International Journal of Scientific Research Updates

Journal homepage: https://orionjournals.com/ijsru/

ISSN: 2783-0160 (Online)

(RESEARCH ARTICLE)

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The use of p-toluenesulfonic acid in the removal of biofilm in chronic ulcers

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International Journal of Scientific Research Updates, 2024, 08(02), 077–089

Publication history: Received on 27 August 2024; revised on 12 October 2024; accepted on 15 October 2024

Article DOI: https://doi.org/10.53430/ijsru.2024.8.2.0060

Abstract

Background: Bacterial biofilms represent a topic of great concern due to their ability to cause chronic, sometimes fatal infections. Considering the prevalence and pathogenicity of biofilms in chronic ulcers, an observational study was designed to evaluate the anti-biofilm capabilities of p-toluenesulfonic acid, using it in the chronic ulcers of patients belonging to the Diabetic Foot clinic.

Methods: Preliminary analysis of an observational study on patients attending the diabetic foot clinic with leg ulcers for at least 4 weeks. 17 patients treated with p-toluenesulfonic acid on a weekly basis for a maximum of 16 weeks. The primary outcome was 100% granulation tissue formation at week 16 and 50% reduction in initial chronic ulcer size.

Results: The average treatment time was 10 ± 4.8 weeks. 8 patients healed, 7 are undergoing treatment, 1 required reoperation due to post-operative complications and 1 spontaneously dropped out of the study after 6 weeks. In the sample of healed patients, the average treatment time was 10.5 weeks with 100% granulation present at the 6th week. The reduction in the overall size of the ulcer and of the granulation area is not significant in the total sample. The data improves in the sample of healed patients even if it does not reach significance (lesion p-value 0.094, granulation p-value 0.13). Applying the analysis to the delta of the two variables, a measurement of the area that coincides with the margins of the ulcer, a p-value of 0.069 is obtained.

The behaviour of the sample does not change when stratified by the dressings applied after treatment with p-toluenesulfonic acid.

Conclusions: Treatment with p-toluenesulfonic acid promotes tissue granulation regardless of the type of dressing subsequently applied. The data on the evolution of the size of the lesions were not significant due to the smallness of the sample but in the sample of healed persons, the formation of granulation after an average of 6 applications transforms the "chronic" ulcer into an "active" ulcer, promoting closure within the subsequent 4 weeks. The appearance of granulation confirms healing. Elimination of biofilm with p-toluenesulfonic acid can reduce healing times and the risk of reinfection.

Keywords: Biofilm; P-Toluenesulfonic Acid; Wound; Wound Care; Diabetic Foot; Chemical Debridement

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1 Introduction

Biofilm can be defined as an "aggregate of microbial cells immobilised in a matrix of extracellular polymers that acts as an independently functioning, homeostatically regulated ecosystem"[1].

The presence of layers of glycoprotein material adhering to the ulcer bed plays a fundamental role in the pathogenesis of the infection.

Biofilms are protected foci of infection and bacterial resistance within the ulcer. They protect bacteria from the effect of antibiotic and antiseptic agents. They represent highly organised bacterial colonies that allow micro-organisms to interact with each other, enabling the exchanging of nutrients and metabolites [2].

Micro-organisms can live separately from each other (planktonic form), floating in the liquid support that contains them, or in aggregate form (sessile form) through the production of biofilm. Bacterial growth and proliferation in chronic ulcers constitute a point of particular relevance. In chronic wounds the most frequently isolated pathogenic bacteria are Gram-positive, Gram-negative and anaerobic. It has been observed that depending on the pathogenesis of the chronic lesion there are important differences in the microbial species responsible for infection and that this microbial flora changes over time. Experimental data on the role of bacterial load in determining wound infection are not unique. In the presence of highly pathogenic bacteria, such as beta-haemolytic streptococcus, low microbial loads are still relevant in the genesis of infections. Generally, however, the infection develops in the presence of a significant infectious load, i.e. a concentration of micro-organisms greater than 105 colony forming units (CFU) per gram of tissue [3][4].

The infection causes an increase in exudate, the composition of which is such as to slow down and block the proliferation of keratinocytes, fibroblasts and endothelial cells. In chronic wounds, the exudate contains high concentrations of inflammatory mediators and MMPs (metalloproteinases) capable of degrading the extracellular protein matrix. This exudative component, if abundant, creates electrolyte imbalance, alters the barrier function of the perilesional skin and causes a slowdown in wound healing [5].

More recently, Moser et al. confirm that the immune response is unable to eliminate the biofilm and instead accelerates collateral damage to the host tissue through persistent inflammation, continuous oxidative damage, fibroblast senescence and lack of factors necessary for wound healing.[6] Wound biofilm (chronic infection) involves an initial phase in which the microbial load stimulates the recruitment of neutrophils, but as the biofilm matures, proinflammatory cytokines are formed (for example, interleukins and TNF-α), contributing to continuous oxidative stress and determining degradation of growth factors.[6] In support of this theory, it has been demonstrated that biofilm prevents the formation of granulation tissue and re-epithelialisation, confirming its ability to stop wound healing [7][2].

