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Pilot Study on Second-Generation Platelet Concentrates for Treating Chronic Osteomyelitis: A Modern Bio-Regenerative Surgery Approach

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1. Abstract

Aim: The supposition is that utilization of advanced fibrin which is enriched with leukocytes with platelets content (A-PRF) for osteomyelitic ulcer in the diabetic limb patients, enables prompt salvage from there debilitating pathology. In this test, the focus was to normalize the application of Platelet Rich-Fibrin in people with osteomyelitic disease, to utilise this 2nd generation platelet paste as a facilitator of effective injury therapy.

Methods: The researchers have been using peripheral blood in the production of (A)-PRF (1300 g × 8 min) matrices in 7 subjects (every one of them diagnosed including diabetes), which presented osteomyelitic disease with skin lesions for at least twenty-four weeks. Membranes, combined with the compression-derived sururnatant fluid, have been introduced in through the skin lesion to the bone, post surgeries removal. The development of injuries in the course of time was has been parsed.

Results: These seven subjects all tested positive on the "Probe-to-Bone" testing, detected by MRI corticoperiosteal plication and / or areas of cortical-spongeous ulcer-adjacent osseolysis. Presence of Gram + germs was documented in our subjects in 52% of cases. Gram + cocci, as St. Aureus (15.6%), -haemolytic Streptococci (12.1%), S. Viridans (7.1%) and Gram - bacillus species including Pseudomonas (10.6%), Proteus (7.8%), Enterobacter (5.7%) were also featured in cultures. Candida Albicans was reported to be present in 2.8%. CBC demonstrated no relevant alterations.

As of today, skin wounds have been cured in 6 of the seven subjects processed (one case for more than 5 years) without any evidence of infectivity or repetition.

Conclusion: Outcomes observed in our cases recommend which the PRF membranes might be a medical alternative in this challenging disease to treat.

2. Keywords: Chronic osseomyelitis; Buffy coat; Augmentation factors; Platelet-rich fibrin; Thrombocyte concentrate

3. Introduction

Choukroun's Platelet-Rich Fibrin (PRF) represents a 2nd generation platelet aggregate, essentially an improvement from PRP (Platelet Rich Plasma) [1]; it is consequently a novelty in the therapeutical options of multiplatelet gelatin using a facilitated approach for the processing and very little artificially produced bio-chemical alterations. In contrast to additional platelet aggregates, this procedure it does not need anti-coagulants, thrombin or any other gelling reagent, making blood only a natural physiological centrifugation derivative with no additives. PRF may indeed be processed by the simple activation of the intrinsic coagulation steps with no clotting factor or coagulant agent [2]. Although cytokines derived from platelets and leukocyte are playing an extremely major factor in the biological makeup of this biomatrix, the fibrin scaffold matrix is definitely the main component in the therapeutical capability of L-PRF. In the course of few minutes, absence of anticoagulant agents allows the majority of platelets in the specimen to be activated, triggering the clotting process.

The term Osteomyelitis (OM) referred in particular to an infection of the bone marrow, as distinct from osteitis in which the infectious process involves periosteum or cortex area, due to wounds or penetrating ulcerations. Despite such differences, both conditions are medically documented and handled in a very analogous mode. Considerable literature has been published on the OM diagnosis during the course of the years and, above all, how OM plays a role in ulcers of the diabetic limb (DFUs). OM complicating the diabetes foot usually results from a proximal injury or a foothold ulceration [3].

The incidence globally ranges between 1:1,000 and 1:20,000, in general population, in Italy 19,000 cases/year, in Europe 100,000 events/year. The male and the female proportion is 2:1.

Osseous & articular bacterial contaminations are distressful for subjects and very demotivating for both them and the clinical professionals who assist them.

The high outcome rate of treatment with antibiotics in the big predominance of infectious diseases not yet attained in this disorder. Various forms of OM require multiple medical and surgery treatment strategies. These disease types including, in order of decreasing incidence: OM associated to an adjacent focus of an infection (post-traumatic injuries, surgical intervention, or prosthetic supplementary replacement); those resulting from vascular malfunction (in diabetic limb infections); and ultimately OM of hematogenous derivation. Chronic OM is combined with avascular necrotic bone disease and the development of sequestration (dead bone); an operative procedure is recommended for correction in combination with antibiotic drug therapy.

