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BIOMICROSCOPY HIGHLIGHTS THE EFFICACY OF SKIN GRAFT ON MICROVASCULAR PARAMETERS IN NON-DIABETIC ULCERS

Medicine

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ABSTRACT

Objective. Laser Doppler flowmetry (LDF), transcutaneous partial pressure of oxygen (pO2) and carbon dioxide (pCO2), and Biomicroscopy (BM) are exams used in daily clinical practice to assess capillary and microvascular functionality. Skin graft (SG) is a well-established intervention for the treatment of chronic lower leg ulcers. The sequence of improvements at the microvascular and macrovascular level, letting to a successful outcome after SG, is not yet been determined. This observation aims to determine which parameters of capillary functionality could show how SG takes to a positive outcome. **Methods.** In this trial, cutaneous perfusion, district microcirculatory reserve, and variation of cutaneous perfusion induced by the postural vasoconstriction reflex (VAR) were measured with LDF as the number and diameter of capillary loops at 1 cm, 2 cm, and 4 cm from the big toe nailfold and the ulcer edge. Cutaneous gas exchanges were detected through pO2 and pCO2, and capillary density and average diameter loops were recorded with BM. Because of the exploratory nature of this study, consecutive patients' afferent to our structure receiving SG for the treatment of non-diabetic chronic non-relapsing ulcers on the lower limb, were assessed before and 5 days after SG. All measures were collected by the same operator and SG was performed in a standardized manner. Paired non-parametric statistical tests were performed to compare basal with after SG measurements. **Results.** All 13 patients observed had a clinically successful outcome after SG in terms of pain reduction, absence of SG complications, and absence of secretion. The median number of capillary loops changed at the different evaluation points (e.g. from 19 vs 21 (P=.004) at the big toe nailfold and 16 vs. 22 (P<.001) at 2 cm from the ulcer edge). The median diameter of capillary loops changed at the different evaluation points (e.g. at the big toe nailfold decreased from 24mm to 19mm (P=.004) and from 16mm to 22mm (P=.031) at 2 cm from the ulcer). The median values of pO2 increased from 10 mmHg to 36 mmHg (P=.005) on supine and from 28 mmHg to 3 mmHg (P=.006) at the dependent lower limb None of the LDF measures, or pCO2.**Conclusion.** In this exploratory evaluation, BM measurements reveal, for the first time, a successful outcome after SG for the treatment of chronic lower limb skin ulcers, especially on the big toe nailfold, 1 and 2 cm from the ulcer. The improvement observed at the capillary level seems parallel with the improvement of tissue oxygenation.

KEYWORDS

Laser Doppler Flowmetry; Capillaroscopy; Biomicroscopy; Skin graft; Transcutaneous Oxygen and Carbon Dioxide

Introduction

Systematic vasculopathy is a common complication of diabetes mellitus (DM) that is observed in non-diabetic patients, too. Much of its morbidity is attributable to the macrovascular and microvascular complications related to hypertension and hyperglycemia.

Laser Doppler flowmetry (LDF) uses the frequency shift of low-power (typically in the region of 1 to 5 mW and single mode) laser light produced by the Doppler effect to assess tissue blood flow. Depending on the laser wavelength employed, LDF can assess blood flow in the nutritional capillaries close to the skin surface and the underlying arterioles and venules deeper in the dermis, involved in the regulation of skin temperature. The use of LDF can be standardized, to address the reproducibility of microvascular measurements, and is commonly used to evaluate the effect of surgery at the microvascular level .

The transcutaneous partial pressure of oxygen (pO2) and carbon dioxide (pCO2) is used in clinical practice to determine the degree of O2 availability and CO2 permeability in a sensitive and reproducible manner[6].

Bollinger et al. have shown that a capillaroscopic examination can detect a high frequency of dilatation at the apex of loops which may be the key feature in DM, but these changes do not appear to be linked to DM duration or disease sub-type. Even if these pieces of evidence are mainly experimental and rarely translated to daily clinical practice, they can constitute the clinical rationale for using capillaroscopy in non-diabetic patients to evaluate the evolution of vascular ulcers in lower limbs.