Infections linked to biofilms represent a serious problem as they reduce the effectiveness of therapeutic treatments, the response capacity of the immune system and consequently can increase mortality related to infections or promote their chronicity [3][9].

Gaining insights into the effectiveness of topical wound treatments for biofilm eradication is critical to improving wound management strategies. Despite numerous available agents claiming antibiofilm properties, supporting evidence remains inconclusive [9].

Various products and approaches on the market claim to have antibiofilm effects which can improve wound healing. However, studies regarding this claim are inconclusive [10][11].

P-toluenesulfonic acid is indicated for the local topical treatment of skin areas at high risk of bacterial development thanks to its ability to remove bacterial biofilm with a physical mechanism of action dependent on the dehydrating nature of the product and independent of the bacterial species present [12].

The aim of our prospective study is to evaluate, in clinical practice, the effectiveness of p-toluenesulfonic acid in promoting the growth of granulation tissue through the removal of biofilm from the bed and from the margins of chronic ulcers.

Reactivation of the signs of tissue repair can be documented by the appearance of granulation tissue and by the reduction in the size of the chronic ulcer compared to the start of treatment.

2 Materials and methods

Prospective observational study approved by the Ethics Committee of the Trento Provincial Health Services Company (Determination no. 1489/2022, 28/10/2022).

Patient enrolment takes place at the diabetic foot clinic once the ulcer has been classified as chronic and therefore becoming a candidate for treatment with p-toluenesulfonic acid.

The use of p-toluenesulfonic acid is added to current clinical practice and to the current outpatient flows on a weekly basis (1 medication/week) and is exclusively applied at our specialist clinic.

2.1 Population subject of the study

2.1.1 Inclusion criteria

Adult patients belonging to the diabetic foot clinic of the Rovereto Hospital suffering from chronic ulcers (present for at least 4 weeks) of the lower limbs of vascular, neuropathic or mixed origin treated with p-toluenesulfonic acid according to the methods described below.

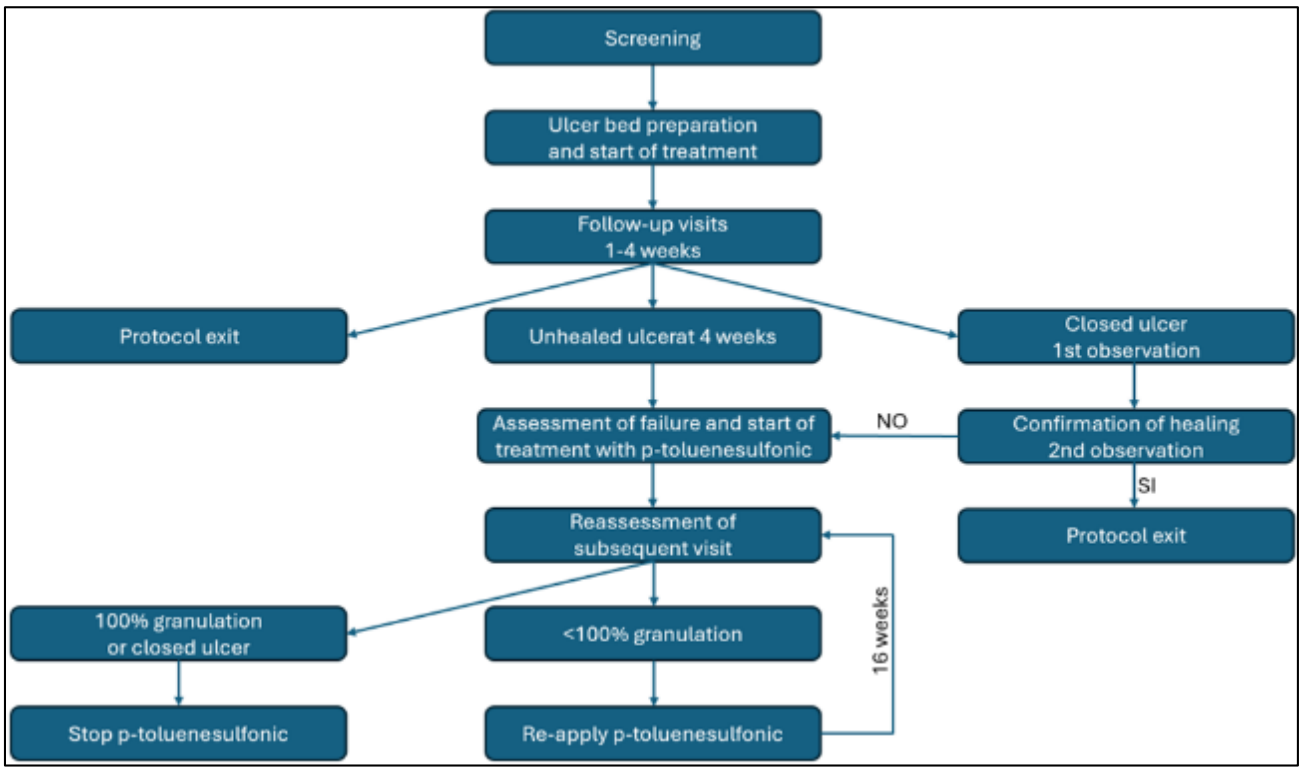
Patient characteristics:

- DMT1 and DMT2.
- ≧18 years of age.
- Chronic ulcer ≥ 4 weeks.
- They may be candidates for lower limb revascularisation.
- They may have already been revascularized in the lower limb.
- They may be awaiting surgery for the application of a dermal substitute or autologous graft.
- They may have undergone targeted or broad-spectrum antibiotic therapy.
- Who are commencing treatment with p-toluenesulfonic acid.