Conversely, acute Osteomyelitis might react to antibiotics only. Overall, a multiple-disciplinary joint approach is needed for most successful results that involve the specialties of Orthopaedic Surgeons, infectious experts, plastic surgeons, as good as vascular surgeons, especially for cases complicated by soft tissue damage [4,5].

The using of 2nd generation of platelets activator generator in DFU with

OM was not familiar to the AA. until a short time ago and was used by them for the first time relatively recently (2018) [6]. This trial presents the outcomes in seven cases with chronic DFU OM of the lower extremities.

A study conducted between 2011 and 2014 that included 150 patients with diabetic feet revealed that there was complete healing in approximately 8 weeks with the use of PFR and in 8 months with PRP treatment, with no recurrence of lesion opening. Another study by Knighton et al. showed that there was 100% healing with PRF over a period of 7.5 to 6.5 weeks [7].

4. Materials and Methods

4.1. Preparing platelet-rich fibrin

Blood amounts were collected after obtaining informed consent from all seven volunteers investigated. All subjects tested and proceedings documented in this trial were executed in adherence with the ethics guidelines of the national institutional and/or research organization and the 1964 Declaration of Helsinki and your subsequent changes. The Ethics Committee declined an ethics request for this trial because blood was not utilized as an identify able resource (Research registry: n°5927) [8].

The facilitators that influence fibrin coagulum creation and pattern comprise genetic elements, intrinsic characteristics (such as anomalous blood plasma concentrations of both thrombin and factor XIII, vessel fluxes, platelet activity, oxidative distress, hyperglycaemia, increased homocysteinemia, pharmaceuticals, and tobacco smoking), and other factors (such as micro gravity), pH, and temperature [8,9]. It was verified that all subjects had chronic osteomyelitis with diabetic inferior extremity ulcers.

The CBC (Complete Blood Count) of the studied cases was even analyzed before beginning tests to validate the standard blood count range. In aim to categorize the quantity and following clinic progress of the reparation, a severe rating was determined by observation of the injury and score the variety of medical, patient and anatomical factors (Wound Severity Score Table 1-4).

Table 1: Patient characteristics

No 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 Injury Grade - Overall Injury Values.

	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 Injury Grade -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 Injury Grade -Wound Measurements.

Size (cm^2)	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

Blood was collected in A-PRF (Advanced Platelet-Rich Fibrin) fiberglass vials without anti- coagulant or gel splitter (9.0 ml A-PRF Serum Vacutainer), for clotting & PRF membranes. Blood was harvested rapidly via fine needle into tubes (mean size 22", fewer than 25" per tube) and immediately (under 1 min) spin-dried, as later described, at over 21°C (range: 21° and 30 °C).

Utilising the L-PRF Wounded Box, compressive clot membrane was created applying light and uniform pressure, and the end membrane always remained uniformly wet and impregnated with serum. The PRF generation procedure is extremely simplified and it demands only one blood source and a DUO Quattro table-top spinner for PRF, engineered for this unique usage (DUO Quattro for PRF) [7-10].

The procedure used is subsequently described: blood specimens are harvested into 9 mL glass cuvettes, free of anti-coagulant or separating gel, and are instantly centrifuged in compliance with the procedure indicated below: 30 sec accelerations, 8 min at 1300 rpm. (189 g), 36 sec decelerations and stop. Post centrifuging, 3 different parts can be distinguished in the tube: RBCs after all, a coagulum of fibrin, which constitutes PRF, in between, and the non-cellular supernatant plasma in the upper part.

