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HUMAN RANDOMIZED CONTROLLED TRIAL

Periodontal regeneration by leukocyte and platelet-rich fibrin with autogenous bone graft versus enamel matrix derivative with autogenous bone graft in the treatment of periodontal intrabony defects: A randomized non-inferiority trial

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Abstract

Background: Aim of the present study was to ascertain if a combination of leukocyte and platelet-rich fibrin (L-PRF) + autogenous bone graft (ABG) may be a clinically "non-inferior" treatment modality as compared with the association of enamel matrix derivative (EMD) with ABG in the management of intrabony defects (IBDs).

Methods: A total of forty-four patients, exhibiting at least one unfavorable intraosseous defect, were treated by L-PRF associated with ABG (22 patients; test group) or EMD+ABG (control group) in each defect. At baseline and 12 months, a complete clinical and radiographic examination was done. Pre- and post-therapy clinical (probing pocket depth [PPD], clinical attachment level [CAL], gingival recession [GR]) and radiographic (defect Bone level [(DBL)] parameters for the different treatments were compared. To guarantee the test treatment's efficacy 1mm was chosen as non-inferiority margin; for clinical relevance, a second non-inferiority margin = 0.5 mm was set.

Results: Clinical and radiographic parameters significantly improved 12 months after surgery in both test and control sites, without inter-groups differences for each measurement. The control group – test group differences for the parameters CAL gain −0.248 mm (−0.618 to 0.122), PPD Reduction −0.397 mm (−0.810 to 0.015), GR Change 0.059 mm (−0.300 to 0.418), DBL Gain −0.250 mm (−0.746 to 0.246) were all within the non-inferiority margin of 0.5 mm.

Conclusion: Our results suggest that the L-PRF+ABG combined treatment of non-contained IBDs produces non-inferior results in terms of CAL gain, PPD reduction, GR increase and DBL gain in comparison with the EMD+ABG combination.

KEYWORDS
clinical trials, dental enamel proteins, grafts, platelet-rich fibrin, regeneration, wound healing

1 | INTRODUCTION

Although the goal of periodontal therapy is to stop the progression of periodontitis, the ideal treatment is to regenerate the lost periodontal tissues. Particularly, the clinician aims at obtaining the healing of deep intrabony defects (IBDs), as it was observed that IBDs are viable predictors of tooth loss.[1] Many surgical techniques have been used to regenerate IBDs including bone grafts (BGs), guided tissue regeneration (GTR), and the use of biological agents [BAs; i.e., enamel matrix derivative (EMD); growth factors; platelet concentrates (PC)].[2] In the presence of non-contained IBDs, the association of a graft with regenerative agents was suggested to produce better results.[3] This may be particularly true in the case of BA lacking space-making properties.

A recent systematic review[3] concluded that the EMD+BG combination may result in additional clinical attachment level (CAL) gain and probing pocket depth (PPD) reduction as compared with EMD alone. A review from Panda et al.[4] reported that PCs with BGs may be advantageously used as a cost-effective adjunct to regenerative therapy. Among PCs, leukocyte- and platelet-rich fibrin (L-PRF) belongs to a group of second-generation blood products prepared by peripheral blood centrifugation without any anticlotting agent, to obtain a dense three-dimensional clot architecture concentrating platelets, fibrin, leukocytes, cytokines, and growth factors.[5]

A non-inferiority trial (NIT), unlike a superiority one, has the sole objective of demonstrating that a new therapy is not worse (within a specified margin) of the comparator standard treatment. The new treatment is considered to be "non-inferior," if the upper confidence limit for the difference between the two therapies is not larger than a prefixed value (known as non-inferiority margin).[6] The usefulness of non-inferiority studies is that the new treatment has some advantages in terms of safety, practicality or economic cost.

L-PRF, because of its ease of use, combined with its low cost and autologous source, represents an interesting BA for regeneration of periodontal IBDs.

