Photodynamic therapy in infected venous and mixed leg ulcers: a pilot experience

Objective: To assess the tolerability and efficacy of photodynamic therapy (PDT) with RLP068, a novel phthalocyanine-derived photosensitiser, in controlling the bacterial load in different leg ulcers, due to vascular pathophysiology.
Method: An observational study of patients with infected leg ulcers of different pathophysiology, admitted to the hospital for a skin grafting procedure, were included. All patients underwent two sessions of PDT at time zero and after 72 hours. A semi-quantitative swab was taken before and 30 minutes after both sessions of PTD. During the time interval between the two treatments the ulcer was covered with foam dressing and compression therapy was applied to all patients. No systemic or topical antibiotics were administered. Statistical analysis of results was performed.
Results: A total of 36 patients (13 males, 23 females; aged 72.4±8.6 years, range: 55-85 years) were recruited; two had ulcers on both legs. PDT was shown to be effective in reducing bacterial load after the first treatment. Before the second PDT treatment (72 hours after first PDT treatment), a slight increase of the bacterial load was observed in all ulcers. However, after the second PDT session, bacterial swab results were negative in all but two ulcers. The procedure was well tolerated in all but four patients, who reported a very severe pain at baseline, which increased during treatment.
Conclusion: In this study, PDT was effective in reducing bacterial load in patients with infected vascular leg ulcers, and allowed successful skin grafting to take place in all patients. The treatment was generally well tolerated. Studies with a greater number of patients and a control group are planned to confirm these results.
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infected ulcers • photodynamic therapy • phthalocyanine • skin grafting • vascular ulcers

The prolonged non-healing phase of a leg ulcer is sometimes due to specific conditions that make healing difficult, such as: arterial involvement, deep vein incompetence or obstruction, diabetes, collagen disease, immune system impairment, calf muscle pump impairment.[1,2] On occasion, it can be as a consequence of mistakes in the diagnostic therapeutic protocol. During this non-healing phase, the ulcer can become larger, the ulcer bed can be covered by fibrin or necrotic slough and infections can frequently occur and recur.It has been demonstrated that the presence of bacteria in the ulcer may further delay wound healing.[3]

In addition to delayed healing, discolouration of granulation tissue, friable granulation tissue, malodour, heavy exudation, pocketing at the base of the wound and wound breakdown are considered clinical signs of infection or critical colonisation.[4,5] When the ulcer bed is covered by a gel-like substance and a shiny layer or green discolouration occurs, the presence of a possible biofilm formation needs to be taken into consideration.[6,7] Hospitalisation of such patients, for surgical debridement and skin grafting, is often considered an option to induce healing of the ulcer, but bacterial load must be reduced or eradicated before any surgical procedure.

The multiplicity of infecting bacteria, either in planktonic form or organised in biofilms, and the increase in multiresistant strains, are part of this complex clinical scenario. New treatment modalities that may overcome these issues are required.

The aim of this study was to assess the tolerability and efficacy of a treatment based on photodynamic therapy (PDT) with RLP068, a novel phthalocyanine-derived photosensitiser, in reducing the bacterial load in patients with infected leg ulcers with vascular pathophysiology, and who were candidates to receive skin grafting.

Methods

This was an observational study of patients with of infected leg ulcers of different pathophysiology. Analysis was performed on patients seen in our clinic during the period September 2016 to March 2017. We included patients >18 years, of either gender, with leg ulcers of vascular aetiology showing signs of critical colonisation or local infection (confirmed by swab samples), that were of at least six months' duration, with an ankle-brachial index (ABI) >0.6.