The fibrin coat is removed from the test tube by with sterilized clamps, and Platelet-Rich Fibrin is produced by the removal of the red coagulum from the end of tube. The percentage of success of this method is rests wholly on the easiness of collecting blood and the velocity of its transference into the spinner [11]. The whole procedure must be conducted at all times out in a sterilized atmosphere and in sterile conditions, because the growth factors contained stimulate tissue regeneration and thus, probably, also bacterial proliferation. This gentle method avoids the extraction and loss of a substantial amount of growth promoters. The GRP- Storage Cases on the commercial market are presented in a range of types and provide, using the squeeze plates, various types of compression, according to the size, giving origin to membranes of different thickness, width and length. The L-PRF Wound Case designed by Authors [10] comprises a metal case with a size of 17.5 × 7.6 × 2 cm and a punctured iron plate with a size of 150 × 68 × 1.5 mm. Another steel plaque acts as a squeeze, 150 × 68 × 1.5 mm, weighing 148 grams. This 2nd conformed to U plate exerts a compression of 142.437 Pa/cm2. In this trial, membrane-producing compression is affixed to the coagulum for 2 minutes. Subsequently, every diaphragm wa sectioned into three areas of equal size: proximal (head), middle (body), and distal (tail) using a cut with a sterile knife. Only the very head section of the diaphragm is used [10,11] and eventually the central part, should it be required.

After the PRF preparation procedure, blood coagulation begins immediately after harvest, instantaneously, as soon as the blood gets in touched by touching the surface of glass pipe, because of the absence of blood thinner agents. If the period required for blood harvesting and the start of spinning (various rpm, g/min) is very extended, fibrin curing is so extensive that only a small clot with no consistency (PRF-like) will result. As a result, the blood sampling should be quick and fast, follows by instant spin, which is a precondition in the PRF exit specifics. The method should produce a layer, with a thickness of approx. 3 mm (3.08±0.5) [11], homogenously moisturized diaphragm with an ooze contains high counts of platelets, leukocytes, vitronectin & fibronectin, also explicit in the forming fibrin mesh as CD34+ haematopoietic staminal elements [11]. Fibrinogen is originally centered at the top portion of the pipe to result in the generation of autologous recirculating thrombin, hence transforming into the fibrin mesh. The end product is a fibrin cluster which contains platelets placed in the middle, precisely between the bottom sheet of RBCs and the top acellular plasma portion.

The resulting The PRF coagule is therefore arranged on the grating in the metallic case of the Wound L-PRF Case and pressure is imposed by the cover of the compressor. This technique formats an autologous fibrin film. The L-PRF case is engineered to generate fixed thickness diaphragms that stay hydrated for multiple hours and allows for the reservoir of exudation of serum found along with the fibrin clots, which contains abundant proteins, including vitronectin & fibronectin [8]. Clot L-PRF apparently is implicated in retarded releasing of growth promotors and of glycoproteins from the matrices (≥7 dd to 28 dd) [9]. Adhesive proteins, such as: fibrinogen (Fg), fibronectin (Fn), vitronectin (Vn), & thrombospondin-1 (TSP-1) are profuse in fibrin patterning. Platelet storage growth factors, fundamental to injury repair, comprise PDGF, and with -AB & -C types; VEGF-A, TGF-β1, EGF (Figure 1), FGF-2, HGF & insulinlike growth factor-1 (IGF-1) also have been found to be present.

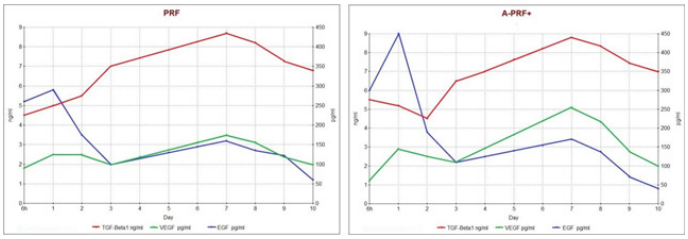

Figure 1: Different levels of growth agents. TGF-β1, VEGF, EGF over time, engendered by L-PRF as well as A- PRF. Statistics analyses of growth agent deliveries by temporal points as average ± standard deviation for PRF and A- PRF+. VEGF, TGF-β1 release, EGF release.

