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THE SECOND-GENERATION PLATELET CONCENTRATES IN THE TREATMENT OF CHRONIC OSTEOMYELITIS: ONE MODERN REGENERATIVE SURGERY

Alessandro Crisci [*1, 2, 3], D'Adamo Raffaele [2], Michela Crisci [4]

[*1] School of Medicine, University of Salerno Italy, 84084 Fisciano, SA, Italy
[2] Unit of Dermosurgery Cutaneous Transplantations and Hard-to-HealWound, "Villa Fiorita" Private Hospital, 81031 Aversa, CE, Italy
[3] Institute for the Studies and Care of Diabetics, Abetaia, 81020 Casagiove, CE, Italy
[4] Faculty of Medicine and Surgery, Vasile GoldisWestern University of Arad, 310025 Arad, Romania

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ABSTRACT

Background: Hypothesis is that the use of fibrin rich in leukocytes and platelets advanced (A-PRF) in ulcer osteomyelitis on diabetic foot, allows recovery from this serious disease. In this study, the goal was to standardize the use of PRF in patients with osteomyelitis, to use this second-generation platelet concentrate, as a facilitator of healing.

Methods: Authors produced and used peripheral blood A-PRF (1300 g × 8 min) membranes in 7 patients (all diabetic) with osteomyelitis and skin lesions for at least 6 months. Membranes, together with the supernatant liquid derived from compression, were inserted into the skin lesion down to the bone after surgical debridement. Evolution of the lesions over time was analyzed.

Results: All seven patients had a positive Probe-to-Bone test, MRI detected cortico-periosteal thickening and/or foci of cortico-cancellous osteolysis adjacent to the ulcer. Gram-positive bacteria were found in our patients in 52% of cases. Cocci Gram +, such as S. Aureus (15.6%), S. □-haemolytic (12.1%), S. Viridans (7.1%), and Bacilli Gram- such as Pseudomonas (10.6%), Proteus (7.8%), Enterobacter (5.7%) are present. Candida Albicans is present in 2.8%. Blood count showed no major changes.

To date, skin lesions have healed in 6 of the seven patients treated (one patient for more than five years) with no signs of infection or recurrence.

Conclusions: Results obtained on our patients suggest that PRF membranes may be a therapeutic option in this difficult to treat pathology.

1. INTRODUCTION

Choukroun's Platelet Rich Fibrin (PRF) [1] is a second-generation platelet concentrate, practically an overcoming of PRP (Platelet Rich Plasma), it is, therefore, a new step in the therapeutic concept of platelet gel with a simplified preparation and small artificial biochemical changes. Unlike other platelet concentrates, this technique does not require anticoagulants, thrombin, or any other gelling agent, which makes the blood no more than a natural

centrifuged with no additives. PRF can be prepared, in fact, simply by stimulating the intrinsic pathway of coagulation without the help of anticoagulants or coagulation factors [2]. Although platelets and leukocyte cytokines play an important role in the biology of this biomaterial, the supporting fibrin matrix is certainly the decisive factor in the real therapeutic potential of L-PRF. Within a few minutes, the absence of an anticoagulant allows the activation of most of the platelets contained in the sample to trigger the coagulation cascade.

Osteomyelitis (OM) refers specifically to bone marrow infection in contrast to osteitis in which the periosteum or cortical surface becomes infected through a wound or penetrating ulcer. Despite these differences, the two are clinically diagnosed or treated very similarly. Much has been written about the diagnosis of OM over the years and, more importantly, how it complicates diabetic foot ulcers (DFUs). OM that complicates the diabetic foot almost always derives from a contiguous wound or foot ulcer [3].

The worldwide incidence is from 1:1,000 to 1:20,000 inhabitants, in Italy 19,000 cases/year, in Europe 100,000 cases/year. Male: Female ratio is 2:1.

Bone and joint infections are painful for patients and frustrating for them and the healthcare professionals who treat them. The high success rates of antibiotic therapy in most infectious diseases have not yet been achieved in this pathology. The various types of OM require different medical and surgical therapeutic strategies. The various types of this pathology include, in decreasing order of frequency: OM secondary to a contiguous focus of infection (after trauma, surgery, or insertion of a joint prosthesis); that secondary to vascular insufficiency (in diabetic foot infections); and finally the OM of hematogenous origin. Chronic OM is associated with avascular necrosis of the bone and the formation of sequestration (dead bone) and the surgical approach is necessary for treatment in addition to antibiotic therapy. On the contrary, acute OM can respond only to antibiotics. In general, a multidisciplinary approach is required for a successful outcome, involving the skills of orthopedic surgery, infectious disease, and plastic surgery, as well as vascular surgery, particularly for complex cases with soft tissue loss [4], [5].

The use of second-generation platelet concentrates in the DFU with OM was not known to the authors until recently and was adopted by them for the first time (2018) [6].

