

ings. One of the therapies adopted in the field of wound care is photobiomodulation, a specific phototherapy using non-ionizing light sources, including lasers, LEDs and broadband light in the visible and infrared spectrum.[5-7] The wavelengths with the most therapeutic applications are in the red (633 nm), and blue (415 nm) ranges.[8] Blue light (B.L.) induces the transition of the inflammatory phase through the modulation of reacting oxygen species, stimulates granulation, angiogenesis, and enhances the re-epithelialization through the activation of fibroblasts and the release of nitric oxide.[8, 9] The Cytochrome C and the Cytochrome C Oxidase, in the electron transport chain, are made sensitive to blue light by the presence of the Protoporphyrin IX chromophore.[10-15] Once activated by blue light, the cytochrome C and the cytochrome C oxidase contribute to strengthening the cellular respiratory process and they increase ATP production. Blue light supports the lesioned tissue with an increase of energy intake, most needed during the proliferation and remodeling phases. The therapeutic action of blue light can also be explained with the Flavins' absorption, which stimulates the production of ROS (reactive oxygen species) a signal transducers of numerous cellular pathways involved in tissue repair.[16, 17] The modulation of ROS stimulates the transition from the inflammatory phase to the proliferation phase, promoting the macrophages' phenotypic transition from M1 to M2 and enhancing angiogenesis.[18, 19] Several studies demonstrate blue light's efficacy and safety in the treatment of skin lesions, inflammatory acne,[20-23] burns,[9] psoriasis vulgaris[24, 25] and diabetic ulcers.[26] Two literature reviews[27, 28] confirm the efficacy of B.L. in wound treatment. A study on Fluorescent biomodulation[29] shows the general positive impact of light in stimulating wound healing processes in Venous Leg Ulcers and Diabetic Foot Ulcers. Despite the above evidences, there are relatively few experiences methodologically rigorous, conducted in daily clinical practice and focused on chronic wounds, treated in an outpatient setting. EmoLED (Emoled Srl, Sesto Fiorentino, Florence, Italy) is a new medical device emitting blue light (410-430 nm); due to its compact size and ease of use it is suitable for the therapy in outpatient settings on daily clinical practice. EmoLED proved to be effective in the enhancement of re-epithelialization of non-healing ulcers in a case series of 20 chronic ulcers treated for four weeks recently published.[30] The Blue Light for Ulcer Reduction (BLUR) study was explicitly designed to provide the practitioner with information on the effectiveness and safety of B.L. for the therapy of lower limb chronic wounds in the standard outpatient practice. BLUR study aimed to assess whether the treatment with EmoLED in addition to the Standard of Care (SoC) enhances the re-epithelialization of chronic wounds more than SoC alone.

Materials and methods

BLUR is a multicenter, prospective, controlled study carried in seven specialized outpatient centers located across Italy.

Patients

Patients with chronic wounds of the lower limb of venous, arterial, or mixed origin, and non-healing surgical dehiscence, were enrolled. To minimize variability the investigator chose, on each patient one wound or part of it that was treated with EmoLED in addition to SoC (Treated Arm) and one wound or part of it that were treated with SoC alone (Control Arm) as shown in Figure 1. The assessment of the wound area was documented by photograms taken at each visit and performed automatically by a dedicated software managed by independent operators unaware of the wound's arm. Each patient has been used as control of him/herself, therefore no masking or randomization was deemed necessary. The protocol defined two cases:

