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Identification of types of wound bed tissue as a percentage and total wound area by planimetry in neuropathic and venous ulcers

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Background: Neuropathic and venous leg ulcers are chronic wounds associated with devitalized tissue and recurrent infection. Management should be guided by accurate tissue assessment, including the use of planimetry, which provides tissue types as a percentage of the total wound bed surface area.

Objective: This innovative study aimed to assess and identify the wound bed tissues, as a percentage, of neuropathic and venous ulcers using digital planimetry, providing support to nurses optimize the management of necrotic tissues and, consequently, to avoid wound infection.

Methods: This cross-sectional study enrolled 24 patients with chronic wounds who were assessed from January to March 2021 at the Wound Outpatients Clinic. The wound photographs were analyzed using Image J 1.53e and a smartphone with WoundDoc Plus® 2.8.2 via digital planimetry. Statistical analyses were performed using the binomial test, t-test, and Mann-Whitney.

Results: Median wound areas ($p=0.3263$) did not differ between the group with 2 or 3 risk factors for delayed healing (Md: 31.7) and the group with up to 1 risk factor (Md: 5.3). A low exudate level was associated with the up-to-1-risk-factor-for-delayed-healing group ($p=0.0405$), while a medium level was associated with the two-or-three-risk-factor group ($p=0.0247$). A heat map displayed the tissue percentages in the wound bed. In the group with 2 or 3 risk factors for delayed healing, 91.7% (11/12) had less than 70% granulation tissue, which was the primary factor for this group ($p<0.0001$). Additionally, 66.7% (8/12) of patients with 2 or 3 risk factors for delayed healing exhibited discolored and/or dark red granulation tissue as the primary factor ($p=0.0130$).

Conclusion: This novel identification of wound area and tissue types as a percentage, using digital planimetry, can play a crucial role in assisting nurses in decision-making related to the appropriate management of devitalized tissues. Furthermore, this measurements may facilitate the conducting of virtual wound consultations and offer valuable support in the development of protocols aimed at preventing infection and biofilm formation in the wound bed.

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Introduction

Chronic ulcers in the lower limbs are wounds below knee level that take more than three months to heal, even with adequate treatment.¹ Patients with wound usually have some comorbidities such as vascular insufficiency,^{1–4} diabetes mellitus (DM)^{1,4–7} and leprosy.^{8,9} As to their etiology, lower-limb ulcers can be associ-

ated with peripheral neuropathies such as DM and leprosy^{5,6,8}; ischemic disease^{5,6} and related to venous insufficiency.^{3,10}

According to a recent meta-analysis, the prevalence of chronic wounds of mixed etiologies and for chronic leg ulcers, in adult general population, were 2.21 and 1.51 per 1000 population, respectively,¹⁰ with higher proportions in patients with obesity, diabetes and cardiovascular disease.^{1,4,7,10} Chronic ulcers are common in older individuals and become more frequent with aging, particularly in those over 70 years of age.^{2,4,10} In addition, this type of wound affects individuals around 70–80 years of age.¹⁰ In relation to sex, the ulcers occurred three times more frequently in the fe-

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male than in male, being associated with post-menopausal period, since studies reported the role of estrogen in skin health.^{2,11,12}

Lack of effective management of devitalized tissue in chronic wounds increases the risk of complications such as infections, amputations and death.^{1,4,13} The prevalence of chronic wound infection in patients in hospital was 3%, which led to delayed healing and the possibility of amputation.^{1,3,7,11}

Risk factors for infection in lower-limb chronic wounds include wound area ≥ 10 cm², presence of slough tissue, and high exudate levels.^{1,14} Patients with diabetic foot ulcer (DFU) had reduced survival and an amputation rate of 29.3% in comparison with patients without diabetes and this may contribute to an upward trend in costs related to these wounds.⁶