By analyzing three pro-inflammatory cytokines (IL-1, IL-6, TNF-), an inflammatory cytokine (IL- 4), and a promoter of vasculogenesis (VEGF), it was evidenced that PRF could also be a critical point in immune stimulation, with capacity to monitor inflammation and proliferation of adult stem cells, containing CD34+ progenitor cells, MSCs (mesenchymal stem cells), SMCs (smooth muscle cells) progenitors, and endothelial progenitors [8-11]. The multipotency about these staminal cells and capacity to improve repairing vascular tissue, due to paracrine mechanisms, also makes them therapeutic vectors in bio-regenerative practice. Further, tissue injury makes very strong chemoactive signaling, affecting stem cells, and providing strong regenerative activity basis. Platelets also have been found to favor recruitment of mature stem cells into the injured tissues and may, thereby, be a substantial device in the performance of cell-regenerative reactions. Active blood platelets release HGF and was referred to enhance MSC recruitment to endothelial cells of the human artery. Human staminal cells proliferation (hMSCs) is related to the amount of platelets in the A-Platelets-Rich Fibrin.

4.2. Hematochemical analysis

Blood specimens were also obtained from each participant to perform a blood count (CBC) using K3E tubes with 5.4 mg EDTA (VacuMed). Depending on previous reports [7-12], three blood samples were harvested from each patient's left brachial vein through an 18-gauge needle, two for PRF generation and one for cellular blood enumeration. The tests were conducted with a Cell Dyn 3500 R cell counter (Abbott Laboratories; Abbott Park, IL, USA). Diagnostic evaluation of OM in the studied subjects was conducted by the Probe-to-Bone (PTB) procedure and then by MRI and bone cell culture for microbiological evidence.

4.3. A-PRF insertion method

After adequate preparation (anticoagulant drug cessation for at last seven days and the use of [LMWHs] subcutaneous low-molecular-weight heparin), every one of the 7 subjects received debridement surgery, under subarachnoideal anesthetic, in the operation theatre, removing non- viable material and any bone chips in the lower part of the wound, also in order to test for bacterial culture. There were no peripheral vessel dilator medications (Iloprost, Alprostadil) adoperated.

Following surgical wound uninfection from a 50% blend of a hydrogen dioxide and iodopovidone and adequate bleeding blockage monitoring with electrocauterization, A-Platelets-Rich Fibrin was first compound in membrane form after coagulum pression for 2 minutes. The compression-derived supernatant was harvested from Wound L-PRF container with a sterilized 10 cc syringe and was accurately injected into the cutaneous injury to the bone along with the portion of PRF located at the proximal extension 1/3 of the matrix A-PRF [10-12].

Before PRF engraftment, the injury was cleaned with hydrogen dioxide as active blotting impedes the effect of growth factors. Medication was accomplished with a oily bandage, sterilized gauze, Germanic cottons, and elastic sticker bandaging. Postoperative medications administered were levofloxacin 500 mg cp, 1 cp daily for five days and (LMWH) low molecular weight heparin (enoxaparin sodium) for seven days, in adding to medications that each subject takes routinely for more conditions. According to the cultivation and antibiograms results, dedicated antibiotics for general use were added for fifteen days. The first bandage was applied after seven days. Subjects were inspected weekly on an ambulatory basis until they healed; if there was no evidence of injury healing, PRF was reapplied five weeks after. All residual PRF was retrieved with liquid and sterilized bandage as at the first fitting. Test subjects persisted under the same upgraded medication scheme among sessions of PRF as was formerly utilized.

Two subjects had to have the proceedings performed a 2nd weather after forty days.

4.4. Injury gravity index

The injury gravity score index was attributed based on the medical and anatomic status and by measured variables of the injury and the patient. Scoring was performed arbitrarily and weighted utilizing standard clinical practice on injury repair. Such general injury criteria are enumerated in Table 2. The anatomic observations of the existence of exhibited bone or the status of plague tendon and the pulse qualities of the pedidial and posterior tibial arteries (and the position relative to the injury) have been noted and achieved (Table 3). The plague was gauged to establish the total injury area, depth, and magnitude of detachment. Wound surface measurement was determined by photographing the lesion and comparing it to a scale stripe measured to the mm and then scanned with measuring system software (IC Measure 2.0.0.133), freely found on the web.

Three dimensions were noted, and the total surface area was an average

of the 3 measurements. Injury timing was based on patient experience history. The test scores allocated to these various wound dimensions can be found in Table 4.

Initial and subsequent wound scores were recorded and stored at each medical visit by two researchers and nurses trained in wound care. These measurements were routinely monitored by the proposing experimenter.