Biomicroscopy (BM) is a simple, non-invasive capillaroscopic test for the in vivo assessment of nutritional cutaneous microcirculation. During BM, the morphology of capillary loops is evaluated by using a magnification system[7–]. This simple technique can provide valuable diagnostic information in the clinical microvascular setting[3]. BM can be performed with various optical instruments, from a standard clinical dermatoscope to a dedicated capillaroscopy system having tailored tissue lighting, a micropositioner for accurate focusing, and image capture and analysis functions with an ad-hoc software (MicroCap, Microlab, Padova, Italy). A drop of immersion oil is added to the nailfold to aid the visibility of the capillary loops. A cold illumination source is used. Normally, the number of capillaries in this area varies between 20 and 30 per mm^2, being about one-half of the finger and toes[7].

A cutaneous ulcer is defined as a consequence of a vascular pathology (arterial, venous, or mixed). To date, even if the short-term clinical efficacy of skin graft (SG) in terms of pain improvement, exudate reduction, and stimulation of granulation tissue is well known the physiopathologic mechanism underlying this tissue recovery remains unknown.

The variation of nutritional bed can be evaluated using BM, and peripheral gas exchanges can be evaluated with transcutaneous partial pressures and microvascular perfusion with LDF even after SG, to assess the clinical outcome of this procedure, but this has never been assessed in the daily practice.

The aim of this observational study was to perform an exploratory evaluation of the presence of biomicroscopic changes after SG. We also estimated how the use of LDF and transcutaneous partial pressures can evaluate the early effectiveness of SG for tissue recovery.

Methods

All patients attending our structure between June 2013 and June 2014, with chronic non-relapsing ulcers on the lower limb that appeared between 6 and 12 months before, were included. All patients took the clinical best medical treatment for their disease. All patients were non-diabetic and non-smokers and in addition had no prior amputations performed. Age, gender, and ulcer aetiology were collected. All patients took Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) as analgesia.

All ulcers were carefully cleaned and bent for at least 2 weeks before SG to reduce possible variability of microvascular parameters due to edema, fibrin, or possible residual infections.

All data were collected by the same operator, to avoid inter-operators' variability, before SG (on the same day or the day before) and 5 days after it, as the first evaluation of the SG outcome was performed 5 days after the intervention, to assess SG outcome and absence of complications (possible graft diastasis, infection) or the presence of pain or exudates.

SG was performed using a homologous (HomT) transplant. Human De-Epidermized Derma (DED), maintained frozen (-27°C) and provided by the "Banca dei Tessuti di Cesena della Regione Emilia Romagna" – Italy, was used for HomT. DED was implanted after being unfrozen in a proper conservation medium. The ulcerated leg was carefully cleaned for a sterilized operative field. The ulcer surface and its periphery (at least 1 cm) were carefully cleaned with a normal ringer solution, too. DED (ready as standard stripes) was then applied as a mono-bed after mesh graft (Mesh-Graft II Tissue Expansion System, Zimmer® Surgical, Dover OH, USA) to ensure surface expandability, tissue repair, and the passage of the exudate through the DED.

All transplants were bent with antiseptic gauze (Holoil garze gel - Ri.Mos, Mirandola - Italy) and fixed gauzes. Fixing bandage was applied using German cotton and cohesive conforming gauze bandage (Peha Haft, Hartmann, Heidenheim - Germany). A non-elastic bandage (Rosidal K, L&R, St. Gallen - Switzerland) was applied, too.

Patients were not anesthetized during SG and the patient's therapy was not changed in the 5 days after SG.

Capillary loops density (measured as units by mm^2) and capillary average diameter (mm) were measured 3 times, using a biomicroscopy instrument (MicroCap, Microlab, Padova, Italy), connected to a computer with specific software for the collection and storage of nutritional capillary parameters on the big toe nailfold, on the feet dorsum, on the ulcer edge and 1, 2 and 4 cm from it, according with Curri suggestions[9]. The average of the 3 measures at each measuring site was included in the statistical analysis.

LDFs were assessed, with a standardized procedure, using Periflux 5000 (Perimed – Italy), after heating the probe to reduce the variability of cutaneous perfusion linked with temperature. LDFs were measured as values and their percent postural changes [(H-D)/H x 100] at the heart (H) level and during dependency (Dep, leg dangling 50 cm below the heart level (VAR) with probe temperature 36°C). In normal subjects, VAR evaluates the variation of cutaneous perfusion induced by the postural vasoconstriction reflex, measuring a 60% reduction of the laser-Doppler signal when compared with the measurements obtained in the normal subject in the supine position[9].