In the United States, Medicare cost projections for all types of wounds ranged from \$28.1 to \$96.8 billion, with higher expenses for outpatient care compared to hospitalization. Additionally, the annual expenditure for the treatment of venous ulcers alone reached \$1 billion.⁴

Detecting complications by use of assessment tools will avoid additional costs associated with chronic wounds. The TIME framework, represented by an acronym that represents tissue, infection or inflammation, moisture balance and edge, can provide a systematic and structured assessment, favoring the healing process of chronic wounds.⁷ Thus, the proper understanding and description of these components enables clear communication among multidisciplinary teams, ensuring holistic care for the patient.⁷ This assessment also allows the professional to be guided to the approaches and implementation of optimal care for each patient.^{7,13}

In order to complement wound assessment, a qualitative tool to obtain wound area is desirable. Measurements methods include the ruler-based techniques, mathematical models, manual or digital planimetry, in addition to stereophotogrammetry and others.^{14,15} Some methods, such as the simple ruler, may pose a risk of contaminating the wound bed, while others have high cost, such as stereophotogrammetry, which uses three-dimensional (3D) measurement.¹⁵

Among the models that use a mathematical algorithm, some are able to provide the contour of wound edge by means of toroidal geometry that decomposes the image in different contrasts by binarization applying Otsu's threshold.^{15,16} The Otsu's threshold is an algorithm which considers that the image has two classes of pixels and calculate the number of black pixels to design a plot of the healing curve and detects the outline of the wound and its total surface area.¹⁷ Stereophotogrammetry is the new technology that uses a laser scanners to generate a 3D image by the collection of points x, y and z co-ordinate system of an object to obtain the area and volume of wound.¹⁸

Still, about the wound measurement methods, the digital planimetry allows to obtain wound surface area by means of a digital photo and a calibration marker placed next to the wound edge followed by use of a computer or smartphone for image analysis based on pixels per centimeter, providing, therefore, the two-dimensional (2D) outline of wound.^{19,20,14}

However, the techniques reported previously, including studies concerning digital planimetry, addressed only the area and volume of the wounds leaving a gap in scientific literature regarding the identification of type and percentage of tissue that will aid vascular nurses to manage devitalized tissues avoiding wound infections. Furthermore, there is no previous research using digital planimetry based on the K-means clustering algorithm which is a color segmentation Red-Yellow-Black (RYB), the tissues as granulation, slough and necrosis, respectively, in percent.²¹⁻²⁵

To fill this gap, our study quantifies, tissue type in neuropathic and venous ulcers by digital planimetry. This facilitates assessment to optimise the management of devitalized tissue, as well as sup-

port for protocol assistance elaboration with the goal to prevent wound infections.

Material and methods

Design study and sample

This cross-sectional study that recruited 24 participants with chronic wounds (neuropathic and venous) ulcers from an Outpatients Wound Clinic located at a National Reference Center in Sanitary Dermatology and Leprosy of the Federal University of Uberlândia. Patients were enrolled after meeting the inclusion criteria and were allocated to one of two groups according to the number of risk factors for delayed healing. The data collection was carried out from January 2021 to March 2021.

Group definition criteria

In this study, two groups were based on the number of wound-related risk factors associated with delayed healing. The risk factors for delayed healing were: wound bed area greater than 10 cm²; discolored and/or dark red granulation tissue; and granulation tissue less than or equal to 70% of the wound bed.^{26,27} The patients with 1 risk factor for delayed healing were allocated to group 1 and patients with 2 or 3 risk factors were allocated to group 2. The risk factors for each patient were assessed on recruitment during the initial wound assessment by a nurse specialized in dermatology using planimetry.