The wound severity score is shown in Table 1 for each subject.

4.5. Description of completed healing

A lesion was categorized as cured when it was totally masked by new epithelium. This was ascertained visually during the assessment of the injury conducted during the routine follow-up programme. At each follow-up, measured and photos of the wound were taken to demonstrate progress. A result of therapy is calculated based on the % change in surface and volume, computed as the measurement minus measurement on the day of the initial grading divided by the initial measurement (IDR) (Figure 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: Daily Re-epithelialization Ratio (IDR)

5. Results

The AA. have generated and utilized A-PRF diaphragms made from venous blood in subjects affected by osseomyelitis, with cutaneous injuries present and documented for at at least six months. Both membranes and liquid, formed by squeezing the Wound L-PRF Case, they were incorporated in the dermal lesion, up to the bone, after the surgery toasted. Lesion evolution was later investigated over time.

The results obtained with this technique are shown in Table One, along with the general characteristics of the patients treated.

The mean age value of the treatment subjects was 65.14 years ± 4.61, including a 5/2 male/female ratio, all subjects had Chronic Osteomyelitis and Chronic Arterial Obstructive Disease diagnosed, non-insulin dependent diabetic illness for 26.00 years ± 8.0 on average. The mean time of osteomyelitis diagnosis was 70.57 ± 22.88 days. The average total Severity Index of treated patients was 26.0 ± 8.0 (Table1).

All subjects tested positive for the Probe-to-Bone assay, and MRI showed corticoperiosteal thickness and/or osteolysis with focal cortical-spongy, contiguous to the ulceration. Osteonecrosis as well was evident with major bone splintering and eroding (Figures 3-5).

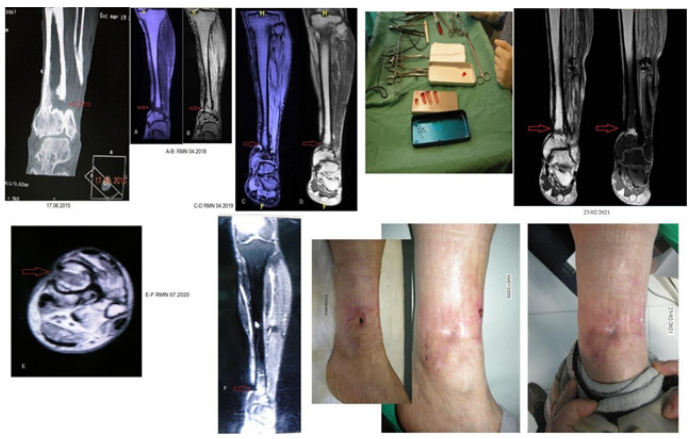

Figure 3: Patient N ° 1. In A-C-D-E-F, MRI at various times in the continuation of the injury until healing, stable for five years. Some bone reconstruction is also appreciably noted at the last MRI.

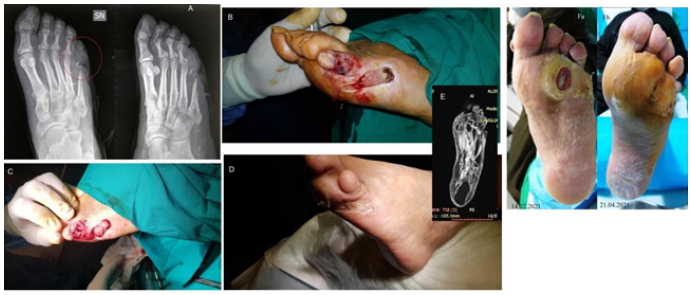

Figure 4: Patient N ° 4. In A radiographic status of the fifth finger, B: intra-operative situation; C: L-PRF grafting deeply into the wound; D: evolution of the injury until recovery after 4 months and a single PRF grafting; E: RNM as of 16.07.20, once recovery is attained; F: 14.02.21 before II intervention