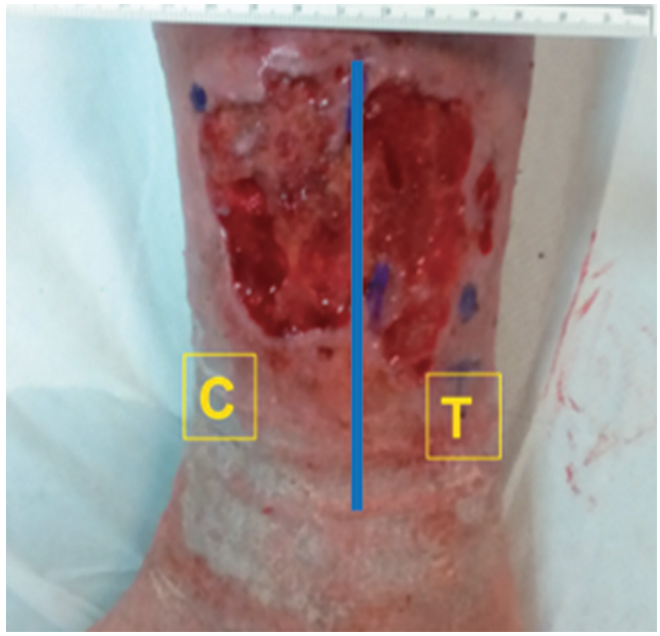

Figure 1.—A lesion is halved according to the main diameter; one part is treated with SoC alone (C) and one part is treated with EmoLED plus SoC (T).

• patients showing one or more wounds >5cm in diameter – the investigator halved the wound according to the larger diameter with a marker. One half of the wound was treated with EmoLED and SoC (treated arm) and the other half with SoC alone (control arm). During the blue light treatment, the control half of the wound was covered with a multi-layered sterile dressing to prevent the exposure to blue light;

• patients with at least two wounds <5cm in diameter – the investigator treated the most severe wound with EmoLED and SoC (Treated Arm) and the other wounds with SoC only (Control Arm). The SoC included ulcer cleansing/debridement, hydrofiber dressing, or hydrofiber with ionic silver in case of signs of infection; in the case of venous and mixed leg ulcers, compression therapy was added when deemed necessary. The wounds or the portions of wounds included in the Treated Arms, in addition to the SoC described above were irradiated with EmoLED for 60 seconds (120 mW/cm^2; 7,2 J/cm^2) at each weekly visit this power density being the limit to avoid photothermal effects.

Study schedule

The investigators collected the patients' data for a period of 10 weeks in 11 weekly visits, from visit V0 (enrolment and first treatment) to visit V10 (end of the study) or Last Observation Carried Forward (L.O.C.F.) if the patients' wound healed before the end of the ten weeks of treatment. From visit V5 on, it was possible not to show up for two non-consecutive visits. At each visit, the investigators evaluated the wound clinical signs: level of exudate, color of the perilesional skin, edges quality, depth, color of the wound bed, presence of granulation tissue, The investigators also took a standardized picture of the wound with an ASUS ZenPad 8.0 (Asus, Taipei, Taiwan) according to a preset procedure. The wound images were analyzed by independent operators at the Institute of Clinical Physiology of the National Research Council (Pisa, Italy) making use of previous experience related to the analysis of ulcers.[31, 32] A custom color-based K-Means[33] algorithm developed in Matlab® (MathWorks, Portola Valley, CA, USA) was applied to segment a wound image in clusters. The software detected a standard reference ruler placed on the wound by the investigator and calculated the conversion factor between the number of pixels and a predefined distance in the ruler (0.5 cm). After selecting the appropriate number of clusters, each cluster area was automatically returned by the software. As for safety, the investigators actively searched for AEs; while the patient reported the wound pain on VAS 0-10 Scale. Independent control of the study was ensured through regular monitoring visits and quality control audits by an qualified independent third party. Table I shows the study schedule.