Digital planimetry, reliability and software for analyzing images

The software WoundDoc Plus®, 2.8.2 Free Trial Version - 2019, designed for android and Apple's iOS mobile, was used for digital planimetry with the goal of determining the wound area (cm²) and percentages of tissues by type (granulation, necrosis, slough, epithelization and others not identified) whose identification was performed by means of color as shown in Figs.1 and 2. The WoundDoc Plus® determines the wound area and tissue type, in percent, using the Red-Yellow-Black system, a tool to evaluate the wound bed tissue characteristics. Red indicates the granulation tissue, yellow is slough and black is necrosis, according to previous descriptions.²¹ In addition, the K-Means clustering algorithm, described by Hartigan and Wong,²² was employed in this application for tissue identification. All digital photographs were visualized and measured using the software Image J 1.53e freeware (National Institutes of Health, Rockville, MD, USA; <http://imagej.net/ImageJ>) for processing and analyzing scientific images.^{28,29} Using of photographs, the surface area of 24 patients were measured using WoundDoc Plus® and Image J 1.53e. Subsequently, the intrarater reliability was obtained through the intraclass correlation coefficient (ICC) showing an excellent reliability with ICC of 0.99 ($p < 0.001$; CI: 0.9981-0.9997) when the areas provided by these software packages were compared, indicating an accurate measurement given by WoundDoc Plus® (supplementary data).

Taking digital photographs and analysis

The digital photographs were taken by a nurse specialized in dermatology, trained for this research protocol using a 13 megapixel Samsung Galaxy M20 camera. The camera position was standardized as 30 cm from the wound bed with its lens axis perpendicular to the wound plane. Two photographs of each wound were obtained with and without flash. A 3 cm paper ruler placed adjacent to the wound was used for calibration. The pictures were obtained after intensive cleaning of the wound bed

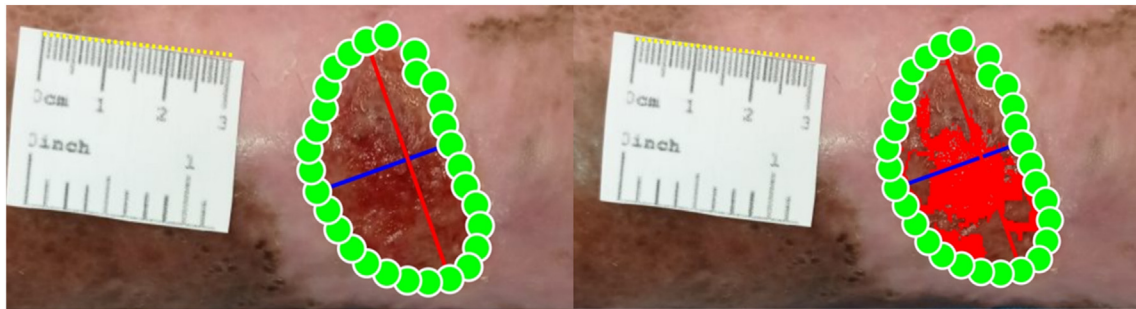


Fig. 1. Total wound area identified by employing a 3 cm calibrator to measure the wound bed using the pixel scale and the percentage of granulation tissue marked by red coloration.