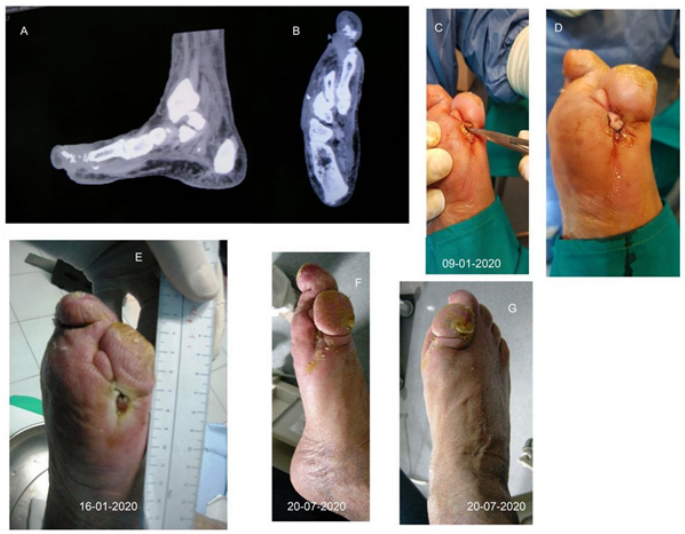

Figure 5: Patient N°5. In A & B MRI status of the 1st digit; C: intra-operative situation; D: L: L-PRF grafting deeper into the wound; E, F, G: evolution of the lesion until recovery at a distance of 6 months and a unique PRF grafting.

Gram-positive bacteria were found in 52% of our cases. Amongst the agents found include Gram-positive cocci as St. Aureus (15.6%), β-haemolytic streptococci (12.1%), S. Viridans (7.1%) and Gram-negative bacillus including Pseudomonas (10, 6%), Proteus (7.8%), Enterobacter (5.7%). Candida Albicans was represented at 2.8% of patients.

The mean time to processing later PRF grafting was 31.43 ± 4.5 days. The average control at the time of writing is 570.6 ± 566.4 day.

6. Discussion

To this date, the cutaneous Osteomyelitis lesions healed in all cured patients (only one person succumbed to heart rate events 2 years after the procedure, but the injury was fully resolved), without any proof of infections or recurrence. In one of the subjects (N°1) we were observing some bone remodelling five years after the cutaneous lesion healed (Figure 2F). The authors' reported utilization of PFR in the therapy of foot skin lesions, obtaining the aforementioned benefits, with moderate stress in terms of operative technique, and financial cost to the health care institution where the subjects were treated. In addition, the operative

risk to which patients are exposed is also small.

DFU therapy is associated with substantial financial expense, and the cost increases with prolonged hospitalization and the need for surgical removal. The presence of osteomyelitis is a critical factor in high costs, longer length of stay, long-term use of antimicrobials, and the need for amputation. In addition, of all the factors that influence scar characteristics, the one that seems to be of greatest importance is the time required for wound healing [13]. A large body of literature supports the hypothesis that closure within 21 days minimizes scarring. Therefore, one of the most important areas of research in burns and wound healing is to elucidate the pathophysiology of wound healing, shortening the time to closure, the risk indicators associated with scarring, and the conversion of this knowledge into therapeutic solutions. The use of PRF in wound therapy as an accelerator of repair seems to justify its use. The inclusion of leukocytes in PRF should be carefully evaluated especially when the biomaterial is used and when scar formation is a major concern.

One factor to consider is the inclusion of leukocyte cells. Marx's (1988) opinion that the optimal level of standard platelet concentration was 3-4 times in PRP formulations recommends avoiding a high platelet concentration first and foremost but also the inclusion of leukocytes. So far, this has been a source of contention: some researchers, including AA. argue that leukocytes should be included as they stimulate wound debridement, wound healing and subsequent tissue regeneration, while showing concerns about the unexpected accentuation of inflammation [14]. Therefore, further questions and further analysis will be necessary to obtain definitive conclusions.

In this trial, in which all seven cases were tested by "Probe-To-Bone" with positive results, MRI showed corticoperiosteal thickness and/or focal areas of cortical-spongeous osseolysis with decreased signal strength near the skin ulceration. Oedema caused by to inflammatory sepsis and soft tissue suppurations were also found in the vicinity (Table 4). In our patients, as frequently happens in chronic lesions, several several microorganisms were found simultaneously: bacteria are the most frequent pathogens, but fungal infections have also been determined.