Endpoints

The primary endpoint is the comparison of residual wound areas, *i.e.,* the area of treated wounds compared to the residual area of control wounds at the 10th week or at the last observation carried forward (LOCF). The residual wound area is defined as the per cent ratio of the wound area measured at the end of the ten weeks or at the LOCF, compared with the wound the area at the time of enrolment. Residual wound area = [(area at the end/area at enrolment) ×100]. A smaller residual wound area is then an expression of a more extensive re-epithelialization. The secondary endpoints are: 1) treatment safety in terms of occurrence and importance of adverse events and side effects; 2) pain reduction, measuring at each visit with the VAS Scale method, the patient's pain; 3) trend of reduc-

TABLE I.—*Study schedule.*

Assessment	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Inclusion/exclusion criteria	1										
Enrolment	1										
Demography	1										
Patient consent	1										
Anamnesis and objective examination	1										
Wound assessment	1	1	1	1	1	1	1	1	1	1	1
Treatment	1	1	1	1	1	1	1	1	1	1	
% Reduction of the treated lesion		1	1	1	1	1	1	1	1	1	1
Adverse events		1	1	1	1	1	1	1	1	1	1
Difference in healing time of the two areas		1	1	1	1	1	1	1	1	1	1
Pain reduction		1	1	1	1	1	1	1	1	1	1
Safety		1	1	1	1	1	1	1	1	1	1

V: visit.

tion of the wound area over time, the wound mean residual area of both arms was measured at each visit; and 4) healing rate, evaluation of the difference in healing time of the two arms.

Statistical analysis

The statistical planning and data analysis were conducted at the Institute of Information Engineering of the University of Pisa.

Sample size

Based on the scientific literature,[34] a variability of 30% in re-epithelialization at the tenth week has been estimated. The worst-case was represented by a standard deviation (S.D.) of the percentage reduction of the area of venous ulcers of 53%.[35] A population of 83 patients was needed to achieve a beta error of 20% and an alpha error of 5% in assessing the difference between treatments. All the patients with sufficient data to allow the wound image computer analysis were evaluated.

Primary endpoint

The comparison of the residual wound areas, has been analyzed both considering the wounds of each patient as paired data and consolidating the wounds in two separate groups (treated group *vs.* control group).

Secondary endpoints

The secondary endpoint were the following: 1) E.A.s were listed and described; 2) pain was reported by the patients using a VAS 10 Scale. Patients who completed seven and ten weeks of treatment were analyzed. The data were analyzed using the Friedman test, a non-parametric equivalent of ANOVA, which makes it possible to assess whether there are differences in the value reported in the different measurements made at a group level; 3) trend of the reduction of the wound area over time – the trend was analyzed with the Wilcoxon test which compared the area under the curve (A.U.C.) of the treated and control groups obtained from the values of the residual wound areas at the various follow-up visits; 4) healing rate – the analysis was performed first obtaining Kaplan-Meyer curves (K.M.) from the healing time analysis. The K.M. curves express the probability of healing at each follow-up visit. The significance of the difference of the curves was evaluated by applying the non-parametric Wilcoxon test with signs, which considers the coupled nature of the data (paired Prentice-Wilcoxon).

Analysis by subgroups

An analysis of the population divided by etiology was carried out considering three groups: 1) venous leg ulcers; 2) arterial and mixed venous/arterial; and 3) other wounds (surgical dehiscence).

Ethical consideration

The study was conducted following the Helsinki Declaration, the guideline ISO 14155/2011 and the Italian Regulations on clinical trials. The Ethics Committee of the Coordinating Centre granted the study authorization on December 4, 2017, and the Italian Ministry of Health approved the study on August 8, 2018. All participants provided written informed consent before enrolment. The study is registered on clinicaltrial.gov under the number NCT04018924.

Results

The study was conducted between April 2018 and September 2019.

Population characteristics

A total of 91 patients were screened, and 90 were enrolled in the study (119 wounds), 84 completed the study (6 drop out for unrelated reasons). Sixty-seven patients were evaluable with the software image analysis and their results have been reported. Sixty-eight patients manifested a relevant pain level at enrolment and have been evaluated for the assessment of pain at the seventh week, 45 of them were also evaluable for the assessment of pain at the 10th week of treatment. At the enrolment, the two sexes were equally represented, and the population was predominantly (61.1%) >70 years old. About one-third of patients showed more than one lesion longer than 5cm in one dimension, in the majority of cases (83/90 patients) in the lower limbs. The most frequent etiology was venous leg ulcers (63.02%) and 50 out of 119 wounds had been present for more than 24 months, with an average age of 67.8 months (Table II). The most frequent comorbidities were arterial hypertension (41.1%), type 1 or 2 diabetes mellitus (20.0%) and obesity (8.8%).