This study describes the results obtained on seven patients with chronic DFU osteomyelitis of the lower limbs.

2. MATERIAL AD METHODS

2.1. PREPARATION OF PRF

Blood samples were collected with the informed consent of all seven investigated volunteers. All participant involvement procedures in this study were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its subsequent amendments. The Ethics Committee waived an ethics request for this study because the blood was not used as an identifiable source [7] (Researchregistry: n°5927).

Factors affecting the formation and structure of the fibrin clot include genetic factors, acquired factors (such as an abnormal concentration of thrombin and factor XIII in plasma, blood flow, platelet activation, oxidative stress, hyperglycemia, hyperhomocysteinemia, drugs, and cigarette smoke) and other parameters (such as microgravity, pH, temperature) [7], [8]. It was confirmed that all donors are carriers of chronic osteomyelitis from diabetic ulcers of the lower extremities.

The CBC (Blood Cell Count) of donors was also studied before starting the experiments to confirm the standard range of blood cell counts. In order to quantitatively classify and follow the clinical course of repair, a wound severity score was established by observing the wound and scoring the various clinical, anatomical, and patient variables (Wound Severity Score Tabb.1, 2, 3,4).

Blood was collected in A-PRF (Advanced PRF) glass tubes without anticoagulant or gel separator (A-PRF Vacutainer serum 9.0 ml tubes), for clot production and PRF membranes. Blood was quickly collected with a needle into tubes (22" mean value, less than 25" per tube) and immediately (within 1 min) centrifuged according to the description at a temperature above 21°C (between 21° and 30°C). Using the L-PRF Wound Box, the compression process of the membrane in the clots is performed through light and homogeneous compression, and the final membrane always remains homogeneously wet and soaked in serum. The PRF production technique is very simple and required only a blood sample and a DUO Quattro table-top centrifuge for PRF specially designed for this application (DUO Quattro for PRF) [6], [7], [8], [9].

The protocol followed is as follows: the blood samples are collected in 9 mL glass tubes, without anticoagulant or separation gel, and are immediately centrifuged according to the following schedule: acceleration of 30 sec, 8 min at 1300 rpm (189 g), 36 sec of deceleration and stop. After centrifugation, three parts are located in the tube: the red blood cells at the bottom, a fibrin clot representing the PRF in the middle, and the acellular plasma at the top. The fibrin clot is extracted from the test tube with sterile forceps and the PRF is obtained by removing the red clot from its lower end. The success of this technique depends entirely on the ease of blood collection and the speed of transfer in the centrifuge [10]. The entire process must always be carried out in a sterile manner, because the growth factors contained stimulate tissue regeneration and, therefore, probably, also that of bacteria. This delicate method avoids the extraction and loss of a significant amount of growth factors. The PRF-Boxes on the market are available in a variety of shapes and exert, through the compression plate, different pressures based on the weight, giving rise to a membrane of variable thickness, width, and length. The Wound L-PRF Box designed by the AA. [10] it consists of a 17.5 × 7.6 × 2 cm metal container containing a perforated steel plate of 150 × 68 × 1.5 mm. There is a second steel plate that acts as a compressor, 150 × 68 × 1.5 mm, with a weight of 148 grams. This second U-shaped plate exerts a pressure of 142.437 Pa/cm2. In this study, compression to produce the membranes was exerted on the clot for 2 minutes. Each membrane is separated into three equal-sized areas: proximal (head), center (body), and distal (tail) through a sterile scalpel cut.

Only the proximal part of the membrane was used [9], [10] and the central part only if necessary.

With the PRF preparation procedure, blood clotting begins just after collection instantly when it comes into contact with the glass surface of the tube, due to the lack of anticoagulant. If the time required for blood collection and the start of centrifugation (various rpm, g/min) is extremely prolonged, the polymerization of fibrin is so widespread that only a small part of the clot without consistency (PRF-like) will be obtained. Consequently, blood collection must be quick and easy, followed by immediate centrifugation and is a prerequisite in the PRF output specification. It is formulated to produce a thick about 3 mm (3.08±0.5) [10], homogeneously hydrated membrane with an exudate rich in platelets, leukocytes, vitronectin, and fibronectin, expressed in the fibrin network that forms, as well as CD34+ hematopoietic stem cells [11]. Fibrinogen is initially concentrated in the upper part of the tube until it causes the formation of circulating autologous thrombin which transforms it into the fibrin network. The result is a fibrin clot containing platelets located in the center, right between the lower layer of red blood cells and the upper plasma acellular part. The PRF clot thus obtained is then placed on the grid in the metal box of the Wound L-PRF Box and covered with the compressor lid. This procedure forms an autologous fibrin membrane. The L-PRF box is designed for the production of constant thickness membranes that remain hydrated for several hours and allows the recovery of the serum exudate expressed by the fibrin clots, this is rich in proteins, such as vitronectin and fibronectin [7]. The L-PRF clot appears to be responsible for a slow release of growth factors and glycoproteins from the matrix (≥7 dd to 28 dd) [8]. Adhesive proteins: fibrinogen (Fg), fibronectin (Fn), vitronectin (Vn) and thrombospondin-1 (TSP-1) are abundant in the fibrinous structure. Growth factors stored in platelets and which are essential for wound repair include PDGF, with -AB and -C; are also present as VEGF-A, TGF-β1, EGF (Fig. 1), FGF-2, HGF and insulin-like growth factor-1 (IGF-1).