Patients <18 years, those with ulcers without signs of critical colonisation or infection, or with ulcers of immunological or diabetic origin; patients receiving

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Table 1. Patient demographics

Patients, n	36
Patients who completed the study, n	32
Patients who did not complete the study, n	4
Males/females, n	13/23
Age, years (range: 55–85)	72.4±8.6
Venous pathophysiology, n	19
Arterial pathophysiology, ABI>0.6, n	8
Mixed pathophysiology, n	9
Ulcer size, cm^2 (range: 20–120)	60±25
Ulcer duration, months (range: 6–28)	13±6
Baseline bacterial load, CFU/cm^2 (range: 20,000–1,100,000)	392,778±380,395
Most common bacteria	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Baseline pain, VAS (range: 0–10)	4.4±2

ABI—ankle-brachial index; VAS—visual analogue scale

cortisone or immunosuppressive treatment; patients with a duration <6 months or ABI <0.6 were excluded.

All treated patients were fully informed on the proposed treatment and signed an informed consent, including permission for the taking and use of photographs. Ethical committee approval was not requested as it is not required in Italy for spontaneous clinical pilot studies with CE-marked products.

Treatment

All patients followed the same procedure. On day zero (D0), semi-quantitative swab samples from ulcer beds were taken in order to measure bacterial load. After cleansing of the ulcer bed with Ringer's solution, a semi-quantitative swab was performed in a clinically chosen $1cm^2$ area by pressing and rotating the swab tip uniformly. Skin area surrounding the wound was marked in order to exactly identify the same area for further swabbing procedures. Swabs were immediately transferred to the laboratory and cultured for aerobic bacteria.

Immediately after the swab procedure, all patients received the treatment consisting of the PDT with the photosensitiser RLP068 (VULNOFAST plus, a topical Medical Device CE0373 marked; Molteni Therapeutics s.r.l., Italy) twice over a four-day period before skin grafting surgery. The photosensitiser RLP068, formulated as a sterile liquid contained in a single-use vial, was applied according to the manufacturer's instructions for use of this medical device. It was evenly distributed on the ulcer bed so as to form a thin film to ensure complete coverage of the area to be treated. The treatment was then applied to the whole wound surface. Any excess of product was removed using a sterile gauze. After 30 minutes of contact, the treated area was illuminated for eight minutes by a portable LED light source (VULNOLIGHT, a Medical Device CE0123 marked, Molteni Therapeutics s.r.l., Italy) able to deliver a red light at 630nm, wavelength, supplying a total energy of $60J/cm^2$ (with a maximum deviation of $5J/cm^2$).

A new swab sample was collected from the same ulcer area 30 minutes after illumination. The treated ulcer surface was then washed with saline solution to remove the residues of the photosensitiser, and covered with an absorbent foam dressing. An inelastic compression bandage was applied to the patient's leg from the base of the toes to the area under the knee.

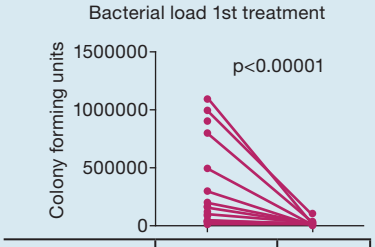
The whole treatment, including pre- and post-treatment swab, was repeated after 72 hours, i.e. on day four (D4) before the grafting procedure.

Ulcer-related pain was evaluated using a visual analogue scale (VAS), where zero represented 'absence of pain' and 10 represented 'very severe pain'.

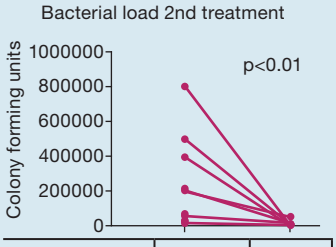
Statistical analysis

Data are reported as medians and interquartile range (IQR). Results before and after treatments in the same individual were compared by means of the Friedman test, a non-parametric test for repeated data. Differences with a $p<0.05$ were considered statistically significant. The graphs and the statistical evaluations were generated by using Graph Pad Prism, version 6, software (Graph Pad, US).