Primary endpoint

The primary endpoint was the comparison of the residual wound area in the treated and the control group. The residual area was defined as the per cent ratio of the wound area at the 10th week or at the LOCF and the wound area

TABLE II.—*Characteristics of the wounds: etiology, duration, position and dimensions at the enrollment.*

Etiology	Number of wounds (patients)
Venous	75 (56)
Mixed and arterial	25 (18)
Other	19 (16)
Duration of the wounds	**Number of wounds (patients)**
≤12 months	58 (42)
13≤ months ≤24	11 (8)
25≤ months ≤48	15 (12)
49≤ months <120	15 (12)
≥120 months	20 (16)
AVERAGE 67.83 (±113.50) months	
MEDIAN 20.5 (range 586) months	
Position	**Number of lesions (patients)**
Lower limb. (leg)	112 (83)
Lower limb. (foot)	6 (6)
Abdomen	1 (1)
Initial dimensions	**Number of patients**
More than one lesion <5 cm	29
At least one lesion ≥5 cm	61

at the enrolment. The residual wound area (Figure 2) was significantly smaller in the group treated with EmoLED in addition to the SoC than in the group treated with the SoC only (42% *vs.* 63%). The group treated with EmoLED in addition to the SoC has a more extensive re-epithelialization at LOCF than the group treated with the SoC alone. The pair data analysis (Figure 3) shows a distribution of results of the whole population between -27.9 and +5.2 (P=0.029) where negative numbers mean a positive outcome of the treated lesion *versus* its control.

Secondary endpoints

Treatment safety

Five SAEs and one AE were recorded during the study. All the SAEs/AEs were related to the patients' comorbidities and were not related to the treatments administered.

Pain

Friedman's test evaluation showed that at the eighth week of treatment the pain (measured with VAS scale) was significantly reduced compared with baseline 3.9 *vs.* 5.3 respectively ($P=9\times10^{-9}$) in the sixty-eight patients showing significant pain levels at enrolment (VAS≥4). Friedman's test showed that the pain value was significantly lower than baseline 4.6 *versus* 7.2, respectively ($P=2\times10^{-7}$) also in the forty-five patients ending therapy at the 10th week.

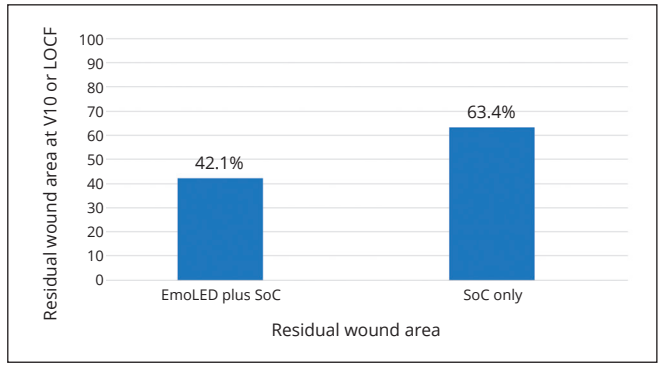

Figure 2.—Residual wound area at the last visit expressed as percentage of the initial area: wounds treated with EmoLED plus SoC and wounds treated with SoC only. Whole wounds population.

