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## Clinical efficacy of cord blood platelet gel in chronic limb-threatening ischaemia patients: a case series

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**Abstract.** The cord blood platelet gel (CBPG), rich in growth factors and chemoattractants molecules, is a useful tool to treat lesions with tissue loss as it accelerates remission and ensures the functional recovery of the limb. The results obtained in a series of 10 patients treated at the “Fondazione IRCCS Casa Sollievo della Sofferenza” in San Giovanni Rotondo (Italy) with CBPG in the treatment of revascularized patients with chronic limb-threatening ischemia (CLTI) are presented here. In the period 2017-2021, they were treated with surgical toileting of the ulcers and application of CBPG. As a general scheme, CBPG was applied once every 21 days for 3 times; 8 out of 10 patients observed had diabetes mellitus, of these 4 were on insulin therapy. One patient underwent a major amputation; two patients died for non-infectious causes. Studies in the literature show that CBPG is safe for patients affected by CLTI and undergoing revascularization, promoting faster healing. Our results appear to overlap this data, also showing CBPG as an effective therapeutic option for the treatment of vascularized patients with foot lesions for CLTI, reducing the risk of major amputation. In perspective, multicenter randomized studies are needed to evaluate immediate and late results.

**Keywords:** chronic limb-threatening ischemia, diabetic foot, human umbilical blood, platelet, thromboangiitis obliterans, regenerative medicine.

### INTRODUCTION

Chronic limb-threatening ischemia (CLTI) is the end stage of peripheral artery disease (PAD). Moreover, it is a highly morbid disease, including significant mortality rates, limb loss, pain, and diminished health-related quality of life among those affected (Goodney et al.). For this reason, the management of CLTI in patients with foot lesions requires, after revasculariza-

tion interventions, a long painful and expensive wound care. In the last years, CBPG has been successfully used for regenerative medicine in skin lesions (Piccin et al. 2017; Bisceglia et al. 2020), oral mucositis (Gelmetti et al. 2018) and orthopedics (Caiaffa et al. 2020).