Aim of this study was to ascertain if a L-PRF+autogenous bone graft (ABG) combination may be a "non-inferior," treatment as compared with the association of EMD with ABG in IBDs management.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This was a prospective, randomized, and controlled clinical trial (Figure 1)[7] designed to evaluate the clinical and radiographic outcomes 12 months after treating non-contained IBDs by a combination of L-PRF and ABG [test sites (TSs)] or a combined treatment by EMD*+ABG [control sites (CSs)]. EMD was chosen as the active comparator (AC) as it is currently the most investigated among BAs for IBDs treatment.[3,8]

A NIT was planned to verify whether the use of L-PRF instead of EMD in association with ABG, each one used with its specific application technique, leads to not inferior therapeutic results.

An AC was present, but we did not include a third experimental arm, a group of IBDs treated using open flap debridement (OFD) alone, as it is widely accepted in NITs.[9]

The estimate of the AC effect was assumed from the meta-analysis of Matarasso et al.[3] by the lower bound (LB) of a 95% confidence interval (CI) of the mean of EMD+ABG, whereas an OFD estimate from the corresponding 95% CI LB was adopted by Venezia et al.[8] The difference between estimates was chosen as the benchmark of the added benefit of the AC and as a ground to evaluate the assay sensitivity.

2.2 | Study population

Forty-four patients (15 males) aged 42 to 64 years (mean: 53 ± 12) participated in the study; they were selected among 282 patients, affected by stage III–stage IV periodontitis,[10] seeking treatment at the Unit of Periodontology of the "G. D'Annunzio," University of Chieti-Pescara, Italy, between October 2017 and February 2018. The inclusion criteria were: (1) no systemic diseases; (2) no medications affecting periodontal status during the previous 6 months; (3) not pregnant or lactating; (4) never-smoker or former-smoker ≥10 years; and (5) the following dental and periodontal factors: a full-mouth plaque score (FMPS)[11] and a full-mouth bleeding score (FMBS)[12] <20% at the time of surgery, no periodontal therapy in the 2 previous years, no dental mobility, ≥20 teeth, vertical bone loss detected by radiographic examination [alveolar crest level (ACL) – bottom of the defect (BD) distance = defect bone level [(DBL)] ≥4 mm at baseline and a PPD ≥5 mm when evaluated 12 weeks after non-surgical therapy and no inadequate endodontic treatment at the experimental sites. Only predominantly 1-, combined 1-2 and 2-wall defects, circumferential defects involving at least three dental surfaces or teeth with a defect angle ≥36°[13] were considered in this study (unfavorable IBDs). The architecture of the candidate defect was investigated by circumferential bone probing during non-surgical therapy and had to be confirmed at the surgical intervention. Each patient participated in the study with a single experimental site. The participants volunteered for the study after they received verbal and written information and signed a consent form approved by the ethical committee of the G. D'Annunzio University of Chieti. The study protocol