2.1.2 Exclusion criterion:

- Acute injuries < 4 weeks.

2.2 Study flow-chart



2.2.1 Procedure

- Phase 1: medical history collection, foot inspection, pulse check, in case of non-palpable or weak pulses, flow evaluation with Ultrasonic Pocket Doppler and complete lower limb ultrasound Doppler. Pain assessment according to NRS scale (*Figure 1*).
- Phase 2: preparation of the ulcer bed with cleansing and disinfection of the ulcer according to the common "Wound Bed Preparation" standards.
- Phase 3: Application of 1-2 ml of p-toluenesulfonic acid (C-DEB) allowing it to react for 10 seconds; washing with plenty of saline and rubbing with sterile gauze. In case of reagent material still present, surgical debridement with spoon-scalpel-curette or other *(Figure 2.)*
- Phase 4: Evaluation of the lesion after debridement with iconographic documentation using photos functional to the analysis of the areas using CAD software: measurement of total area and granulation area which we will subsequently call "lesion" and "granulation" *(Figure 3).*
- Phase 5: After the use of p-toluenesulfonic acid (C-DEB), application of any paraffin-based gauze/active hydrogel-based/hydrocolloid-based/silver-based/VAC therapy dressing.
- Phase 6: re-evaluation on a weekly basis according to the study's flow chart.

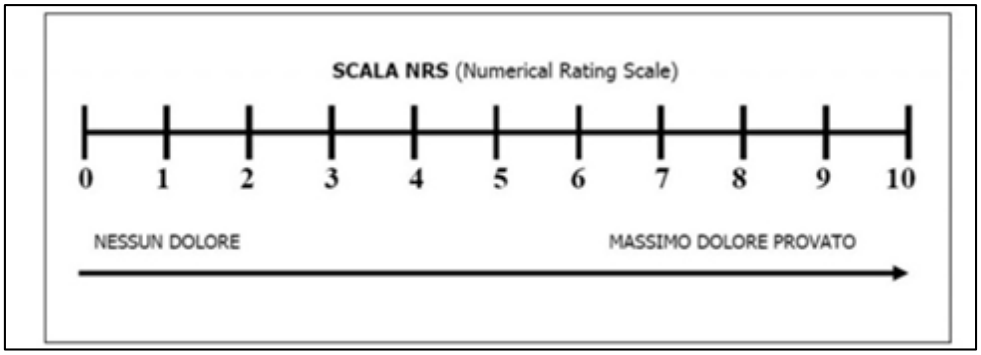


Figure 1 Behavioural Pain Scale (BPS) by Payen et al. (2001)

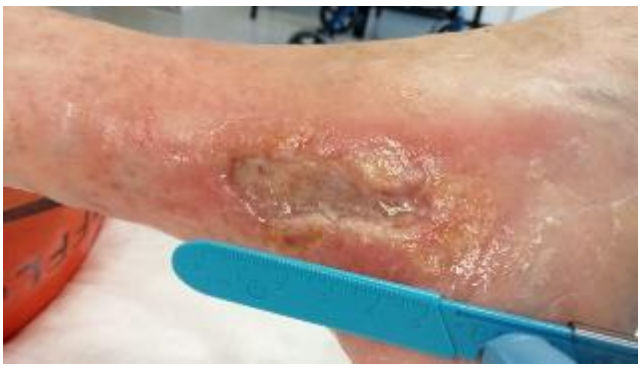
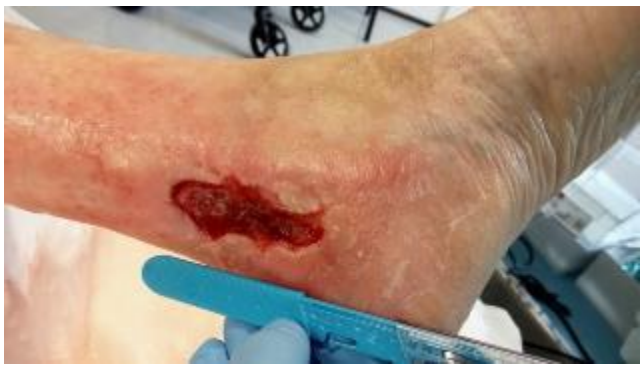
A: p-toluenesulfonic acid application	B: ulcer after debridement
	

Figure 2A Biofilm reaction to p-toluenesulfonic acid; B: ulcer bed after p-toluensulfonic acid removal