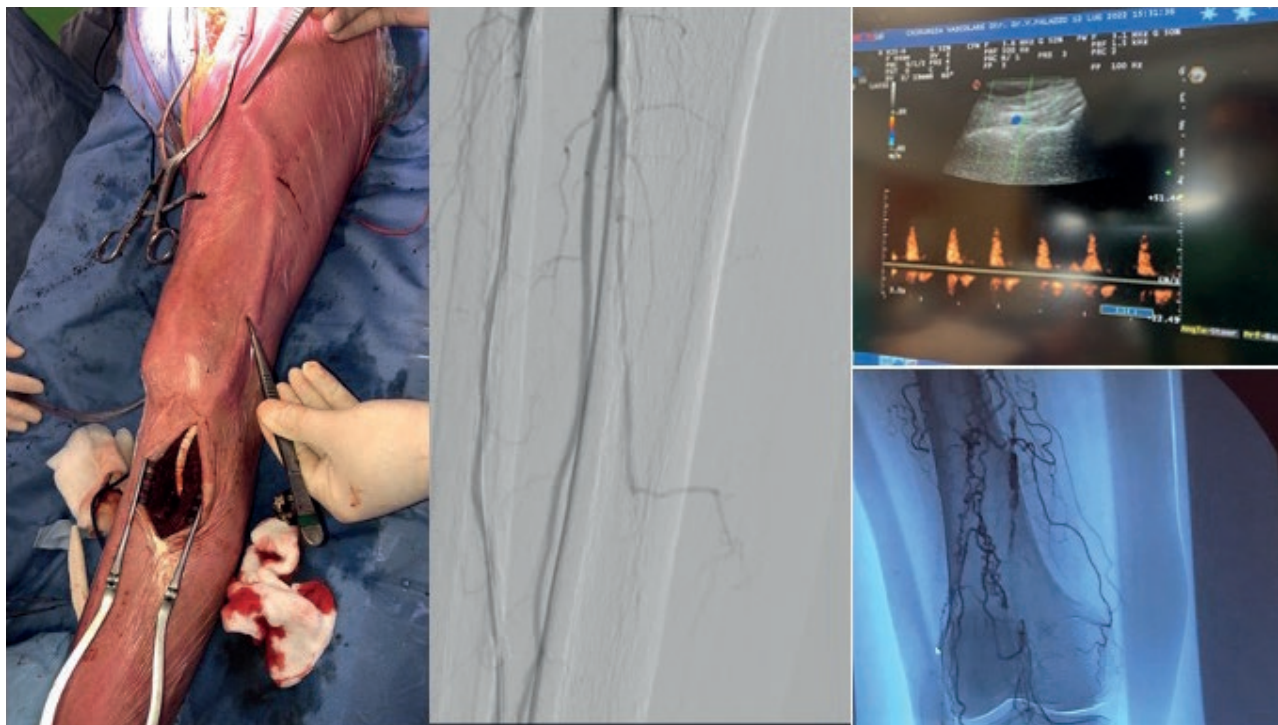
## PATIENTS AND METHODS

We report the case of 10 patients suffering from CLTI treated with vascularization intervention, ulcer's debridement and/or minor amputation and then cord blood platelet gel application (CBPG) in the Department of Vascular Surgery of the "Fondazione IRCCS Casa Sollievo della Sofferenza", located in San Giovanni Rotondo (Italy) between 2017 and 2021. As many as 8 patients had a type II diabetes; 2 patients had Buerger disease, also known as thromboangiitis obliterans; all patients had non-infected wounds prior to CBPG application and no local and systemic clinical signs of sepsis. Exclusion criteria included ongoing chemotherapy and oral corticosteroid therapy, a history of malignant tumors with a disease-free interval of 3 years or less. Informed consent was obtained from the patients prior to CBPG application and after approval of the Ethics Committee of our Hospital. Demographic characteristics, comorbidities, WiFi score, the type of treatment and outcome are listed in Table 1. Vascular lab testing after arterial revascu-

larization demonstrated improved tibial artery flow in all patients with improved pulsatility and peak systolic velocity > 40 cm/s (Figure 1). Thereafter, each patient is addressed to standard wound care (normal saline solution and the surrounding skin was cleansed with betadine solution) and CBPG wound applications. First of all, it is determined the count 'ABO' cord group of the patient because the CBPG should be hemocompatible with the patient's blood (as well virological markers for HIV, HCB and HBV). The CBPG was applied topically on the wound and covered with sterile gauze. The CBPG protocol in our Department provides for one application each 21 days for 3 times. At the end of the protocol, patients underwent follow-up in the vascular lab on a monthly basis. Our clinical outcomes were: the survival of the patient and the preservation of the limb. CBPG is prepared from Cord Blood (CB) units collected after the informed consent has been collected from the mothers. The units were placed into plastic bags containing 25-30 mL of citrate-phosphate-dextrose anticoagulant by trained midwives, before and after placental delivery in natural deliveries and in Caesarean sections, respectively, according to the validated and standard Apulian Cord Blood Bank (located in the Casa Sollievo della Sofferenza Hospital) operating procedures. After storage and transportation at monitored room temperature to the CB banks were performed, the units were processed within 48 hours of collection. Units containing less than

**Table 1** Demographic characteristics, comorbidities, Rutherford stage, the type of treatment, ulcer's characteristics of our cases ( 1 Peripheral artery disease 1=Buerger Disease, 2=type II diabetes; 2 coronary artery disease, 3 chronic kidney disease, 4 Wound-Ischemia-Foot Infection classification system ; 5 below-the-knee femoro-popliteal bypass, 6 Plain Old Balloon Angioplasty, 7 Drug Eluting Balloon, 8 endoarterectomy, 9 Above-the-knee femoro-popliteal bypass, 10 wound healing