Fig 1. Bacterial load before and after first and second treatment with photodynamic therapy with RLP068



	Before	After
Minimum	20,000	0.0
25% Percentile	100,000	0.0
Median	185,000	0.0
75% Percentile	800,000	15,000
Maximum	1.100e+006	100,000



	Before	After
Minimum	0.0	0.0
25% Percentile	0.0	0.0
Median	0.0	0.0
75% Percentile	57,500	0.0
Maximum	800,000	50,000

Results

Patients

We assessed 36 leg ulcers in 34 patients—two patients had ulcers on both legs—12 males, 23 females, aged 72.4±8.6 years (range: 55–85 years) with critically colonised or infected leg ulcers of different pathophysiology and who were awaiting skin grafts.

Ulcer size was $60±25cm^2$ (range: 20–120 cm^2); ulcer duration was 13±6 months (range: 6–28 months). Ulcers had a venous pathophysiology in 19 cases, arterial in eight cases, and were mixed (both venous and arterial) in nine (Table I).

All the ulcers were clinically infected with a bacterial load of 185,000 colony forming units (CFU) (IQR:

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100,000–800,000). There were clinical signs of biofilm in 23 ulcers. Bacterial species most commonly found on swab specimens were Gram-positive such as: *Staphylococcus aureus*, *Staphylococcus lentus*, *Staphylococcus haemoliticus*, *Staphylococcus warneri*, *Enterococcus faecalis* and Gram-negative such as *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morganella morganii*. All patients had previously received multiple antibiotic treatments and in 30 of them resistant bacteria to common antibiotics were found.

Intolerable pain during the first treatment, which requested its immediate interruption, was the cause of four patients, with a painful ulcer at baseline, not completing the treatment.

Results refer to 32 ulcers in 30 patients. After the first treatment the bacterial load became close to zero in 24 ulcers. In the remaining eight ulcers, the bacterial load was reduced by the PDT to zero CFU (IQR: 0–15,000) CFU ($p<0.0001$). Before the second treatment on day four, the samples from the ulcer bed showed bacterial load in 12 ulcers. Bacterial load had also increased in four ulcers that had recorded a level close to zero when assessed after the first treatment. The median CFU was zero (IQR: 0–57,500). Despite this bacterial load increase, there was still a statistically significant reduction compared with the baseline CFU count ($p<0.0001$). At the end of the second treatment, the bacterial load was close to zero in all but two ulcers with 15,000 CFU and 50,000 CFU, respectively ($p<0.0001$) (Fig 1). Clinical signs of biofilm were not visible (Fig 2 and 3). No difference between ulcers of different pathophysiology was reported.

Baseline ulcer pain evaluated by VAS was four (IQR: 3–5). It slightly, but significantly, increased to five (IQR: 4–6.75) during treatment ($p<0.001$). In particular, in four patients with extremely painful ulcers at baseline (VAS ≥8), pain became so intolerable during the first treatment that it had to be stopped. In 13 patients pain remained unchanged during the treatment, and in 19 patients the pain slightly increased but remained in a tolerable range and all these patients were able to complete the treatment (Fig 4a). The pain returned to baseline value within one hour in all patients. It should also be noted that baseline pain decreased on day four to VAS 3 (IQR: 2.25–4), probably due to better infection control. The second treatment was better tolerated, with a lower pain increase compared to the first treatment, VAS 4 (IQR: 3–4), which produced a lower statistical significance ($p<0.05$) (Fig 4b).

Fig 2. Venous leg ulcer (VLU) before and after treatment with photodynamic therapy (PDT) with RLP068. At baseline (**a**) and four days after two treatments with PDT (**b**). Improvement of ulcer bed and disappearance of biofilm may be clinically evaluated



Fig 3. Mixed ulcer before and after treatment with photodynamic therapy (PDT) with RLP068. At baseline (**a**) and four days after two treatments with PDT (**b**). Improvement of ulcer bed and disappearance of biofilm may be clinically evaluated



Discussion

Conventional antimicrobial drugs have been effective in treating infections. However, at the same time, their overuse has contributed to the rise of antimicrobial resistance.[8] Efforts to develop new antibiotics are expensive and have been relatively unsuccessful as, due to the way in which antibiotics work, sooner or later microorganisms develop resistance,[9,10] making such new drugs ineffective.