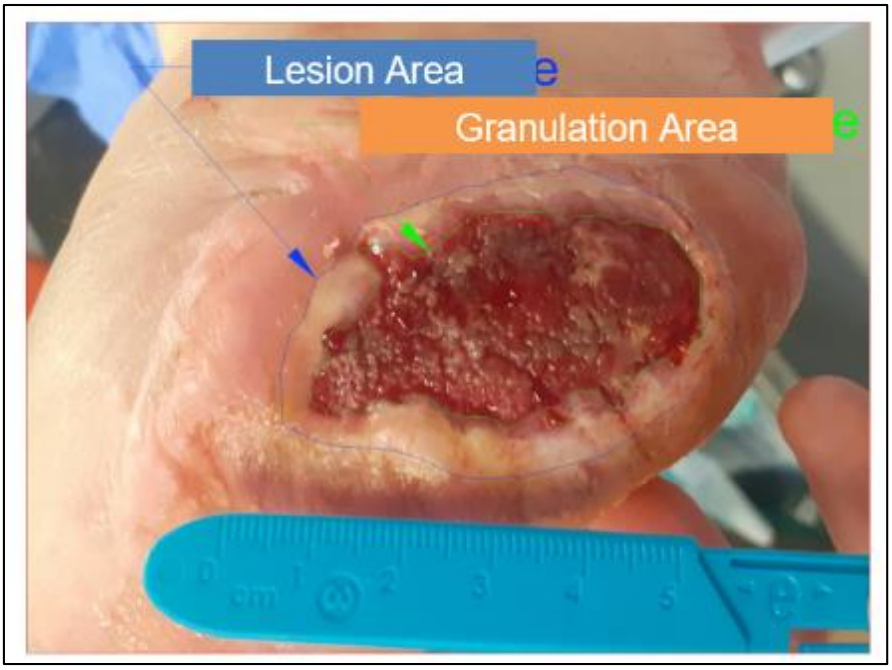


Figure 3 Example of perimeter for area calculation with CAD software

2.3 Statistical analysis

Absolute and relative frequencies (percentages) are calculated to describe the distribution of categorical variables (including the proportion of patients who will have 100% granulation at 16 weeks and the proportion of those who will have a 50% reduction in lesion area). Continuous variables (such as the % of granulation tissue developed, the number of product applications, the % reduction of the lesion area) are represented through maximum mean and standard deviation. The association between lesion characteristics (such as the type of ulcer) or patient characteristics (such as the presence of comorbidities) and the achievement of 100% granulation at 16 weeks or a 50% reduction in the lesion area is evaluated using Fisher's exact test. Comparisons of numerical variables at different times (e.g. T0 and final T) are conducted through the t-test for two paired samples for means. Values less than 0.05 are considered statistically significant.

In the subgroup of healed patients, two survival analyses were conducted using the Kaplan-Meier method, using first the halving of the granulation size as the outcome, then the presentation of a 100% granulation area.

Statistical analyses are conducted using SAS System Software v9.4 (SAS Institute Inc., Cary, NC, USA).

3 Results

We analysed the data of 17 patients with T2DM with chronic ulcers present for at least 4 weeks.

One patient required reoperation due to post-surgical complications in the first week of observation and was therefore excluded from the analyses.

Of the remaining 16 patients, one patient spontaneously dropped out of the study after 6 weeks, i.e. 6 applications of p-toluenesulfonic acid.

Of the 15 remaining patients with an average age of 67 years, 14 were male and 1 female, 5 presented neuropathic type ulcers, 6 mixed, 3 venous, 1 post-traumatic (*Figure 4*).

6 patients had been vascularised in the previous 8 months.

5 patients were treated with a silver-based dressing, 6 with gauze containing active hydrogel, 3 with fatty gauze (*Table 1*).

The average treatment time (equivalent to the total number of applications) was 10 ± 4.8 weeks (i.e. 10 ± 4.8 applications). During this observation, 8 patients healed, 7 are still undergoing treatment.

The average number of applications in the healed group was 10.5 or 10.5 weeks.

The mean size of the lesion area was 652.0 mm2 (SD 928.6) for the total group and 521.9 mm2 (SD=986.6) for the healed group, the mean size of the central granulation area was 350.6 mm2 (SD 655.5) for the total group and 281.3 mm2 (SD=674.1) for the healed group.

5 patients underwent 16 weeks of treatments (ID 004-006-008-009-013) presenting a reduction of chronic ulcer by 9.1% in terms of lesion and 1.0% in terms of granulation. Of these, 2 healed, 3 maintained chronic ulcers, of which one required revascularisation surgery.

Patients ID 017-018-023 are still undergoing treatment with an average reduction of 36.6% in terms of lesion and 12.5% in terms of granulation.

Patient ID 011 with post-traumatic ulcer underwent treatment for a total of 6 applications, and required revascularisation surgery due to worsening of the locoregional condition, and therefore left the study after 6 applications.

Patients with venous ulcers complained of pain at the ulcer site with an average intensity of 8.7 (SD=4.7) at the start of treatment and an average intensity of 4.3 (SD=3.3) at the end of treatment.

All the others had no pain. No adverse reactions were observed.