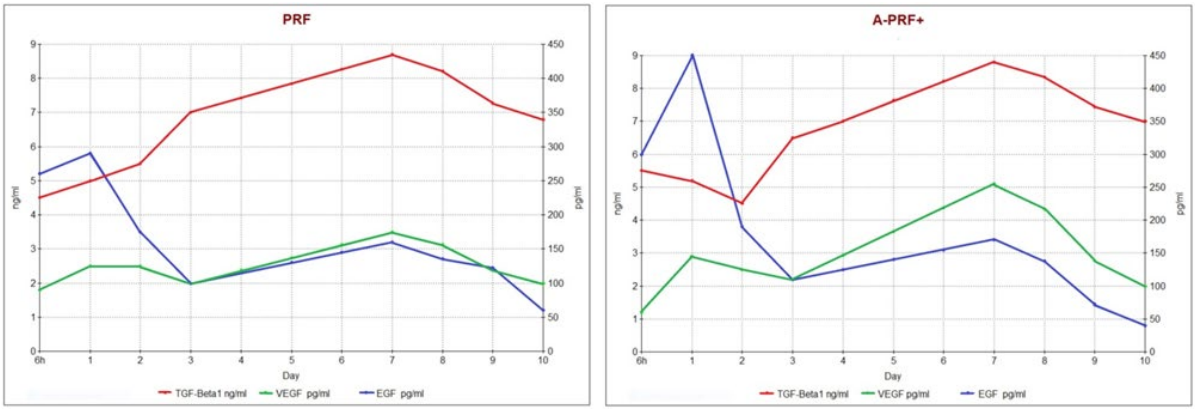

Figure 1: Different concentrations of growth factors TGF-β1, VEGF, EGF over time produced by L-PRF and A-PRF.

Statistical analysis of the growth factor releases by time points as the mean ± standard deviation for PRF and A-PRF+. VEGF, TGF-β1 release, EGF release, (*p < 0.05), (***p < 0.0005), (****p < 0.0001).

By analyzing three proinflammatory cytokines (IL-1 β, IL-6, TNF-α), an inflammatory cytokine (IL-4) and an angiogenesis promoter (VEGF), it was shown that PRF could also be a crucial point in immune modulation with ability to control inflammation and proliferation of adult stem cells, including CD34+ progenitor cells, MSCs (mesenchymal stem cells), SMCs progenitors (smooth muscle cells) and endothelial progenitors [8], [9], [10], [11]. The multipotency of these types of stem cells and their ability to increase vascular tissue repair and due to paracrine mechanisms also make them therapeutic vehicles in regenerative medicine. Furthermore, tissue damage generates strong chemo-attracting signals for stem cells, providing the basis for their regenerative activity. Platelets regulate the recruitment of adult stem cells to injured cells and can, therefore, be a substantial mechanism in the execution of regenerative cellular responses. Activated platelets release HGF and have been described to promote the uptake of MSC in human artery endothelial cells. The proliferation of human stem cells (hMSCs) is proportional to the platelet concentration in the A-PRF.

2.2. BLOOD CHEMISTRY ANALYZES

Blood samples were also taken from each patient to perform a complete blood count (CBC) using K3E tubes with 5.4 mg EDTA (VacuMed). According to previous studies [6], [7], [8], [9], [10], [11] three blood samples were taken from each patient's left brachial vein through an 18 gauge needle, two for PRF production, and one for cell blood counting. Tests were performed with a Cell Dyn 3500 R (ABBOTT) cell counter. The diagnostic evaluation of OM in the patients studied was carried by the Probe-to-Bone (PTB) method and then through an MRI and culture of bone cells for microbiological evidence.