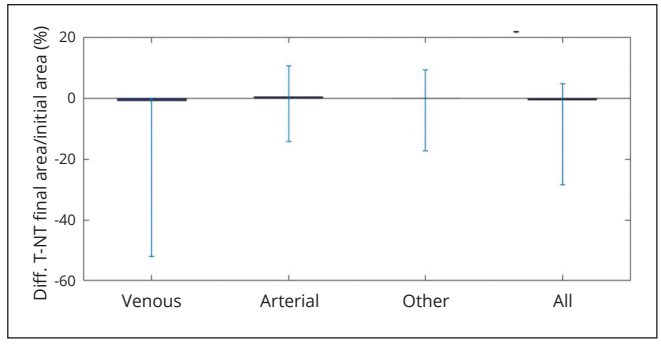

Figure 3.—Pair data analysis of the whole population and the three separated sub group considered. Events in favor of the treated group carry the negative sign.

Trend of the reduction of the wound area over time

Sixty-four of the 67 patients had enough data to process the A.U.C. Treated and control group progressively reduced the wound area, the difference is close to the statistical significance (P=0.076).

Healing rate

Sixty-seven patients were evaluable for the processing of K.M. curves expressing the probability of healing at each follow-up visit. Even if the difference did not reach the statistical significance, the treated group appeared to have a higher probability of healing at the 10th week (0.238 *vs.* 0.176; P=0.16). Furthermore, treated wounds that reached full re-epithelialization are 41,7% more than control (17 treated wounds and 12 control).

Sub-group analysis

In the sub-group analysis, the results of non-venous wounds were hampered by limited sample size and are not reported in this section.

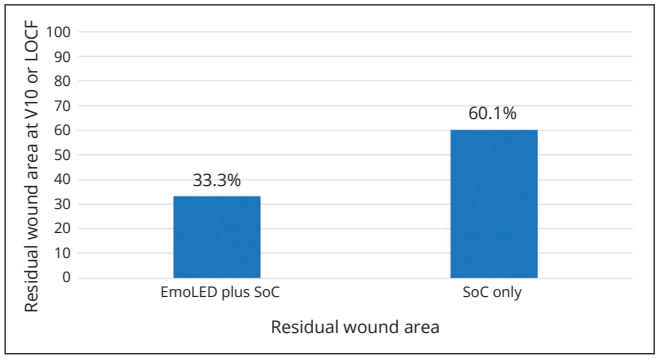

Figure 4.—Residual wound area at the last visit expressed as percentage of the initial area: wounds treated with EmoLED plus SoC and the wounds treated with SoC only. Wounds of venous origin only.

Primary endpoint

The analysis of the subgroups showed that the venous wounds (40 patients) had a marked response to the treatment with EmoLED. More specifically the pair data analysis (Figure 3) of venous ulcers population shows a distribution of results between -51.1 and + 0.2 (P=0.007) providing the evidence of a better outcome in the Treated wounds respect to its Control for almost every patient. The data is also confirmed by the significant prevalence of the treatment *versus* control in the group analysis showing a lower residual wound area at L.O.C.F. than the group treated with SoC alone, 33.3% *versus* 60.1% respectively (Figure 4).

Secondary endpoints

Pain

wounds of venous origin (43 patients) had a significant reduction of pain at VAS at the 7th week *vs.* baseline (3.9 *vs.* 5.3; $P=7.6\times10^{-5}$) and at the 10th week (30 patients; 4.7 *vs.* 7.2 $P=7.6\times10^{-5}$).

Trend of the reduction of the wound area over time

In the wounds of venous origin, the A.U.C. of wounds treated with EmoLED plus SoC was smaller than the A.U.C. of wounds treated with SoC alone. The difference is remarkably close to statistical significance (P=0.06). As for healing rate, no statistical difference was observed for the wound of venous origin (40 patients, P=0.11).