Traditional management of clinical wound infection consists of systemic or local treatment, or a combination of both. Local treatment is able to offer the advantages of immediate effect, lower rates of systemic side-effects,[11,12] and no interference with the endogenous bacterial flora far from the site of infection.

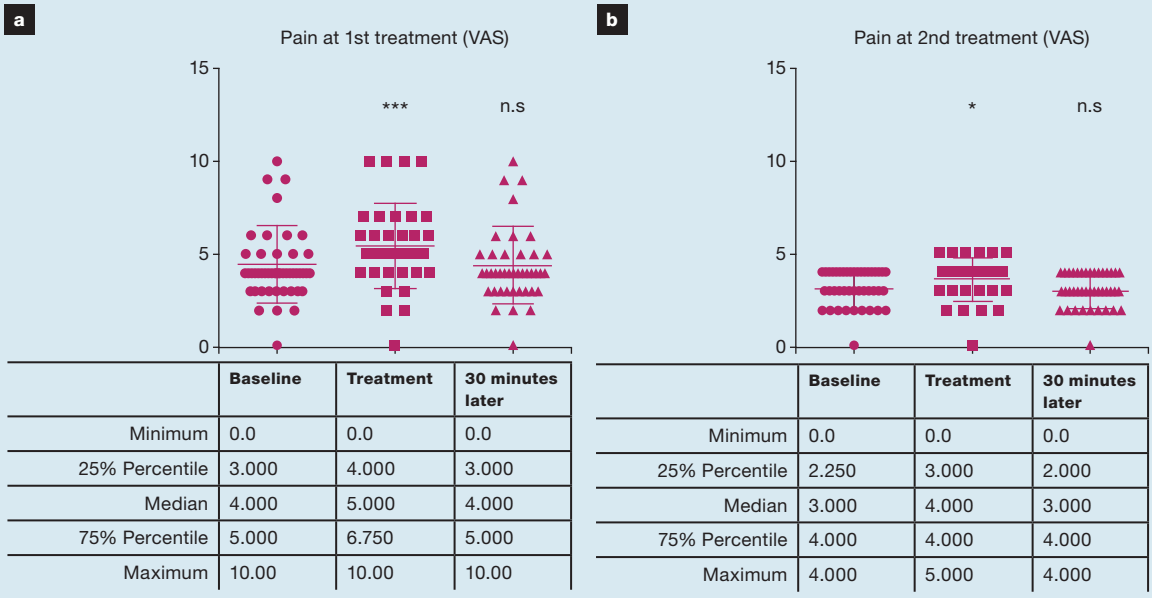
In this context, PDT may be a useful tool to treat infected wounds; in fact, the following advantages can be claimed from previously published findings:[13,14]

- Rapid and effective action
- Broad spectrum of antimicrobial activity, including wild type and resistant strains
- No induction of resistance by multiple PDT[15] due to the multitarget interference with bacteria exerted by reactive oxygen species (ROS), such as singlet oxygen, superoxide anions and hydroxyl radicals produced

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Fig 4. Pain evaluated by means of visual analogue scale (VAS) in baseline conditions, during and 30 minutes after the first (**a**) and second (**b**) treatment with photodynamic therapy with RLP068



	Baseline	Treatment	30 minutes later
Minimum	0.0	0.0	0.0
25% Percentile	3.000	4.000	3.000
Median	4.000	5.000	4.000
75% Percentile	5.000	6.750	5.000
Maximum	10.00	10.00	10.00

	Baseline	Treatment	30 minutes later
Minimum	0.0	0.0	0.0
25% Percentile	2.250	3.000	2.000
Median	3.000	4.000	3.000
75% Percentile	4.000	4.000	4.000
Maximum	4.000	5.000	4.000

during the photodynamic process of light activation of RLP068
- No interference with normal tissues or bacterial flora outside the lesion
- No systemic absorption of the photosensitiser.