2.3. A-PRF GRAFTING PROCEDURE

Each of the seven patients, after appropriate preparation (suspension of anticoagulant drugs for at least 7 days and subcutaneous low molecular weight heparin) underwent surgical debridement, under subarachnoid anesthesia, in the operating room, with the removal of non-tissue vital and possible bone fragments at the bottom of the lesion, including to perform the planned bacterial culture tests. Peripheral vasodilator drugs (Iloprost, Alprostadil) were not used. After disinfection of the surgical lesion with a 50% mixture of hydrogen peroxide and iodopovidone and appropriate control of hemostasis with electrocautery, A-PRF was prepared in the form of membranes after compression of the clot for 2 minutes. The supernatant derived from the squeezing was collected from the Wound L-PRF Box with a sterile 10 cc syringe and was carefully inserted into the skin lesion down to the bone together with the PRF fragment constituting the proximal third of the A-PRF membrane [9], [10], [11]. Prior to PRF grafting the lesion was washed with hydrogen peroxide because the active bleeding prevents the action of growth factors. The dressing was performed with greasy gauze, sterile gauze, Germanic cotton, and adhesive elastic bandage. Post-surgical drug therapy was with levofloxacin 500 mg cp, 1 cp per day for 5 days, and low molecular weight heparin (enoxaparin sodium) for 7 days, in addition to the drugs that each patient regularly takes for other pathologies. Based on the results of the culture and the antibiogram, specific antibiotics for general use were added for 15 days. The first dressing was performed after 7 days. Patients were examined every week on an outpatient basis until they recovered. If there were no signs of wound healing, PRF was reapplied 5 weeks later. All PRF residues were removed with water and sterile gauze on first application. Patients continued with the same updated dressing regimen between PRF sessions as had been used previously.

Two patients had to perform the procedure a second time after 40 days.

2.4. SEVERITY SCORE OF THE WOUND

A wound severity score was assigned based on the clinical, anatomical situation, and by measuring the wound and patient variables. The scores were arbitrarily assigned and weighted using the traditional clinical experience on wound healing. These general wound parameters are listed in Table 2. Anatomical considerations such as the presence of exposed bone or tendon wound location, and pulse quality of the pedidial and posterior tibial artery (and location relative to wound location) were recorded and obtained (Table 3). The wounds were measured to determine the total area of the wound, the depth, and extent of the detachment. The measurement of the wound

surface was determined by photographing the wound and comparing it with a strip graduated to the millimeter and then analyzed with a measurement software (IC Measure 2.0.0.133), found free on the web.

Three measurements were made and the final surface was the average of the three measurements. The duration of the injury was determined by the patient's history. The scores assigned to these various wound measurements are found in Table 4.

Initial and subsequent wound scores were recorded and tabulated at each clinic visit by two clinical investigators and wound healing nurses. These determinations were periodically checked by the proposing investigator.

The wound severity score is shown in table 1 for each patient.

2.5. DEFINITION OF SUCCESSFUL HEALING

One wound was classified as healed when it was completely covered with new epithelium. This was determined visually during the wound assessment performed during the routine follow-up program. At each visit, measurements and photographs of the wound were taken to document the progress. The treatment result is based on the percentage change in surface area and volume, calculated as measurement minus measurement per day of initial assessment divided by initial measurement (IDR) (Fig. 2).

$$\text{Index Daily Re-epithelization (IDR)} = \frac{\text{EstT0 (cm}^2\text{)} - \text{EstTX (cm}^2\text{)}}{\text{EstT0 (cm}^2\text{)}} / X$$

EstT0(cm²)= Area Extension at Time 0
EstTX(cm²)= Area Extension at Time X
X= days since the start of therapy

EXCELLENT=IDR≥0.037 Repair at 100%;
GOOD =IDR>0.019 Repair > 50%;
MEDIOCRE=IDR<0.019 Repair <of 50%;
INSUFFICIENT=IDR<0.004 Repair<of 10%;
PEGGIORATED = IRG≥0.004 Extension > by 10%;

Figure 2: Index Daily Re-epitelialization (IDR)

3. RESULTS

The authors produced and used A-PRF membranes created from peripheral blood, in patients with osteomyelitis, with skin lesions standing for at least 6 months. The membranes, together with the liquid derived from the compression of the Wound L-PRF Box, were inserted into the skin lesion, down to the bone, after surgical debridement. The evolution of the lesions was subsequently analyzed over time.

The results obtained with this technique are shown in table 1, together with the overall characteristics of the patients treated.

Table 1: Patient characteristics

№ of subject	Age (years)	Gender	Admission length (days)	Comorbidities	DDY	Wound size (cm) L×W×H	Wound Location	Treatment time	Follow-up(days)	Result	Total Severity Score
1	68	Male	103	PAD, Osteomyelitis	25	2 × 2 × 5	Lower third left leg	40	1825	Spontaneous closure	25
2	71	Female	64	PAD, Osteomyelitis, HTN	40	1 × 1 × 2	Right V° finger foot	25	365	Spontaneous closure	24
3	63	Female	56	PAD, Osteomyelitis, ESRD	24	2 × 2 × 2	Right Plantar	32	180	Spontaneous closure, deceased for CAD	40