Cutaneous perfusion and microcirculatory reserve were recorded on the plantar surface of the big toe, on the II inter-metatarsal space (dorsum foot), 1 cm from the ulcer edge, and on the third medium of the lower limb. Measurements were performed, with the lower limb supine or depending, at 36°C. Maximal microvascular cutaneous perfusion was measured with laser-Doppler, too, as the variation of cutaneous flux after maximal point-source stimulation, via probe heated to 44°C, with the supine lower limb, to obtain local vasodilation and estimate residual maximal local perfusion.

Cutaneous perfusion was measured as Perfusion Units (PUs), and VAR as percent variation of perfusion (%).

pO2 and pCO2 (mm Hg) were evaluated using Periflux 5000, Perimed - Italy. The Clark-Severinghaus electrode was heated to 44°C to maximize local vasodilatation for improving measure precision and reproducibility through the reduction of the variability of cutaneous functional and metabolic values temperature-linked. Heating allowed the maximal extraction of gases. We measured pO2 and PCO2 when the leg is depending to maximize gas extraction from peripheral districts, too.

A sample size of 10 subjects was calculated assuming a standard deviation (SD) approximately equal to the expected mean variation of the primary outcome variable (capillary loops density and capillary average diameter), a .05 level of significance, a power of .80, and a two-tailed paired samples parametric test. As the outcome was expected to be not-normally distributed, the use of non-parametric tests was forecasted. Thus, according to Lehmann recommendations, an additional 25% of subjects were included, obtaining a total sample size of 13.

Being a preliminary observation, having the main objective of assessing the possible modifications of microvascular parameters to evaluate the outcome of SG, an open-non-controlled design was chosen.

Descriptive statistics included mean ± SD, median, minimum and maximum. All graphs were designed using the Box-Whisker plots where the ends of the whisker are set above the third quartile and below the first quartile. If the Minimum or Maximum values are outside this range, then they are shown as outliers. Variations between baseline and endpoint were compared with both paired Mann-Whitney U-test (u-P) and t-test (t-P). Because of the observational nature of the study, only comparisons with a $P<.05$ for both tests were considered statistically significant, to maintain a more conservative approach.

All statistical analyses were performed with Microsoft Excel 2010. Mann-Whitney U-test was performed using the excel worksheet from the Handbook of Biological Statistics.

Because of the small sample size, no subgroup analyses were performed.

All patients gave their informed consent to be included in this study and the possible disclosure of the results belonging to this observation. The local ethical review board was advised about this data collection for the study proposal.

Results

A total of 13 (2 males, 11 females) non-diabetic patients with advanced lower leg ulcers were observed. Their age ranged between 28 and 78 years (mean 65.6±16.3). 4 patients had lesions of arteriopathic etiology (Leiriche stage 2b), 6 phlebologic (deep venous insufficiency), and 3 both arteriopathic and phlebologic (Leiriche stage 2a, deep venous insufficiency). All ulcers were located in the distal third of the leg. Ulcers area ranged between 10 and 300 cm^2; due to the nature of this study, the link between ulcer size and the outcome was not considered. Two of the arteriopathic patients had also polycythemia treated with onco-carbide. None of the observed patients had critical ischemia as none of them had acidosis.

All patients had a clinically satisfactory outcome post SG, according to expectations, so none of them continued the use of painkillers 5 days after SG or showed exudates, or had any complication, including graft diastasis or infection. None of the patients dropped out and, thus, all needed data were collected (no missing data).

The number of capillary loops was significantly increased at the big toe nailfold (median 19 vs 21 u - P=.004 - 18.4±2.4 vs 21.0±1.4; t-P<.001), 1 cm from ulcer edge (median 16 vs 31; u-P<.001 - 16.0±1.1 vs 32.2±4.8; t-P<.001), 2 cm from ulcer edge (median 16 vs 22; u-P<.001 - 15.9±2.0 vs 22.3±1.7; t-P=.008) and 4 cm from ulcer edge (median 16 vs 22; u-P<.001 - 16.9±2.0 vs 22.5±1.2; t-P<.001) (Fig. 1). The diameter of capillary loops was significantly reduced at the big toe nailfold (median 24mm vs 19mm; u-P=.004 - 24.2mm ± 2.6mm vs 19.0mm ± 1.6mm; t-P<.001), on the ulcer edge (median 39mm vs 28mm; u-P<.001 - 39.8mm ± 5.3mm vs 28.6mm ± 3.7mm; t-P<.001), 1 cm from ulcer edge (median 16mm vs 31mm; u-P<.001 - 32.2mm ± 4.8mm vs 23.4mm ± 2.7mm; t-P<.001) and 2 cm from ulcer edge (median 16mm vs 22mm; u-P<.031 - 26.4mm ± 4.3mm vs 23.2mm ± 2.7mm; t-P=.008) (Fig. 2).