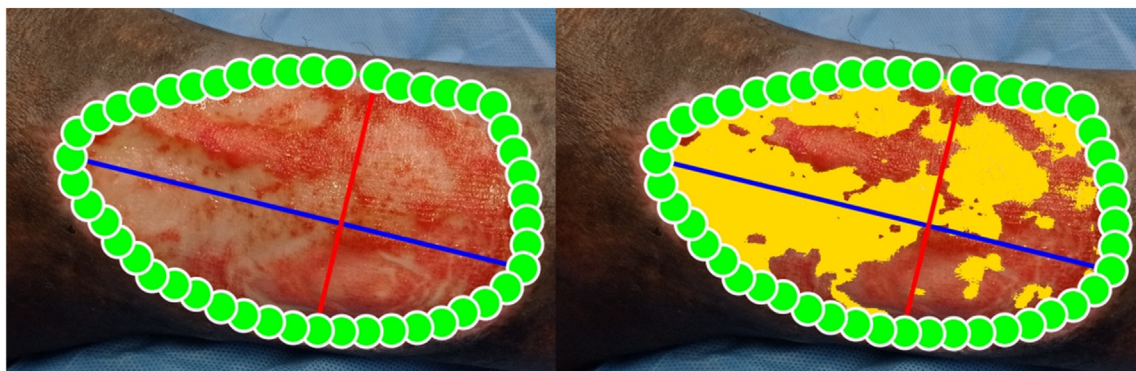


Fig. 2. The software images show the original picture of the wound and identification of slough by colour and in percentage of a venous ulcer located in the medial malleolus of the right leg by means of yellow coloration.

with saline solution until complete removal of the foreign bodies and residual exudate on wound bed. For data collection, four wound views were considered: anterior, posterior, lateral and medial. As to the percentages of tissues, for those wounds which required more than one view, the final values were calculated by means of the sum of percentages divided by the number of views. The sum of tissue percentages for all wounds was equalled 100%.

The digital planimetry of wound photographs were analyzed by another nurse also specialized in dermatology using a personal computer for Image J 1.53e and a smartphone for WoundDoc Plus®, 2.8.2.

The methods for pictorial analysis of chronic wounds are similar for the two software programs. The method for analysing the wound photographs by the software Image J 1.53e has been described in another study.²⁹ The lines drawn along the border of the wound and the straight line along the calibrator, in the software Image J 1.53e, were implemented using the digitalizing tablet One by Wacom®, model CTL472L.

With regard to the software WoundDoc Plus® 2.8.2, after the photograph file was opened inside, a line was drawn along the border of the wound using a Capacitive Stylus Pen ifundom®. The next step was to draw a line along the calibrator (image of 3 cm). Then the software calculated the distance in pixels of the segment, in this case in centimeters (cm).

Based on distance in pixels, the software automatically recalculated the wound area in cm², the longest length and the widest width. Afterwards, the software obtained the total area and the percentages for each type of tissue after clicking on the bottom "beta" that is shown in Supplementary Figure 1.

All wound photographs were assessed clinically to confirm whether the software classified the type of tissue correctly. In those wounds whose granulation tissue was indicated by the soft-

ware as epithelial (pink color) or other tissue not identified (grey color), the percentage was modified by an expert who adjusted the real percentages, as depicted in Supplementary Figure 1.

Inclusion and exclusion criteria

The inclusion criteria were: patients with a chronic wound (venous or neuropathic) in lower extremity and older than 18 years attending the Wound Outpatients Clinic. Exclusion criteria were: individuals with acute or chronic wounds associated with other etiologies such as a burn, injuries, pressure injuries, oncology-related wounds, and arterial ulcers.

Data collection

The epidemiological and wound variables were recorded by using an instrument for data collection and by means of physical examination. The etiology of wounds was diagnosed by physicians (neurologist and dermatologist). The main demographic and epidemiological variables used in the study were: sex; ethnic group; age; body mass index (BMI); comorbidities (hypertension, diabetes, previous leprosy treatment) and social habits, smoker, alcohol consumer [more than 300mL/day of beer; more than 60mL/day of wine; more than 30mL/day of spirit]. The variables associated with wounds were: wound area; type of tissue in percent; number of wounds; etiology; location; exudate level and type; odor in wound bed; wound edge characteristics, presence of edema and wound duration. The exudate levels were described according to a prior research.³⁰ The odor was assessed qualitatively by a specialist nurse in dermatology, before and after dressing change, according to presence or absence of odor in wound bed. All patients had wound in lower extremity for a time greater or equal to six months.

Table 1
Demographic and epidemiological characteristics of patients with chronic wounds in lower limbs.