4	60	Male	46	PAD, Osteomyelitis, HTN	20	1 × 3 × 5	Left V° finger foot	27	120	Spontaneous closure	15
5	66	Male	45	PAD, Osteomyelitis, ESRD	15	1 × 2 × 5	Right I° finger foot	33	211	Spontaneous closure	20
6	58	Male	94	PAD, Osteomyelitis, HTN	23	1 × 1 × 5	Left toe plantar	33	388	Spontaneous closure	35
7	70	Male	86	PAD, Osteomyelitis, HTN	35	2 × 2 × 5	Right toe plantar	30	905	Spontaneous closure	23
Average ± D.S.	65.14 ±4.61	5/2	70.57 ±22.88		26.0 ±8.0	1.4 ×1.8×4.1		31.43 ±4.5	570.6 ±566.4		26.0 ±8.0

*improvement is defined as limb recovery within one and a half years but without wound closure. DDY: year of diabetes diagnosis; PAD: Peripheral arteriopathy; ESRD: end-stage renal disease; HTN - hypertension; CAD: Coronary Arterial Disease;

Table 2: Total Wound Score-General Wound Parameters

	None	Mild	Marked	Total
Periwound eritema	0	2	5	7
Periwound edema	1	2	4	7
Wound purulence	0	3	4	7
Wound fibrin	0	2	5	7
Limb pitting edema	1	2	4	7
Limb brawny edema	0	3	4	7
Wound granulation	0	0	0	0

Table 3: Total Wound Score-Anatomic Considerations

				Dorsalis		Posterior	
Exposed Bone	Score	Exposed Tendon	Score	Pedis Pulse	Score	Tibial Pulse	Score
Yes	10	Yes	7	0-1+	5	0-1+	5
no	0	No	0	2+	2	2+	2
				3-4+	0	3-4+	0

Table 4: Total Wound Score-Wound Measurements

Size (cm2)	Score	Depth (mm)	Score	Undermining (mm)	Score	Duration	Score
<1	0	<5	0	<2	3	<8 wk	0
1-2	1	5-10	3	2-5	5	8 wk-6 mo	1
2-5	3	10-20	7	>5	8	6 mo-1 yr	2
5-10	6	>20	10			2-3 yr	5
10-30	8					5-10 yr	7
>30	10					10 yr	9