The PDT treatment with RLP068, performed in this case series, was able to show its effectiveness in controlling the bacterial load in vascular, hard-to-heal leg ulcers due to its unique mode of action. The differences between the baseline bacterial load values and the values after the first PDT treatment, and before and after the second PDT treatment were all statistically significant.

The application of the photosensitiser RLP068, and the subsequent illumination, caused changes in the wound microenvironment due to inactivation of the bacteria but, probably, also to other positive activities described for PDT.[16–18]

The results of this case series confirm the outcomes of a previous randomised, placebo controlled, trial study (the DANTE study; Diabetic ulcer Antimicrobial New Topical treatment Evalution)[19] regarding the effect on the bacterial load—although the two studies were performed in different patients populations. In the DANTE study, PDT was performed in patients with infected diabetic foot ulcers (DFUs) as a single application of the photosensitiser compound RLP068, activated by red light. The procedure used in the DANTE study included the simultaneous administration of a systemic antibiotic and showed a very significant decrease of the local bacterial load, significantly greater in the combined treatment groups than in the placebo-antibiotic arm, resulting in a rapid reduction of bacterial load of the lesion.[19]

However, in our clinical experience, two PDT sessions were performed in a 72-hour time period. No simultaneous antibiotics were administered. Nonetheless, the PDT treatment was effective in reducing the bacterial load. After the second treatment, no clinical sign of biofilm was observed. It should be noted that no instrumental biofilm evaluation was performed and the presence of biofilm was assessed based only on visual inspection and clinical experience.

Regarding pain, this pilot demonstrated different results compared with the DANTE study performed on DFUs, where pain is somehow reduced/abolished by the underlying diabetic neuropathy.

Vascular leg ulcer can be painful and painkillers may be requested for pain control. In these cases, the PDT treatment may slightly increase the baseline pain. However, in this study, this usually returned to its baseline values within about one hour. It is also worthwhile to mention the high levels of procedural pain in patients with baseline severe pain before pain killer administration. In these patients, despite a significant reduction in baseline pain after pain killer administration, the procedural pain can still remain strong—this happened in four of our patients. In such patients, a careful risk/benefit assessment should be undertaken in order to evaluate eligibility for the procedure or, as an alternative, consider pre-treatment with painkiller medication.

Limitations

The results presented in this analysis of our clinical experience should be carefully evaluated, taking into consideration the limitations related to the lack of a control group and the limited number of cases.

Despite the lack of a control group, we are not aware of any antiseptic/antibacterial procedure able to control

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the bacterial load of infected wounds with just two applications in 72 hours.

Conclusions

PDT, with the new photosensitiser phthalocyanine-derivative RLP068, represents a promising wound management procedure, which is effective in controlling bacterial load with just two applications in a 72-hour time period. This enabled, in this study, skin grafting to be performed in chronic patients with infected ulcers. Efficacy was demonstrated without adding any systemic antibiotic therapy or local antiseptics.

Reflective questions

- In which wounds is the use of photodynamic therapy (PDT) most effective?
- What is the likelihood of inducing bacterial resistance by using PDT?
- What are the advantages, if any, of using PDT over other treatment methods?

The procedure was well tolerated in the majority of patients, though not tolerated in patients who reported severe pain before starting the treatment.