Patient (year)	PAD <sup>1</sup>	Age	CAD <sup>2</sup>	CKD <sup>3</sup>	HbAc1 (mmol/L)	WiFi score <sup>4</sup>	Revascularization surgery strategy	Foot lesion	Outcome
1 (2017)	2	86	No	No	53	222	F-P bypass BK <sup>5</sup>	Gangrene I finger, 6 cm length medial ulcer	1th-toe amputation <u>72-month-follow-up died for cardiovascular disease</u>
2 (2021)	2	74	Yes	Yes	54	131	F-P bypass BK	Two dorsalis ulcers	1th trans-metatarsal amputation
3 (2019)	2	62	Yes	No	51	222	PTA stenting	Forefoot amputation stump dehiscence	Complete WH <sup>10</sup>
4 (2020)	2	78	No	No	53	221	POBA <sup>6</sup>	I finger amputation stump dehiscence	Complete WH
5 (2018)	2	73	Yes	No	99	121	DEB <sup>7</sup>	10 cm length plantar ulcer	Complete WH <u>50-month-follow up died for cardiovascular disease</u>
6 (2020)	2	83	Yes	No	57	231	POBA	I-II-III finger amputation stump dehiscence	Complete WH
7 (2020)	2	81	Yes	No	97	332	EA <sup>8</sup>	Infected heel ulcer	<u>Tigh amputation</u>
8 (2018)	2	72	Yes	Yes	55	221	F-P bypass AK <sup>9</sup>	External heel ulcer	Complete WH
9 (2020)	1	73	No	No	No diabetic	121	F-P bypass AK	Intergidital ulcers	Complete WH
10 (2018)	1	41	No	No	No diabetic	221	F-P bypass AK	Acral ulcers	Complete WH



**Figure 1.** Vascular interventions realized in our case series are finalized to improve tibial distal arterial flow.

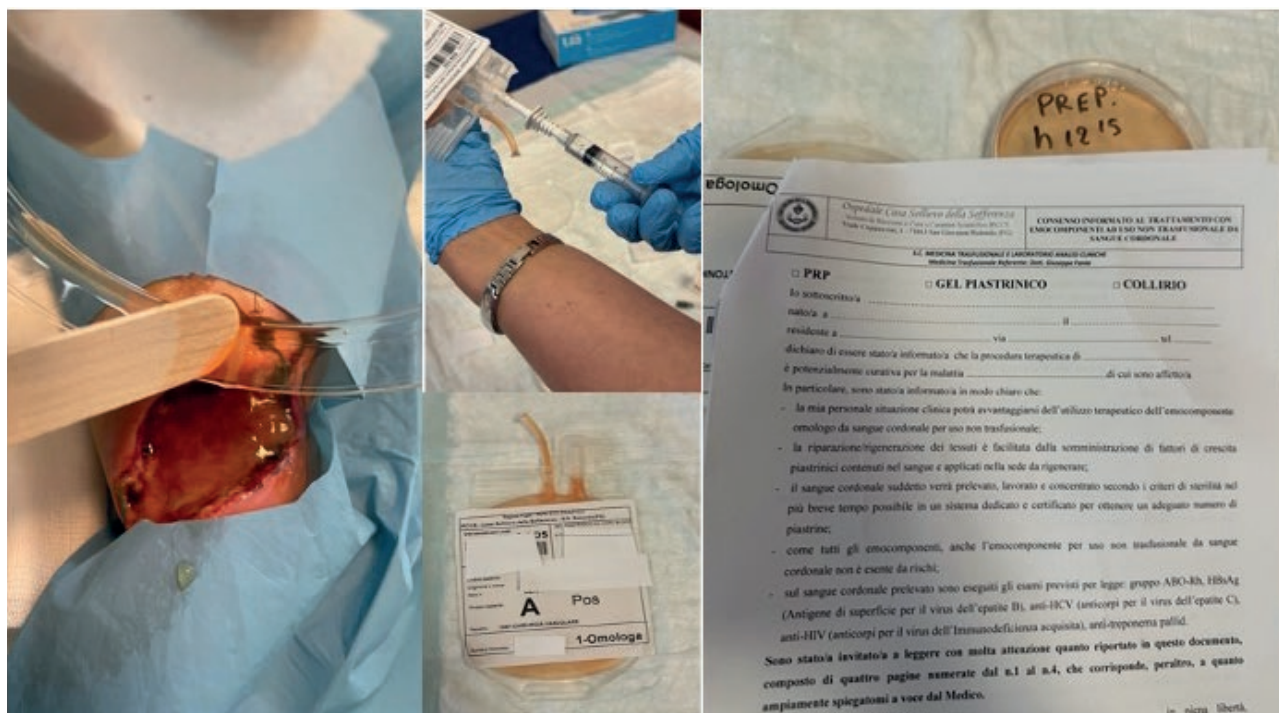
$1.5 \times 10^9$  nucleated cells (which are not routinely banked for allogeneic hematopoietic transplantation purposes), a platelet count of  $150 \times 10^9/L$  or greater and a volume of 50 mL or greater were processed into CBPG within 48 hours of collection by the blood bank staff. The units are centrifuged at 200 to 210 x g for 10 to 15 minutes, and the platelet-rich plasma is collected in a transfer bag, which is centrifuged at 1800 to 2600 x g for 15 minutes. Most of the supernatant platelet-poor plasma is then removed, and the platelets are resuspended at a concentration of  $1 \pm 0.2 \times 10^6/ mL$ . The platelet concentrate, with an average volume of approximately 10 mL, is finally transferred into a storage bag and cryopreserved without cryoprotectant in a mechanical freezer at a temperature below  $-24^\circ C$ . At the time of use, the platelet concentrate is thawed, and a platelet gel is formed by the addition of 10% calcium gluconate in a 1:3 ratio (Figure 2).