Laser Doppler parameters did not change significantly five days after skin graft (Fig. 3, Fig. 4) as VAR (Fig. 5), pCO2 (Fig. 6), while pO2 significantly improved on the ulcer both on supine (median 10 mmHg vs 36 mmHg; u-P=.005 - 14.5 mmHg ±14.0 mmHg vs 34.5 mmHg ± 14.5 mmHg; t-P<.001) and dependent lower limb (median 28 mmHg vs 53 mmHg; u-P=.006 - 24.6 mmHg ±13.7 mmHg vs 44.9 mmHg ±16.9 mmHg; t-P<.001) (Fig. 7).

None of the patients reported any clinically relevant adverse event during the observation period.

Discussion

SG is used to support lower limbs ulcers recovery.

Our observation shows, for the first time, that BM measures, including an increased number of capillary loops density and reduction in capillary diameter in some sites, improved 5 days after SG, while these improvements were not observed for LDF and pCO2 measurements. BM improvements parallel with pO2 increase on ulcer edges.

BM assessments on the big toe and 1 and 2 cm from the ulcer edge seem to be the most sensitive to show post-SG amelioration of tissue metabolism of the peri-wound area, according to the observed

increased number of capillary loops 4 cm from the ulcer edge and the reduced capillary diameter on the ulcer edge.

These results could be linked with the observation that BM investigation can anticipate the risk of skin necrosis in patients with arterial insufficiency.

The absence of any significant improvement observed with LDF, including VAR and 44° perfusion might be due to the small sample size and the short term of observation, which, together with the absence of specific interventions on the macrovascular system, have not allowed a significant modification of macrovascular flow.[16,17]

All basal microvascular measures showed high variability in the degree of tissue metabolic damage; these parameters, observed at baseline in the area around the lesion, were higher than those observed on the ulcer edge. According to transcutaneous partial pressure measurements, the ulcer was hypoxic and had a reduced residual capillary functionality associated with ischemic neuritis, being responsible for pain and exudate production. The feedback loop including edema, linked with the altered hemodynamic equilibrium of the arterio-venular regulation of local perfusion, the reduced capillary diameter, and number, worsening local oxygenation, might be linked with ulcer chronicity.

Edema improvement reduces capillary diameters and improves capillary visibility, allowing measuring a higher number of capillary loops on the big toe, 1 and 2 cm below the ulcer, being the most sensitive measurement zones.

Thus, in the peri-ulcerative zone, decreased peri-vasal edema improved the visibility of capillary loops, together with their reduced diameter, and, together with increased tissue oxygenation, reduce functional capillary-feeding impairment.

Even if SG are widely used in clinical practice, the reasons for forecasting their successful outcome remain unknown. The results belonging to this observation suggest that SG seems to improve this microvascular imbalance highlighted by BM measurements. Obviously, SG does not increase total local perfusion but optimizes local flow distribution reducing tissue local edema and exudate production in the peri-lesional area.

These changes in microvascular patterns improve local nutritional functionality, linked with the observed increased local oxygen availability, which might explain the improvement of trophic lesions after SG. The observed reduction in pain suggests the hypothesis that homologous SG inhibits the release of vasoactive substances responsible for generating pain, improving the ulcer recovery process.
This is an observation, performed on a few patients from a single center by a single operator, to minimize assessors and intervention variability. The small sample size reduces the possibility of generalizing the outcomes. Furthermore, some of the patient's characteristics, concomitant treatments, and the data of follow-up visits were not recorded. The relation between hypoxia and SG outcome was not evaluated, too.

These results need to be confirmed in a properly designed study to verify the usefulness of BM measurements in the evaluation of SG outcomes.

Conclusion

This experience shows that BM evaluations reveal a successful outcome, in terms of re-perfusion, at the capillary level paralleling with the improvement of tissue oxygenation after SG for the treatment of chronic lower limb skin ulcers. Further randomized, prospective studies are needed to confirm hypotheses belonging to this preliminary observation.

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