		2 or 3 risk factors for delayed healing		Up to 1 risk factor for delayed healing		Total		Z**	p-value
		n	%	n	%	n	%		
Sex	Male	6	25.0	5	20.8	11	45.8	-0.4	0.6820
	Female	6	25.0	7	29.2	13	54.2		
Ethnic group	White	4	16.7	4	16.7	8	33.3	-0.86	0.3865
	Brown	3	12.5	5	20.8	8	33.3		
	Black	5	20.8	3	12.5	8	33.3		
Age	Average (SD $\pm$)	63.1	(± 9.6)	67.8	(± 11.3)				0.2878#
	Median (Min./Max.)	65	(45/78)	67.5	(52/86)				
Body Mass Index (BMI)	Underweight	2	8.3	1	4.2	3	12.5	0.61	0.5371
	Normal	3	12.5	4	16.7	7	29.2	-0.44	0.6534
	Overweight	2	8.3	4	16.7	6	25.0	-0.94	0.3458
	Obese	5	20.8	3	12.5	8	33.3	0.86	0.3865
Type 2 diabetes	Yes	3	12.5	2	8.3	5	20.8	-0.5	0.6152
	No	9	37.5	10	41.7	19	79.2		
Arterial Hypertension	Yes	5	20.8	5	20.8	10	41.7		*
	No	7	29.2	7	29.2	14	58.3		
Previous leprosy multidrug therapy	Yes	7	29.2	8	33.3	15	62.5	-0.42	0.6733
	No	5	20.8	4	16.7	9	37.5		
Smoking history	Yes	2	8.3	0	0.0	2	8.3	-1.47	0.1396
	No	10	41.7	12	50.0	22	91.7		
Alcohol history	Yes	5	20.8	3	12.5	8	33.3	-0.86	0.3865
	No	7	29.2	9	37.5	16	66.7		

Note: ** Binomial test; *p-value equals to 1.000 (equal percentages); # Student's t test.

Statistical analysis

The normality of continuous data was tested using the Shapiro-Wilk test. The *t* test and Mann-Whitney were employed to compare differences between groups for average and sum of ranks, respectively. The binomial test was utilized to verify the association between groups and epidemiologic/demographic/clinical characteristics of participants and wound variables through difference of percentages. A heat map was designed to show the percentage distribution of wound tissue types per patient. The intra-rater reliability between the same total areas for each wound obtained from different software was performed by intraclass correlation coefficient (ICC). The program GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis considering an alpha error of 5%.

Ethical considerations

This study was approved by the Research Ethics Committee at the Federal University of Uberlândia – Brazil (CAAE: 29441020.0.0000.5152). Written informed consent was obtained, respecting the patient's rights.

Results

The present study characterized the epidemiological and clinical profile of 24 patients with chronic wounds in lower extremity, and determined measurements and percentages of predominant tissues in the wound bed by digital planimetry. Comparison of descriptive data between the two groups found no statistically significant differences (Table 1).

Table 2, shows the variables associated with chronic wounds in lower limbs for both groups. We highlighted the associations between low exudate level ($p=0.0405$) and hydrated edges ($p=0.0247$) in the group with up to 1 factor for delayed healing. The wound duration did not have significant differences between percentages when the two groups were compared according to intervals as shown in Table 2.

Nearly all patients in group 2 had a wound with less than 70% granulation tissue. This variable reached statistical significance ($p<0.0001$) (Table 3). Secondly, the discolored and/or dark red granulation tissue (high suspicion of biofilm) was seen as an important risk factor for delayed healing in the group with 2 or 3 factors (66.7%; 8/12; $p=0.0130$) (Table 3). Conversely, although the wound area greater than 10 cm² did not have significance by groups comparison, this factor prevailed in the group with up to 1 risk factor for delayed healing (50%; 6/12; $p=0.4076$) (Table 3).

Fig. 3 is a heat map displaying measurement and profiling clinical characteristics of the wound bed according to percentages of tissue type of granulation, necrosis, slough, epithelial and other tissue in which the grey scale represents their percentage variation.