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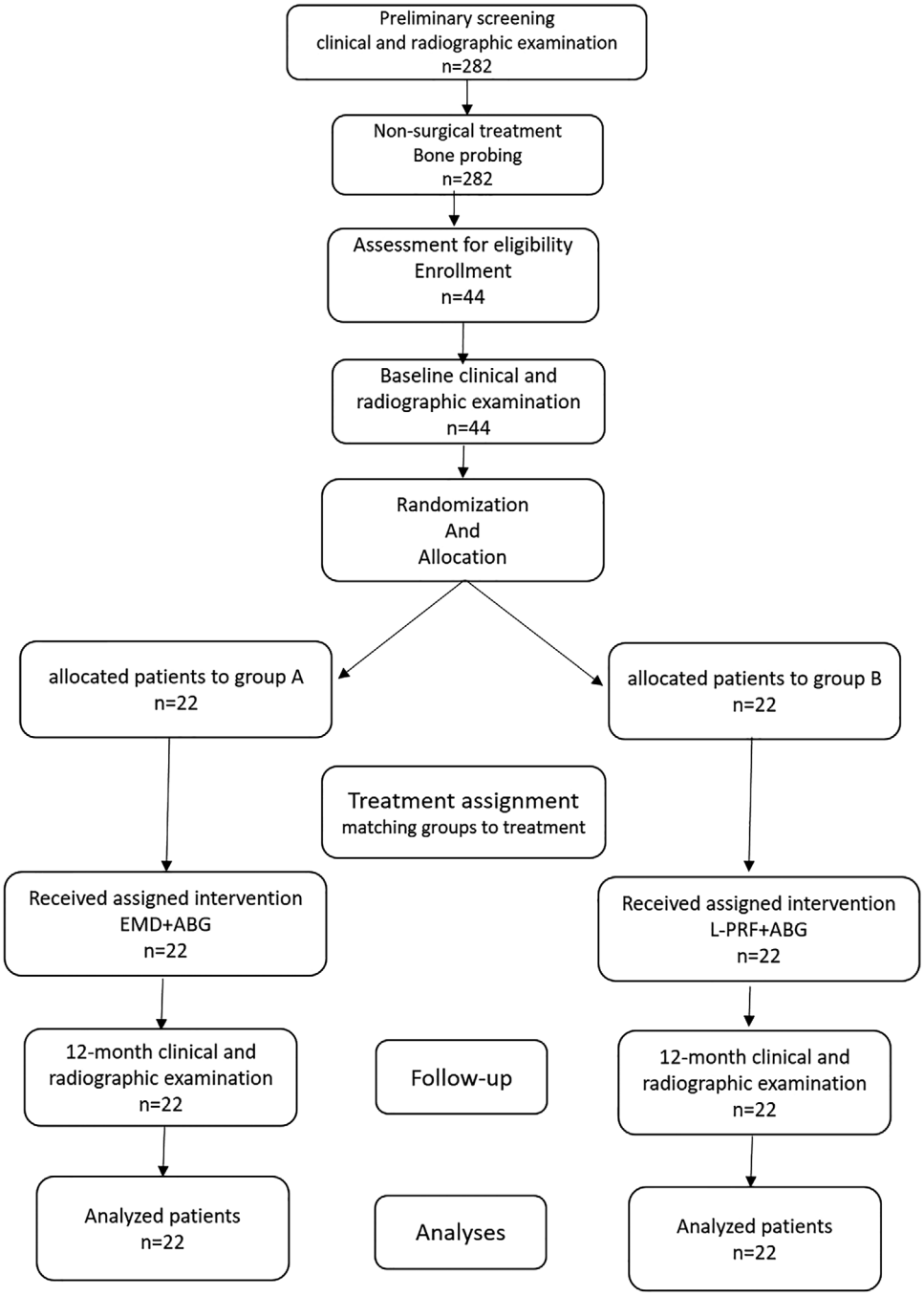

FIGURE 1 CONSORT diagram showing the study layout

was in accordance with the Declaration of Helsinki of 1975, as revised in 2013. The study took place from February 2018 to April 2019. Four months before the surgical treatment, all 44 patients underwent scaling and root planing (SRP) by ultrasonic instruments* and Gracey curettes† and motivational instructions on oral home care. This study is registered at clinicaltrials.gov as NCT03510780.

2.3 | Non-inferiority margin

A reliable estimate of the expected CAL gain from an EMD+ABG treatment was drawn from the meta-analysis by Matarasso et al.[3] The median CAL gain was M = 3.76 mm (SD = 1.07) with 95% CI 3.48 to 3.83 mm. Prudently[14] the lower bound (3.48 mm) was chosen as an estimate of EMD+ABG CAL gain. From Literature,[15] an estimate of CAL gain attainable by OFD alone for papilla preservation flaps is 2.48 mm (CI 1.44 to 3.52 midpoint). The differential effect of using EMD amounts about 1mm.

* Cavitron Select, DENTSPLY, Rome, Italy.

† Hu-Friedy, Milan, Italy.

In accordance with the 95% to 95% method,[16] a magnitude equal to the AC's expected effect (1 mm) was chosen for the M_1 margin, to guarantee the new treatment's efficacy. For clinical relevance, a second M_2 margin was set to preserve at least a further 50% (0.5 mm) of the comparator's effect. Both M_1 and M_2 were adopted for the secondary outcomes too.