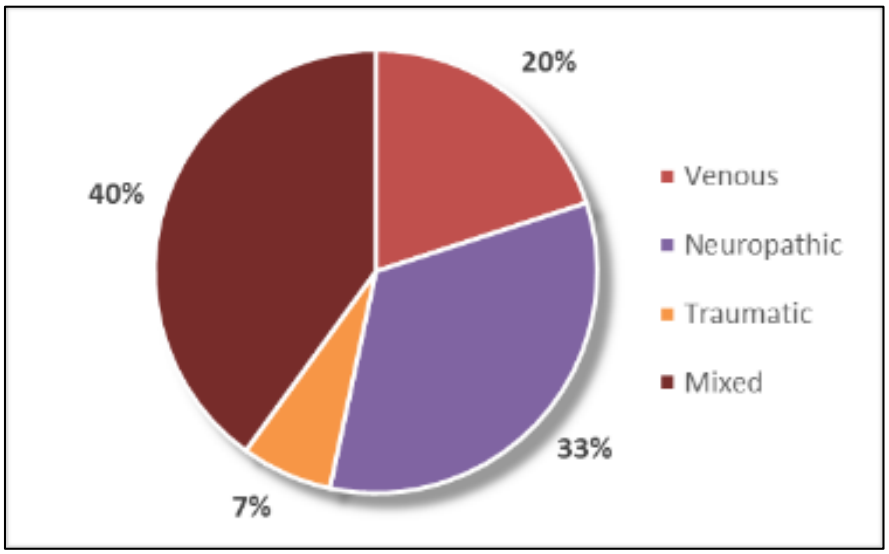


Figure 4 Lesion type distribution in the study population

The reduction in the overall size of the ulcer and of the granulation area was not significant in the total sample (*p*-value 0.43 lesion, *p*-value 0.26 granulation). The data improves in the healed sample even if it does not reach significance (*p*-lesion value 0.094, *p*-granulation value 0.13). By applying the analysis to the delta of the two variables, i.e. to the measurement of the area that coincides with the margins of the ulcer, a *p*-value of 0.069 is obtained.

The behaviour of the sample under examination stratified by dressings applied does not show specific differences, in particular patients treated with silver dressings do not have a different behaviour compared to those treated with only fatty gauze or hydro actives (Tables 2-3).

Table 1 General characteristics of the study population

N° of patients = 15		
Gender (n, %)	Male	14 (93%)
	Female	1 (7%)
Age at T0 [mean (SD)]	67.0 yrs (12.5)	
Recovery (n, %)	Yes	8 (53%)
	No	7 (47%)
Type of diabetes (n, %)	Type 1	0 (0%)
	Type 2	15 (100%)
Type of ulcer (n, %)	Venous	3 (20%)
	Neuropathic	5 (33%)
	Traumatic	1 (7%)
	Mixed	6 (40%)
Type of treatment at T0 (n, %)	With silver	5 (33%)
	Idrogel	7 (47%)
	Paraffin gauze	3 (20%)
Weeks of treatment [mean (SD)]	10.3 weeks (4.8)	
Revascularisation peripheral arterial disease	Yes	6 (40%)
	No	9 (60%)
Time elapsed since vascularisation [mean (SD)]	8.0 months (6.8)	
Lesion at T0 [mean (SD)] mm2	652.0 (928.6)	
Granulation at T0 [mean (SD)] mm2	350.6 (655.5)	
Pain at T0 (n, %)	Yes	5 (33%)
	No	10 (67%)
Pain at final T (n, %)	Yes	3 (20%)
	No	12 (80%)
Pain at T0 [mean (SD)]	2.4 (3.9)	
Pain at final T [mean (SD)]	1.3 (2.6)	

Table 2 Development of total lesion size and granulation area and calculation of the difference

All patients	**T0**	**T final**	***p*-value**
Granulation (mm2)	350.6	323.3	0.438
Lesion (mm2)	652.0	490.9	0.268
Delta (lesion - granulation) (mm2)	301.4	167.7	0.069

In the subgroup of healed patients, the average treatment time was 10.5 weeks with presentation of 100% granulation within the 6th week (*Figure 5*).

Figures 6 and 7 show the survival analyses with the Kaplan-Meier estimator for the halving of the size and the presentation of 100% granulation areas in the healed group.

Table 3 Behaviour of the subgroup of patients medicated with a silver dressing

Medication with Ag	**T0**	**T final**	***p*-value**
Granulation (mm2)	905.8	821.0	0.443
Lesion (mm2)	1562.8	1225.8	0.349
Delta (lesion - granulation) (mm2)	657.0	404.8	0.196