Discussion

Our study has demonstrated advances in wound planimetry analysis by means of novel findings of wound bed tissues identification as percentage, a fundamental approach for the patient's follow-up, providing data to nurses the management of devitalized tissues, decision-making in relation to debridement, as well as support for protocol assistance elaboration with the goal to prevent wound infections. Moreover, we characterized the sample according to demographic, epidemiological and chronic wound features by describing characteristics of the group with 1 risk factor and characterization of exudate levels and wound edges.

In relation to the sex, the female was more prevalent in our sample, in line with previous cross-sectional study.³¹ Similar results were discussed in other studies which reinforces the higher prevalence of ulcers in this sex.^{2,3,32,33}

In terms of age, older participants prevailed in both groups. This is consistent with a study reported by Australian researchers that individuals with chronic ulcers below the knee had a mean age of 71.1 years.³ Another key fact that explains the higher chronic wound percentage in the older person may be an age-associated inflammatory prolonged response and skin composition changes in this individuals entailing delayed reepithelization.³⁴

Although diabetes and hypertension were not analysed in this study, diabetes is a factor known to affect wound healing due to

Table 2

Variables associated with chronic wounds in lower limbs.

		2 or 3 risk factors of delayed healing		Up to 1 risk factor of delayed healing		Total		Z **	p-value
		n	%	n	%	N	%		
Wound area (cm ²)	Average	33.1		25.3					
	SD (±)	30.0		46.2					
	Median	31.7		5.3					0.3263#
	Minimum	2.0		1.4					
	Maximum	72.7		143.2					
Number of wounds	1	8	33.3	9	37.5	17	70.8	-0.44	0.6534
	2	4	16.7	3	12.5	7	29.2		
Etiology	Neuropathic ulcer	3	12.5	3	12.5	6	25		*
	Venous ulcer	9	37.5	9	37.5	18	75		*
Location	Right plantar	2	8.3	2	8.3	4	16.7		*
	Left plantar	1	4.2	1	4.2	2	8.3		*
	Right leg	5	20.8	5	20.8	10	41.7		*
	Left leg	4	16.7	4	16.7	8	33.3		*
Exudate levels	Low	4	16.7	9	37.5	13	54.2	-2.04	0.0405
	Midium	6	25	1	4.2	7	29.2	2.24	0.0247
	High	2	8.3	2	8.3	4	16.7		*
Exudate type	Serous	10	41.7	8	33.3	18	75	0.94	0.3458
	Serosanguineous	0	0	1	4.2	1	4.2		
	Purulent	2	8.3	3	12.5	5	20.8	-0.5	0.6152
Odor	No	6	25	8	33.3	14	58.3	-0.82	0.4076
	Yes	6	25	4	16.7	10	41.7		
Wound edge	Hydrated	1	4.2	6	25	7	29.2	-2.24	0.0247
	Maceration	4	16.7	2	8.3	6	25	0.94	0.3458
	Dehydration	3	12.5	4	16.7	7	29.2	-0.44	0.6534
	Rolled	4	16.7	0	0	4	16.7		
Lower extremity edema***	Yes	4	16.7	5	20.8	9	37.5	0.42	0.6733
	No	8	33.3	7	29.2	15	62.5		
Wound duration	≥ six months – five years	5	20.8	4	16.7	9	37.5	0.42	0.6733
	> 5 years – 10 years	3	12.5	1	4.2	4	16.7	1.09	0.2733
	> 10 years	4	16.7	7	29.2	11	45.8	-1.22	0.2191

Note: ** Binomial test; *p-value equals 1.000 (equal percentages); *** Edema close to the wound involving the whole lower extremity; # Mann Whitney U test.

Table 3

Risk factors for delayed healing by group of patients with chronic wounds.