## RESULTS

As many as 7 out of 10 patients had complete wound healing (WH). Two patients died of cardiovascular disease (table 1). Unfortunately, 1 out of 10 patients (patient n. 7) underwent a major amputation because of a gangrene of the limb at the 25-month follow-up.

## DISCUSSION

Few are the clinical trials in the Literature (Samarikanova et al. 2020, Volpe et al. 2017) on the CBPG application to the diabetic foot, and our case series presents the results on foot lesion also in thromboangiitis obliterans, which is a nonatherosclerotic, inflammatory and thrombotic involvement of distal vessels of the extremities, most commonly affecting young male smokers. After vascular intervention, in selected patients, other adjuncts therapies can be used, including spinal cord stimulation (De Caridi et al 2016 feb, De Caridi et al. 2016 Apr) and vacuum-assisted wound (VAC) therapy (De Caridi et al 2016 Jun.; De Caridi et al. 2016 Oct.) and recently bioengineered tissues or skin substitutes and growth factors (Mannari et al. 2002, Serra et al 2013), have been used to improve WH. Impaired WH in PAD patients is due to poor angiogenesis, diminished leukocyte migration, early fibroblast senescence, extended inflammatory phase and decreased skin tensile strength, which enables wound recurrence. The interest of our group is focused on CBPG factors for the complex implication on wound healing: Platelet-Derived Growth Factor, Insulin-Like Growth Factor 1, Fibroblast Growth Factor, Epithelial Growth Factor, Vascular Endothelial Growth Factor, Transforming Growth Factor  $\beta_1$ ,



**Figure 2.** CBPG activation for implantation on patient's ulcers.

Hepatocyte Growth Factor and cytokines (IL-1, IL-6, IL-4)(Leme et al, 2022). In addition, CPBG seems to play a role in pain relief, as observed in diabetic patients, through immunomodulation and by stimulating neural regeneration in damaged fibers (Rosenberger et al. 2019; Bouhleb et al. 2007; Sandireddy et al. 2014, Rubio et al 2017; Rah et al 2017, Tsuda et al. 2019).

### CONCLUSION

CBPG treatment protocol in foot lesions of CLTI patients after vascularization intervention in our experience appears safe and effective. Multicenter randomized studies are needed to evaluate late results, in particular for a national protocol of outpatient treatment.

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