Current successful management of chronic osteo-myelitis comprises surgical intervention, antibacterial therapy, hyperbaric oxygen (OTI), active antibacterial stimulation (ITSB). Surgical therapy is the pillar of treatment [17]. The goal is the cancellation of the infection and the functional restoration of the bone segment being treated. With the surgical procedures adopted up to now for care of osteomyelitis, the necessity for eliminating the infection is to remove the bone and all affected contiguous tissues down to healthy viable tissue. Sometimes, however, minor removal may be sufficient, affecting neither the stability nor the function of the treated extremity, but frequently, after excision of the infected bone, appropriate surgical reconstruction is required. Along with the contaminated tissues, it is also recommended to completely removing all methods of internal fixation (plaques, screws, nails, clips, etc.) present in the infection zone and re- stabilizing by external fixing. All the present surgical methods can give excellent results, but with regard to extremely long and demanding therapies, they are at risk of complications and failure. The use of A-PFR in osteomyelitis pathologies followed by us has given the results reported in this study with a moderate commitment both in terms of surgical technique and economic for the health facility where the patient is treated. In addition, the surgical risk to which the subject is exposed is also low (our subjects were all treated under subarachnoid or local anesthesia).

Ultimately, the impact of PFR on bone cells may not be due to the effects of an individual growth component, but to synergistic effects of multiple platelet-derived growth factors.

More medical, histological, and statistics trials are obliged to understand the utility of this new procedure. However, there is no question, it is impossible to ignore that, as produced by an autologue blood specimen, the PRF generated is low and only a restricted quantity can be utilized. This circumstance restricts the routine using PRFs in major osteomyelitis injuries. Although the possible uses of PFR are varied, to date no precise information on how the biomatrix works can be found, its biology, efficacy and limitations is needed to maximize its application in the regular clinic practice.

7. Conclusion

Ultimately, A-PFR is mechanically robust, able to withstand cargoes, has a dual capacity to extend under stress, and maintains surgical staples so that two or more diaphragm can be stitched together (deforms significantly before ripping; modulus: 0.2 MPa; stress: 140%; fracture force:

3.2 N.mm) [17-21]. The membrane showed a fracture strength comparable to the rupture of an entire aorta and much higher than standard PRP clots [22-24].

Use of A-PFR in subjects with OM from a diabetic limb ulceration will certainly improve our comprehension of wound care, particularly in the remodeling treatment of chronic skin injuries. The objective was to normalize the uses of (L)-PFR in subjects with osteomyelitis to utilize this 2nd generation platelets concentrated, encouraging healing procedures.

The evidence presented in these 7 patients indicates that PFR matrix may be a possible therapeutical option in this troublesome disease. Based on this remark, we are planning to design a prospective randomized clinical study to validate the therapeutic effect of A-PFR and its products, such as i-PFR, as well as its anti-microbial effect. The important reasons to explain the likely variability which can be observed in the outcomes might be ascribed to the different categories of platelet aggregates applied (PRP, PFR) which may differ in the preparations (gel or liquid), as well as in the amount of thrombocytes, leukocyte concentration, and mesh density, fibrin, in the mode of activation that can occur spontaneously, through adherence to biological tissues or, can be induced by thrombin and also with calcium chloride. The AA. recommend the utilization of leukocyte and platelet-containing formulations in a combined fashion after surgical debriding to decrease microbiome count (by eliminating bacteria and by inhibiting biofilm production) and to promote heal.

However, 2nd generation concentrates of platelets (PCs) possess weak influences on tissues bio- regeneration alone, these bio-materials may be capable of to enhance the effectiveness of the initial or primary treatment, such as surgery or drugs, an improvement in the immune system response to antigens is observed [15]. PC therapy is also considered a "substitutional therapy".

In these cases, PCs provide substances necessary for biological tissue regeneration, including growth agents and scaffolding materials, that cannot be provided by surgery or drugs.

The AA. hope that this work will be one of the basis for future investigations to fully exploring the participation of leukocytes in (L)-PFR preparing to achieve an optimized preparation to both control infection and effectively promote injury healing in particular in cases of chronic osteo- myelitis.

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