		2 or 3 risk factors for delayed healing		Up to 1 risk factor for delayed healing		Total		Z	p-value
		n	%	N	%	n	%		
Less than 70% granulation tissue	Yes	11	91.7	1	8.3	12	50.0	4.08	<0.0001
	No	1	8.3	11	91.7	12	50.0		
More than 10cm ² of wound area	Yes	4	33.3	6	50	10	41.7	-0.82	0.4076
	No	6	50.0	8	66.7	14	58.3		
Discolored and/or dark red granulation tissue	Yes	8	66.7	2	16.7	10	41.7	2.48	0.013
	No	4	33.3	10	83.3	14	58.3		

hyperglycemia in which ischemia inhibits angiogenesis contributing to infection and increasing the risk of amputation.³⁵ These comorbidities were associated with non healing as reported in a previous study.³²

Most of the participants enrolled, 62.5%, had undergone previous multidrug therapy for leprosy. Brazil recorded 18,318 new cases of leprosy in 2021, a disease related to neural damage. The occurrence of plantar ulcers was associated with loss of skin sensitivity caused by *Mycobacterium leprae* and cell-mediated immunity as reported in another studies.^{36,37} Even though our results referred to wound area > 10 cm² did not have association with either group, some studies showed this risk factor was a predictor for infection and delayed healing of venous ulcers.^{3,38}

Our sample had predominantly ulcers of venous etiology. A survival analysis study, that enrolled 250,000 patients older than 50 years, identified increased mortality for patients with venous ulcers.³⁹ Low exudate level was found in the group with one risk factor for delayed healing. However, this association may be influenced by wound area, since the median in this group is lower than in the group with 2 or 3 factors. High levels of exudate

can trigger complications related to moisture and may indicate infection.¹³

In our study the duration of the wound ranged from six months to greater than 10 years. A prior study attributed aging as the responsible for delayed wound healing, since it is associated with declined regenerative capacity, cell senescence, loss of cellular plasticity in aged skin and damage in keratinocytes and fibroblast heterogeneity.⁴⁰ With respect to odor and devitalized tissue (slough/necrosis), these were more frequent in the group with 2 or 3 risk factors, which had a greater number of individuals with less than 70% granulation tissue. These data suggests that odor may be associated with necrosis and slough tissue which mandates debridement for odor control and preventing infection.^{41,42}

The discolored and/or dark red granulation tissue, present in 47.1% of participants, can be related to a suspected biofilm formation. Previous studies have associated, clinically, the presence of this complex community of microorganisms with discoloured granulation tissue.^{43,44}

According to the heat map, granulation tissue was the most common type of tissue present, followed by slough. Similar pat-