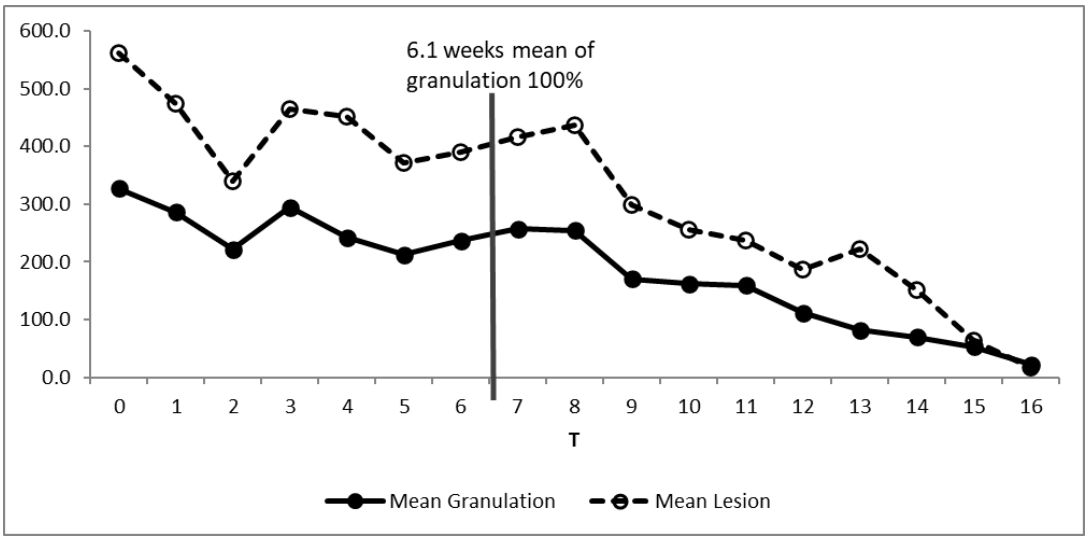


Figure 5 Development of 'lesion' area and 'granulation' area dimensions in the group of cured patients