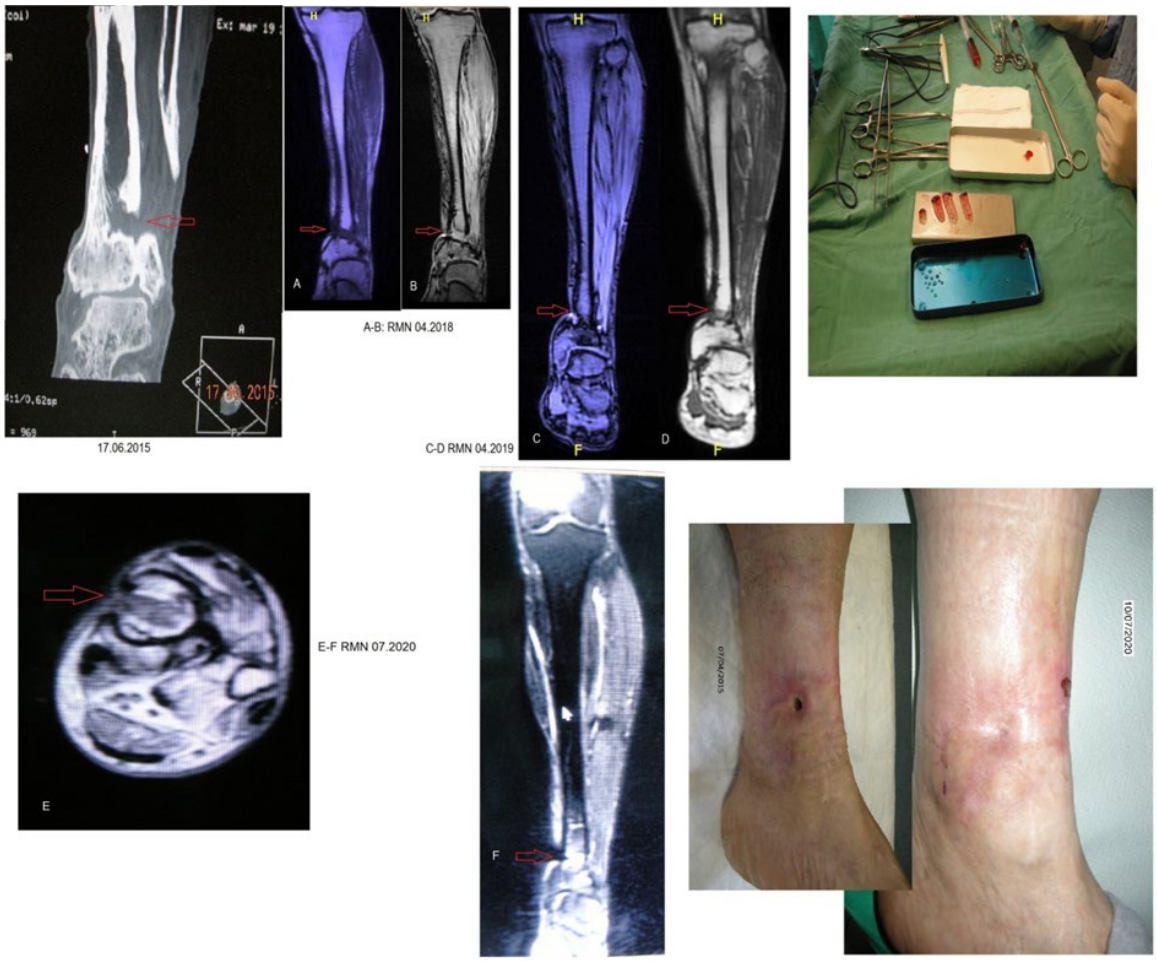

Figure 3: Patient N ° 1. In A-C-D-E-F, MRI at various times in the evolution of the wound until healing, stable after five years. Some bone regrowth is also appreciated at the last MRI.

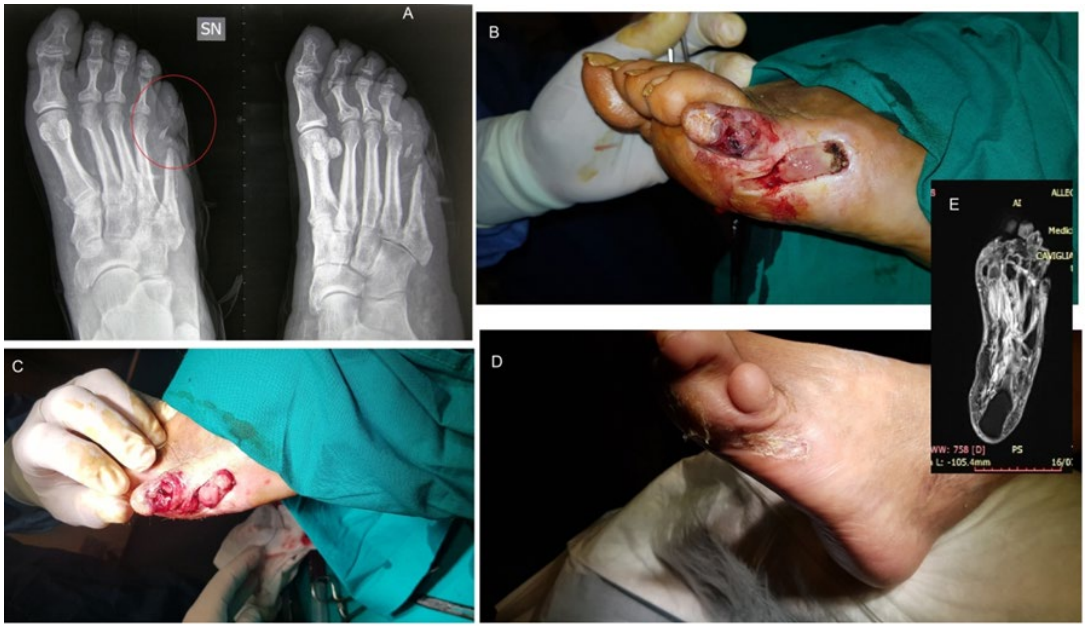

Figure 4: Patient N ° 4. In A situation radiography of the fifth finger, B: Intraoperative conditions; C: L-PRF graft deeply into the lesion; D: wound evolution until healing after 4 months and only one PRF graft; E: RNM as of 07/16/2020 once healing is achieved.