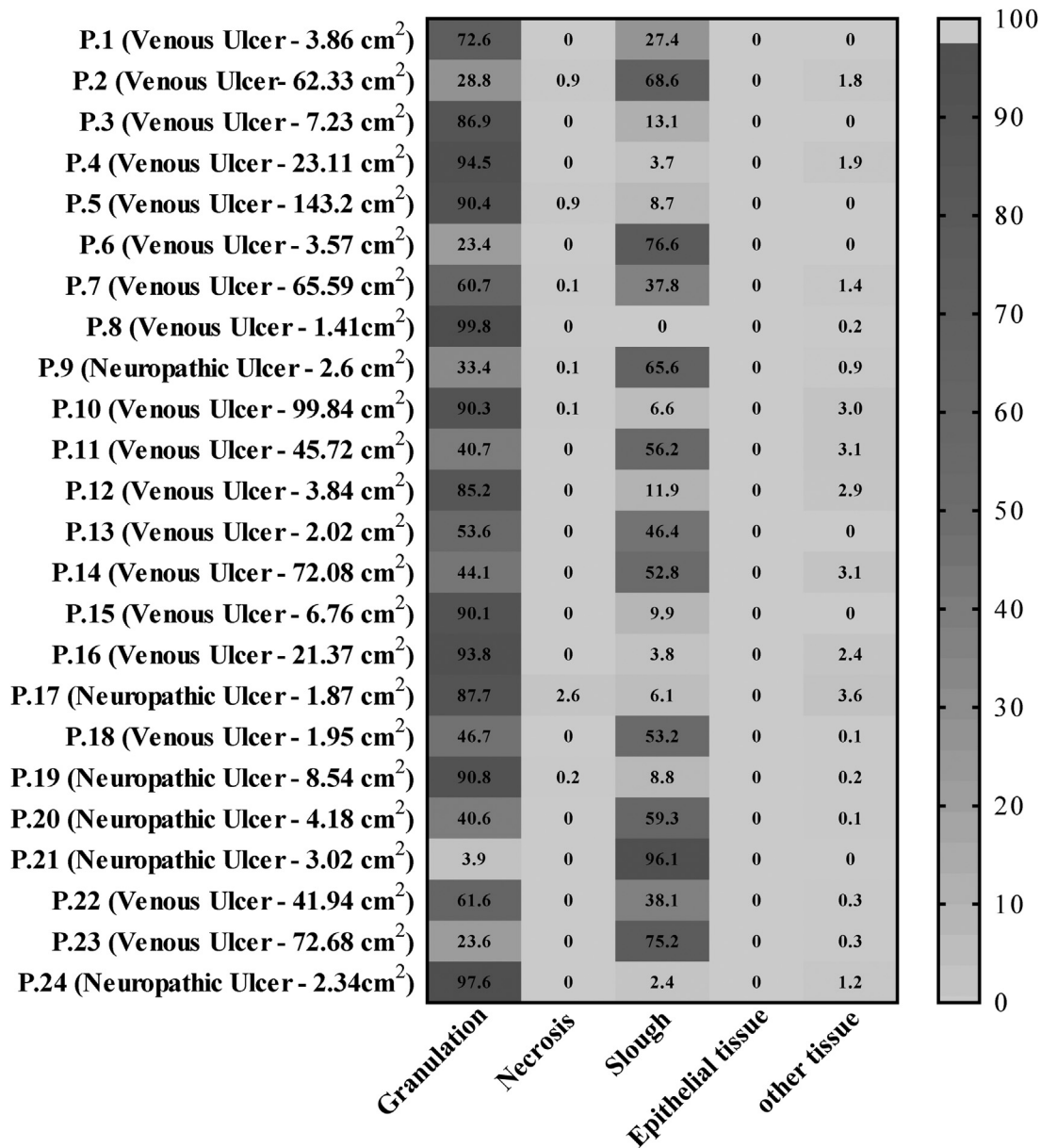


Fig. 3. Heat map of wound measurements showing total area, etiology and percentage by tissue type.

terns were identified in another study, attributing slough tissue as a risk factor for infection in ulcers.³

The main limitation of this research is the small sample size, which does not allow the findings from being extrapolated to the population.

Conclusions

The use of digital planimetry for identifying wound area and tissue type by percentage may assist nurses in providing in-person or virtual wound consultations based on an objective measurement of tissue types. Moreover, serial measurements aim to better assess devitalized tissue, biofilm formation and guide decision-making regarding debridement and specific dressings. Additionally, it supports the elaboration of protocol assistance with the goal of preventing wound infections.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Silvia Bottaro Carvalho Alcântara: Collected the data, Wrote the paper, Other contribution. **Juliano Gonçalves de Araújo:** Collected the data, Other contribution. **Diogo Fernandes Santos:** Conceived and designed the analysis, Performed the analysis, Wrote the paper. **Tathiane Ribeiro da Silva:** Collected the data, Performed the analysis, Wrote the paper. **Isabela Maria Bernardes Goulart:** Conceived and designed the analysis, Performed the analysis, Other contribution. **Andrea Mara Bernardes da Silva:** Performed the analysis, Wrote the paper, Other contribution. **Douglas Eulálio Antunes:** Conceived and designed the analysis, Contributed data or analysis tools, Performed the analysis, Wrote the paper, Other contribution.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.jvn.2023.06.005](https://doi.org/10.1016/j.jvn.2023.06.005).

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