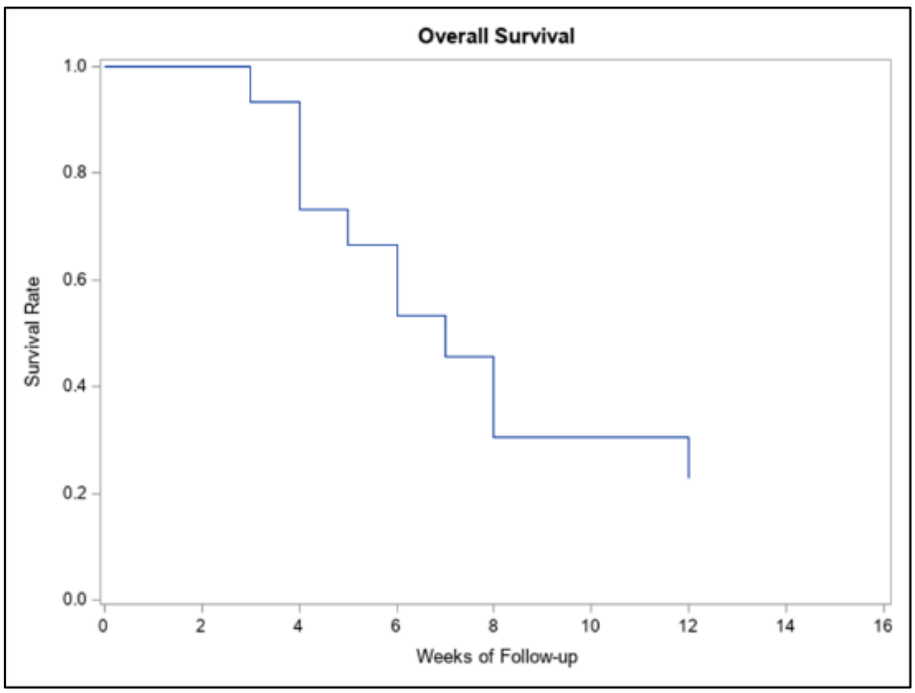


Figure 6 Granulation formation time 100%

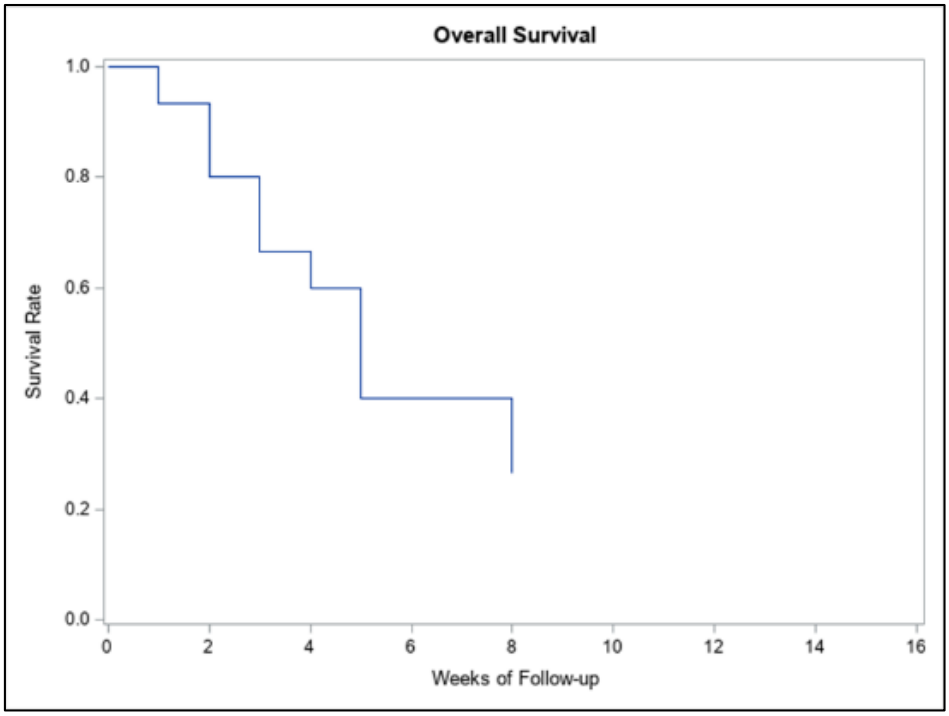


Figure 7 Half-life granulation formation 100%

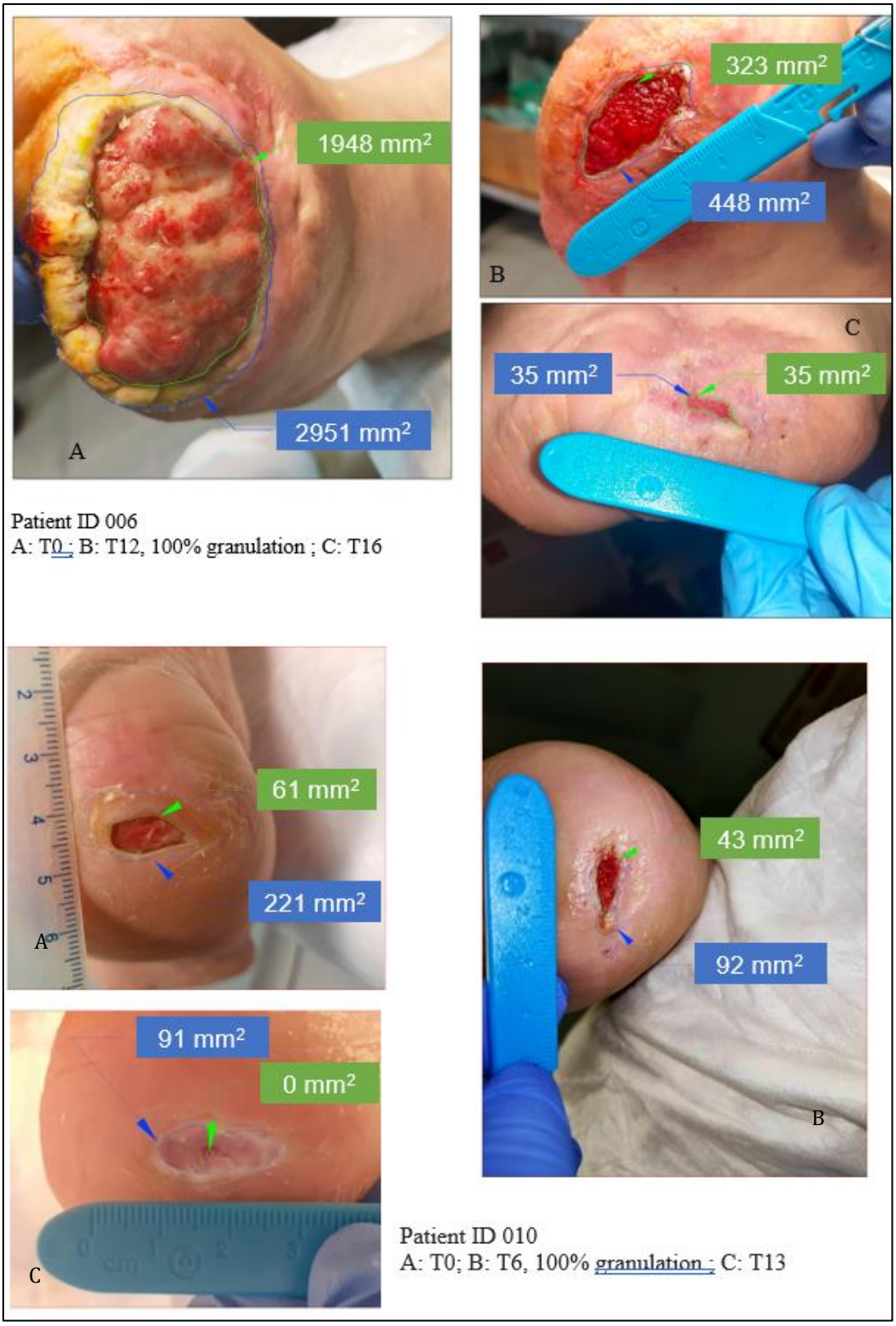


Figure 8 Patient ID 006 A: ulcers at time 0 fist application of p-toluenesulfonic acid; B: ulcer at week 12, 100% granulation; C: ulcer at week 16; Patient ID 010: A: ulcers at time 0 fist application of p-toluenesulfonic acid; B: ulcer at week 6, 100% granulation; C: ulcer at week 13

4 Discussion

Infections caused by antibiotic-resistant bacteria were among the leading causes of death in 2019, with approximately 4.95 million people dying from diseases in which antimicrobial resistance (AMR) played a major role. In fact, it is estimated that 1.27 million deaths correlate directly with antimicrobial resistance [7]. The presence of biofilm with its intrinsic antimicrobial resistance is medically recognised as one of the main causes of chronicity of infections. The Centres for Disease Control and Prevention (CDC) estimates that biofilms are responsible for more than 65% of all chronic bacterial infections, while the National Institutes of Health (NIH) estimates this as being at around 80% [13] [14].