Discussion

The BLUR study evaluated the performance and safety of a new medical device EmoLED, in addition to SoC in the treatment of chronic wounds in a context of current clinical practice. In our opinion the study has two main reasons of interest: first, it fills a gap in clinical studies specifically targeted to the treatment of chronic wounds in daily clinical practice; second, the assessment of a new medical device (EmoLED) particularly suitable for the use in outpatient settings and everyday clinical practice. The study was carried out with rigorous methodology and an independent quality control. The choice to use each patient as control of her/himself reinforced the homogeneity of the population, although it prevented discrimination in pain data and introduced a positive effect in the control area in the case of single wounds divided in two part and treated partially. Although this effect can influence the data to the disadvantage of the blue light treatment, we believe that the off-target effect cannot compromise the conclusions of the study on the superiority of the treatment compared to the SOC only. The reliability of the data is reinforced by the use of a specific image analysis software for the evaluation of the wound area, which represents a remarkably accurate and unbiased assessment method.

Limitations of the study

The main limitations of this study are the inclusion of ulcers of different etiology that may respond to therapy in different manners; this difference is partially overcome by the pair data analysis of the results and the small number of wounds of non-venous origin which hampered a comprehensive stratification by etiology. The wounds treated were indeed chronic and challenging to treat, as demonstrated by the median life of the lesion (20.5 months), with a significant share of wounds over 12 months. Treatment with EmoLED in addition to the SoC for 60 seconds once a week induced a substantial reduction in the area of the wound than the SoC alone, as shown in Figure 5. Study population experienced a marked reduction in pain compared to baseline. This result, although undifferentiated between treatments and controls, strongly suggests an effect of the treatment on pain. The pain reduction was manifest at the week four of treatment, it seems to be correlated to the clinical evidences, reported by investigators, of the resolution of the inflammatory state. The evaluation of results stratified by subgroups showed a particularly marked response of the wounds of venous origin. The EAs observed during the study were unrelated to the treatments and were an expression of the underlying pathologies.

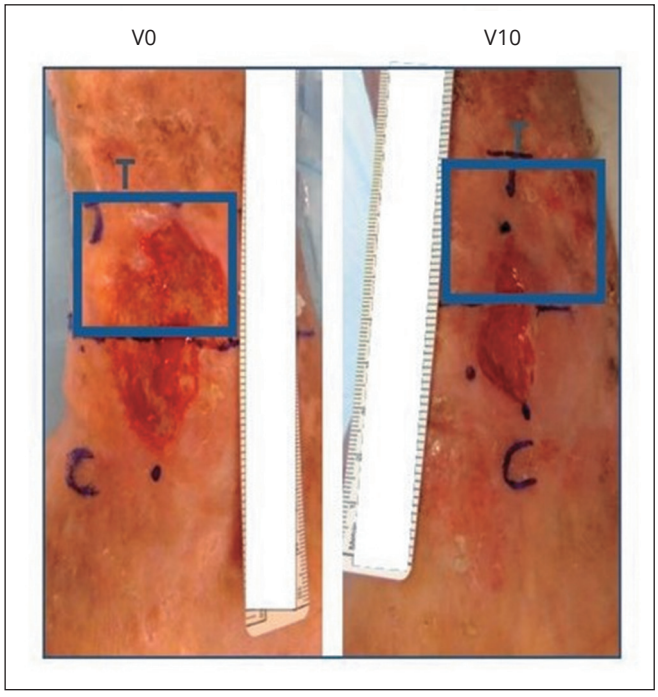

Figure 5.—Improved healing of a wound treated with EmoLED and SoC compared to SoC alone at V0 and V10. Patient age: 72 years; ulcer's age: 12 months. Pathology: chronic venous insufficiency.

Conclusions

The BLUR study shows that treatment with EmoLED in addition to the SoC enhances the re-epithelialization of chronic wounds of different etiology with a statistically significant difference compared to therapy with SoC alone, this result seems to be particularly evident in venous leg ulcers. Since the majority of non-healing ulcers are treated on an outpatient basis, in our opinion, it is crucial the availability of a treatment that can accelerate the re-epithelialization of the wound and that it can be carried out on an outpatient basis. We think that this first experience should be followed by studies on other types of wounds, such as inflammatory wounds, and that the action on pain and the whole inflammation phase, should be investigated in the future, as this parameter was particularly evident from what patients reported and the clinical observations indicate.

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