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

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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-
tation 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 revascularization 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>
<thead>
<tr>
<th>Patient</th>
<th>PAD1</th>
<th>Age</th>
<th>CAD2</th>
<th>CKD3</th>
<th>HbAc1 (mmol/L)</th>
<th>WIFI score4</th>
<th>Revascularization surgery strategy</th>
<th>Foot lesion</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (2017)</td>
<td>2</td>
<td>86</td>
<td>No</td>
<td>No</td>
<td>53</td>
<td>222</td>
<td>F-P bypass BK5</td>
<td>Gangrene I finger, 6 cm length medial ulcer</td>
<td>1st-toe amputation 72-month follow-up died for cardiovascular disease</td>
</tr>
<tr>
<td>2 (2021)</td>
<td>2</td>
<td>74</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
<td>131</td>
<td>F-P bypass BK</td>
<td>Two dorsalis ulcers Forefoot amputation stump dehiscence</td>
<td>1st trans-metatarsal amputation Complete WH10</td>
</tr>
<tr>
<td>3 (2019)</td>
<td>2</td>
<td>62</td>
<td>No</td>
<td>Yes</td>
<td>51</td>
<td>222</td>
<td>PTA stenting</td>
<td>I finger amputation stump dehiscence</td>
<td>Complete WH</td>
</tr>
<tr>
<td>4 (2020)</td>
<td>2</td>
<td>78</td>
<td>No</td>
<td>No</td>
<td>53</td>
<td>221</td>
<td>POBA6</td>
<td>10 cm length plantar ulcer</td>
<td>Complete WH 50-month follow up died for cardiovascular disease</td>
</tr>
<tr>
<td>5 (2018)</td>
<td>2</td>
<td>73</td>
<td>Yes</td>
<td>No</td>
<td>99</td>
<td>121</td>
<td>DEB7</td>
<td>I-II-III finger amputation stump dehiscence</td>
<td>Complete WH</td>
</tr>
<tr>
<td>6 (2020)</td>
<td>2</td>
<td>83</td>
<td>Yes</td>
<td>No</td>
<td>57</td>
<td>231</td>
<td>POBA</td>
<td>Infected heel ulcer</td>
<td>Complete WH</td>
</tr>
<tr>
<td>7 (2020)</td>
<td>2</td>
<td>81</td>
<td>Yes</td>
<td>No</td>
<td>97</td>
<td>332</td>
<td>EA4</td>
<td>External heel ulcer</td>
<td>Tigh amputation Complete WH</td>
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<td>2</td>
<td>72</td>
<td>Yes</td>
<td>Yes</td>
<td>55</td>
<td>221</td>
<td>F-P bypass AK9</td>
<td>Interdigital ulcers</td>
<td>Complete WH</td>
</tr>
<tr>
<td>9 (2020)</td>
<td>1</td>
<td>73</td>
<td>No</td>
<td>No diabetic</td>
<td>121</td>
<td>F-P bypass AK</td>
<td>Acral ulcers</td>
<td>Complete WH</td>
<td></td>
</tr>
<tr>
<td>10 (2018)</td>
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<td>41</td>
<td>No</td>
<td>No diabetic</td>
<td>221</td>
<td>F-P bypass AK</td>
<td></td>
<td></td>
<td>Complete WH</td>
</tr>
</tbody>
</table>
1.5 x 10⁹ nucleated cells (which are not routinely banked for allogeneic hematopoietic transplantation purposes), a platelet count of 150 x 10⁹/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 +/- 0.2 x 10⁶/ 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°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 (Samarakova 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 β1,
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; Bouhlel 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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REFERENCES

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