The process of biofilm formation involves dynamic and complex interactions between bacterial exopolysaccharides (EPS) and the cell surface. The viscoelastic properties and adhesion strength of bacteria facilitate the formation of biofilms and make them resistant to antimicrobial agents.

A crucial challenge in the treatment of biofilms is the use of antimicrobial substances capable of acting by completely removing them in a short space of time. The presence of even microscopic biofilm residues and cellular debris contaminated by the biofilm promotes subsequent colonisation by other bacteria. Furthermore, prolonged exposure to a non-eradicating treatment is known to contribute to resistance to antimicrobial treatments.

The treatment strategy should therefore target both EPS and residual micro-organisms, while safeguarding healthy host cells. Rather than using antimicrobials at high cytotoxic doses with broad action even on healthy cells, a strategy could be to target the environment surrounding the bacterium, i.e. the biofilm matrix, making it inhospitable, for example with a low oxygen tension or with an Extremely acidic or basic pH.

Using this strategy, the biofilm matrix could be degraded by including resident micro-organisms and sparing host cells. The exploration of efficient dispersants to eliminate pathogenic biofilms is still limited.

The development of effective physical treatments for biofilm is a major challenge and a promising field of research.

In the treatment of oral pathologies (peri-implantitis, aphthous stomatitis...) the use of dissecting agents has long been known. An extremely hygroscopic solution composed of isomers of hydroxy methoxy benzenesulfonic acid and hydroxybenzene sulfonic acid, sulphuric acid and water was used in the treatment of recurrent aphthous stomatitis [15] [16] with the aim of facilitating the decontamination procedure, denaturing the adhesion proteins used by the bacteria in surface defects and implant threads [17].

The presence of bacteria in the periodontal soft tissues can cause chronic inflammation and secondary bone reabsorption, and promote the formation of a "pocket" around the surface of the implant or bone. The biofilm that forms on the surface of the implant prevents its osseointegration. To date, there are no human studies that demonstrate, at a histological level, reattachment of the bone to the implant surface after peri-implantitis.

Current evidence suggests that peri-implantitis responds to deep cleaning surgical treatment. However, the use of drying agents in periodontal disease and recurrent canker sores appears promising as an alternative treatment [18].

The use of hydroxy methoxy benzenesulfonic acid and hydroxybenzene sulfonic acid has also been proposed for degradation of the biofilm on the bottom of chronic ulcers and on the perilesional skin with positive results. Methane sulfonic acid (MSA) has recently been used in the treatment of biofilm in chronic ulcers [19] [20].

In our study we tested p-toluenesulfonic acid (95% polyethylene sulfonic acid solution and glycerol) which denatures, dehydrates, and coagulates biofilm and microbial environment in chronic wounds.

The data obtained from the preliminary analysis of our sample allowed us to test the capacity of p-toluensolfoonic acid. The treatment added to normal clinical practice promotes tissue granulation regardless of the type of dressing subsequently applied. The formation of granulation after an average of 6 applications transforms the ulcer into an "active" lesion and accelerates healing times, promoting its closure on average within the following 4 weeks. The data on the trend of ulcer dimensions are not currently significant due to the smallness of the sample when compared only to the absolute value of the areas measured.

Since with the reactivation of the repair processes not only does granulation appear in the central area but the characteristics of the margins of the lesion change, smoothing out and reducing and replacing them with healthy and vital tissue, the dimensional data of the margin can be considered as a significant and independent variable of healing as can be interpreted from figure 2. Figure 2 shows, in fact, that in parallel with the appearance of granulation and subsequent healing, the perilesional area tends to zero.

From preliminary observations, particularly in the healed group, the appearance of granulation confirms subsequent healing. Elimination of the biofilm with p-toluenesulfonic acid leads to complete tissue repair or the appearance of granulation tissue independent of the medications applied.

The use of this device in common clinical practice can reduce the recovery times of our patients and therefore the risk of reinfection

5 Conclusion

Biofilm management is the priority goal in wound care. P-toluenesulfonic acid has the ability to remove bacterial biofilm with a physical mechanism of action dependent on the dehydrating nature of the product and independent of the bacterial species present.

In our experience treatment with p-toluenesulfonic acid promotes tissue granulation regardless of the type of dressing subsequently applied. The appearance of granulation promotes subsequent healing. In the sample of healed people, the formation of granulation after an average of 6 applications transforms the “chronic” ulcer into an “active” ulcer, favoring its closure in the following 4 weeks.

P-toluenesulfonic acid may represent a new way for chemical debridement that can reduce the healing time of ulcers and consequently the risk of reinfection. It is an easy-to-handle approach for health care personnel to introduce into hospital and territorial clinical practice

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that no conflict or potential conflict of interest exists in relation to this clinical study.

Statement of informed consent

Informed consent was obtained from the participant included in the clinical study.

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