Further studies, on a larger population and with a control group, should be undertaken to confirm these initial results. **JWC**

References

1 Margolis DJ, Berlin JA, Strom BL. Risk factors associated with the failure of a venous leg ulcer to heal. Arch Dermatol 1999; 135(8):920–926. https://doi.org/10.1001/archderm.135.8.920

2 Milic DJ, Zivic SS, Bogdanovic DC et al. Risk factors related to the failure of venous leg ulcers to heal with compression treatment. J Vasc Surg 2009; 49(5):1242–1247. https://doi.org/10.1016/j.jvs.2008.11.069

3 Bjarnsholt T, Kirketerp-Møller K, Jensen PØ et al. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 2008; 16(1):2–10. https://doi.org/10.1111/j.1524-475X.2007.00283.x

4 Gardner SE, Frantz RA, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair Regen 2001; 9(3):178–186. https://doi.org/10.1046/j.1524-475x.2001.00178.x

5 White RJ, Cutting KF. Critical colonization—the concept under scrutiny. Ostomy Wound Manage 2006; 52(11):50–56

6 Hurlow J, Bowler PG. Clinical experience with wound biofilm and management: a case series. Ostomy Wound Manage 2009; 55(4):38–49

7 Dietrich LE, Price-Whelan A, Petersen A et al. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of Pseudomonas aeruginosa. Mol Microbiol 2006; 61(5):1308–1321. https://doi.org/10.1111/j.1365-2958.2006.05306.x

8 Barbosa TM, Levy S. The impact of antibiotic use on resistance development and persistence. Drug Resist Updat 2000; 3(5), 303–311

9 Hwang TJ, Gibbs KA, Podolsky SH, Linder JA. Antimicrobial stewardship and public knowledge of antibiotics. Lancet Infect Dis 2015; 15(9):1000–1001. https://doi.org/10.1016/S1473-3099(15)00235-2

10 Spellberg B, Bartlett JG, Gilbert DN. The future of antibiotics and resistance. N Engl J Med 2013; 368(4):299–302. https://doi.org/10.1056/NEJMp1215093

11 Dai T, Huang YY, Hamblin MR. Photodynamic therapy for localized infections—State of the art. Photodiagn Photodyn Ther 2009; 6(3-4):170–188. https://doi.org/10.1016/j.pdpdt.2009.10.008

12 Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci 2004; 3(5):436–450. https://doi.org/10.1039/b311900a

13 Wainwright M, Maisch T, Nonell S et al. Photoantimicrobials—are we afraid of the light? Lancet Infect Dis 2017; 17(2):e49–e55. https://doi.org/10.1016/S1473-3099(16)30268-7

14 Jori G, Fabris C, Soncin M et al. Phtodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. Lasers Surg Med 2006; 38(5):468–481. https://doi.org/10.1002/lsm.20361

15 Dei D, Chiti G, De Filippis MP et al. Phthalocyanines as photodynamic agents for the inactivation of microbial pathogens. J Porphyr Phthalocyanines 2006; 10(03):147–159. https://doi.org/10.1142/S1088424606000181

16 Giuliani F, Martinelli M, Cocchi A et al. In vitro resistance selection studies of RLP068/Cl, a new Zn(II) phthalocyanine suitable for antimicrobial photodynamic therapy. Antimicrob Agents Chemother 2010; 54(2):637–642. https://doi.org/10.1128/AAC.00603-09

17 Percival SL, Suleman L, Francolini I, Donelli G. The effectiveness of photodynamic therapy on planktonic cells and biofilms and its role in wound healing. Future Microbiol 2014; 9(9):1083–1094. https://doi.org/10.2217/fmb.14.59

18 Saw CL. Science against microbial pathogens: photodynamic therapy approaches. In: Science against microbial pathogens: communicating current research and technological advances. Mendez Vilas A (Ed). Formatex 2011; 668–674

19 Mannucci E, Genovese S, Monami M et al. Photodynamic topical antimicrobial therapy for infected foot ulcers in patients with diabetes: a randomized, double-blind, placebo-controlled study—the DANTE (Diabetic ulcer Antimicrobial New Topical treatment Evaluation) study. Acta Diabetol 2014; 51(3):435–440. https://doi.org/10.1007/s00592-013-0533-3



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