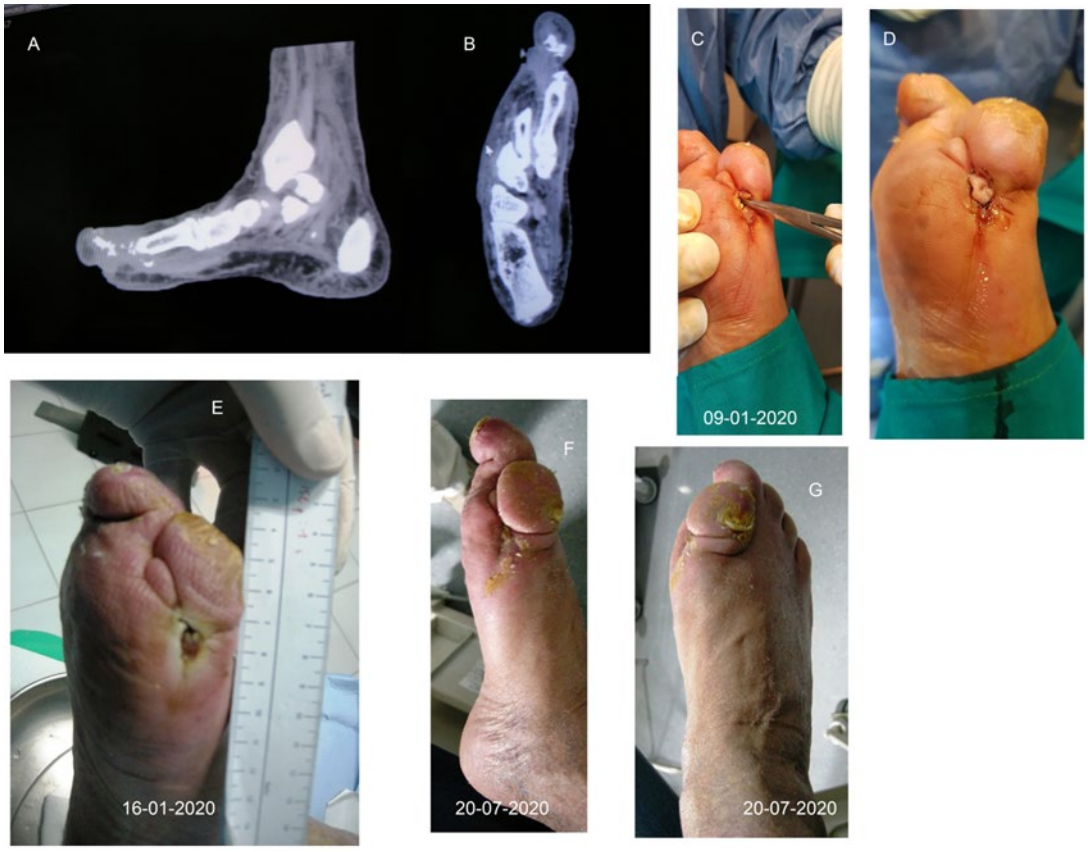

Figure 5: Patient N°5. In A and B MRI situation of the 1st finger, C: Intraoperative conditions; D: L-PRF graft deeply into the lesion; E, F, G: wound evolution until healing after 6 months and only one PRF graft.

The mean age of treated patients is 65.14 years ± 4.61, with a male/female ratio of 5/2, all patients were carriers of Chronic Osteomyelitis and Chronic Obstructive Arteriopathy in non-insulin-dependent Diabetic Disease diagnosed for 26.00 years ± 8.0 in average. The mean duration of osteomyelitis was 70.57 ± 22.88 days. The average total Severity Score of treated patients was 26.0 ± 8.0 (Tab.1).

All patients showed positivity on the Probe-to-Bone test, and Nuclear Magnetic Resonance showed a cortico-periosteal thickening and/or osteolysis with foci of the cortico-spongy, adjacent to the ulcer. Osteonecrosis was also present with severe bone fragmentation and erosion (Figs 3-5).

Gram-positive bacteria were found in our patients in 52% of cases. Among the agents found are Gram-positive cocci such as S. Aureus (15.6%), β-haemolytic streptococci (12.1%), S. Viridans (7.1%) and Gram-negative bacilli such as Pseudomonas (10, 6%), Proteus (7.8%), Enterobacter (5.7%). Candida was present in 2.8% of cases.

The average treatment time after the PRF graft was 31.43 ± 4.5 days. The mean follow-up to date is 570.6 ± 566.4 days.

4. DISCUSSION

To date, skin osteomyelitis lesions have healed in all patients treated (only one patient died of cardiac causes two years after surgery, but the lesion was already completely healed), with no signs of infection or relapse. In one of the patients (N°1) we are observing a certain bone regrowth five years after the healing of the skin lesion (Fig. 2F). The authors' use of PRF in the treatment of skin lesions of the foot achieved the reported results, with moderate effort in terms of surgical technique, and economic costs to the healthcare facility in which patients were treated. Furthermore, the surgical risk to which the patient is subjected is also low.

Treatment of DFU is associated with a significant financial burden, and the cost increases with prolonged stay and the need for surgery. The presence of osteomyelitis is a critical factor for high costs, the longer length of hospital stay, long-term use of antibiotics, and the need for amputation. Furthermore, of all the factors affecting scar quality, the one that appears to have the greatest impact is the time it takes to heal a wound [13]. Extensive literature

supports the claim that wound healing within 21 days minimizes scarring. Therefore, one of the main areas of research on burns and wound healing is to clarify the pathophysiology of the wound healing process, shortening the wound closure time, the risk factors related to the scarring process, and the conversion of this knowledge in therapeutic solutions. The use of PRF in wound healing as an accelerator of wound repair appears to justify its use. The inclusion of leukocytes in the PRF must be carefully evaluated particularly when the biomaterial is used for wound repair and when scar formation is a major concern.

One point to consider is the inclusion of leukocytes. Marx's opinion (1988) that the ideal concentration rate of platelets was 3-4 times in PRP preparations suggests the need to avoid not only highly concentrated platelets but also the inclusion of leukocytes. Until now, this has been a topic of debate: some researchers, including the AA. argue that leukocytes should be included to facilitate wound debridement, wound healing, and subsequent tissue regeneration, while some are concerned about the unexpected exacerbation of inflammation [14]. Therefore, further questions and further investigation will be needed to reach conclusions.

In this study, all seven patients performed the "Probe-To-Bone" test with positive results, MRI detected cortico-periosteal thickening and/or focal points of cortico-cancellous osteolysis with reduced signal intensity in the vicinity of the skin ulcer. Edema due to septic inflammation and soft tissue abscesses were also found (Tab. 4). In our patients, as frequently happens in chronic lesions, several germs have been found at the same time: bacteria are the most common pathogens, but fungal infections have also been identified.

The treatment of chronic osteomyelitis currently consists of surgical treatment, antibiotic therapy, hyperbaric oxygen therapy (OTI), active antibacterial stimulation (ITSB). Surgical treatment is the cornerstone of therapy [16]. The goal is the removal of the infection and the functional restoration of the bone segment being treated. With the surgical procedures adopted up to now for the treatment of osteomyelitis, the possibility of eradicating the infection is to remove the bone and all the adjacent tissues affected up to the healthy vital tissue. Sometimes, however, a small removal may suffice, which does not compromise either the stability or the function of the treated limb, but in many cases, after the removal of the infected bone, a suitable surgical reconstruction is necessary. Together with the infected tissues, it is also advisable to remove all the means of internal synthesis (plates, screws, nails, staples, etc.) present in the infected area, and to resort to a new stabilization by external fixation. All the existing surgical techniques can offer excellent results, but in the face of extremely long and demanding treatments, and at not negligible risk of complications and failures. The use of A-PRF in osteomyelitis lesions treated by us have given the results reported with a moderate commitment in terms of surgical technique and economic for the health facility where the patient is operated on. Furthermore, the surgical risk to which the patient is subjected is also low (our patients were all treated under sub-arachnoid anesthesia).

Finally, the effect of PRF on bone cells cannot be due to the action of a single growth factor, but to the synergistic effects of various platelet growth factors.

Further clinical, histological, and statistical studies are required to understand the benefits of this new technique. However, it cannot be ignored that as obtained from an autologous blood sample, the PRF produced is scarce and only a limited volume can be used. This fact limits the systematic use of PRF in large osteomyelitis lesions. Even if the potential applications of PRF are therefore wide, accurate knowledge of the functioning of the biomaterial, its biology, efficiency, and limits are required to optimize its use in daily clinical practice.

5. CONCLUSIONS

Overall, the A-PRF is mechanically strong, capable of withstanding loads, has a two-fold ability to stretch under tension, and holds surgical sutures so that two or more membranes can be sutured together with surgical stitches (deforms in significantly before laceration) modulus:0.2 MPa; stress:140%; energy at break:3.2 N.mm) [16], [17], [18], [19], [20]. The membrane showed a rupture resistance equivalent to rupture of an intact aorta and much higher than traditional PRP clots [21].

Using A-PRF in cases of OM from a diabetic foot ulcer will certainly improve our understanding of wound healing, particularly in the regenerative therapy of chronic skin lesions. The point was to institutionalize the utilization of PRF in patients with osteomyelitis, to utilize this second-age platelet concentrate, encouraging healing forms.

The results obtained in these seven cases suggest that PRF membranes may be a therapeutic option in this difficult-to-treat pathology. Starting from this experience, we intend to perform a randomized study to confirm the

clinical effect of A-PRF and its derivatives, such as i-PRF, also as a function of its antibacterial action. The important reasons to explain the likely variability that can be observed in the results could be attributed to the types of platelet concentrates used (PRP, PRF) which may differ in the form (gel or liquid), as well as in platelet concentration, leukocyte content, network density. fibrin, in the mode of activation that can occur naturally by contact with tissues or, can be induced by thrombin or calcium chloride.

The authors suggest using formulations containing leukocytes and platelets in combination after debridement surgery to both reduce bacterial load (by killing bacteria and inhibiting biofilm formation) and stimulate healing.

Although second-generation platelet concentrates (PCs) have weak effects on tissue regeneration alone, these biomaterials may be able to maximize the efficacy of primary or initial therapy, such as surgery or drugs, by observing an enhancement of the system response. immune to antigens [14]. PC therapy can also be considered a "replacement therapy".

In both cases, PCs provide the elements necessary for tissue regeneration, including growth factors and scaffolding materials, which cannot be provided directly by surgery or drugs.

The authors hope that this work will be one of the bases for future studies to further explore the contribution of leukocytes in PRF preparation to achieve optimal preparation both to fight infections and to effectively promote wound healing especially in cases of chronic osteomyelitis.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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None.

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