2.4 | Sample size and randomization

Not knowing beforehand whether the collected data would had met the assumptions of an analysis of covariance (ANCOVA), the sample was initially sized to power an analysis of variance (ANOVA).

For a one-tail test, with $\alpha = 0.05$ and a SD = 1.07 mm, 20 patients per group were enough to detect the margin $M_1 = 1$ mm in CAL gain between the groups, having a power $1\text{-}\beta = 0.90$.[17]

Assuming an ANCOVA was viable, the sample size to detect an M_2 margin was calculated. Adjusting for baseline values, setting β to 0.2 and adding two more patients allowed to halve the margin (Δ_{NI} $M_2 = 0.5$).

To compensate for possible dropouts, 44 patients were recruited.

2.5 | Blinding protocol

Each defect was assigned a number and was randomly allocated to one of two groups by a computer-generated table.* To conceal group allocation, opaque envelopes were assigned to the specific experimental site and were opened during surgery after defect's debridement. The matching of groups with treatment was performed by a person, unrelated to experimentation and responsible for keeping and breaking the blinding, and known by him alone.

Three blinding groups were envisaged. The first was composed by one examiner and data collector, trained with a calibration exercise to obtain an adequate intra-examiner reproducibility (repeated probing procedures until the examiner obtained a substantial correlation as measured by Cohen's Kappa ≥ 0.6).

The second one were two expert clinicians (MDT and BF), preliminarily inter-examiner calibrated (Cohen's Kappa ≥ 0.6) whose task was to evaluate all the radiographs, reaching an agreement on the location of both ACL and BD.

Both these groups were always kept unaware of treatment allocation.

The third group, the surgeon, was blinded till allocation envelopes opening. The analyst received data aggregated by groups, and provided two 95% CIs, both for group A minus group B and vice versa; only the correctly matched one was going to be retained after blinding breaking.

* R Core Team (2019).

2.6 | Clinical measurements

Patients underwent complete oral and periodontal examinations 3 months after SRP. These included FMPS, FMBS, PPD, CAL, and gingival recession (GR) at six sites per tooth. Clinical measurements at experimental sites were recorded using a University of North Carolina no. 15 periodontal probe.† The measurements were taken at the pre-experimental screening (first visit), after the non-surgical treatment (baseline), and 1 year after, by the same experienced examiner (MG).

2.7 | Radiographic measurements

Periapical radiographs were taken by a 70-kV intraoral x-ray system‡,§ with an exposure time of 0.12 seconds and a digital sensor¶. Pre-SRP, preoperative and 12-month post-operative intraoral standardized radiographs were taken with the long-cone parallel technique using digital sensor holders# customized to the occlusal surfaces of the candidate/selected experimental teeth with a thermoplastic occlusal bite index, the same used at all visits. DBL was evaluated by a dedicated dental software‡ measuring the linear distance between the most coronal interproximal ACL and the BD.

2.8 | Platelet-rich fibrin preparation

L-PRF was produced according to the protocol developed by Choukroun et al.[18]

Immediately before surgery, 30 mL of blood was collected in three 10-mL sterile tubes without anticoagulant from each patient of both groups, to avoid unblinding, by venipuncture and it was immediately centrifuged‖ at 3000 revolutions/min for 10 minutes.

A structured fibrin clot (L-PRF) formed between the red corpuscle layer and the acellular plasma; it was removed from the tube and squeezed in the L-PRF Box** to form three membranes for each TS: one of these was cut and mixed with the ABG acting as a grafting material.

2.9 | Surgical technique

All the surgeries were performed by the same clinician (MP) (Figure 2). In both groups a simplified papilla preservation flap[19] was raised. Briefly, the buccal incision was intracrevicular with an oblique incision across the papilla. It was

† UNC-15, Hu-Friedy, Chicago, IL.

‡ Carestream Dental LLC, Atlanta, GA.

§ Carestream CS 2200, Carestream Dental LLC, Atlanta, GA.

¶ Carestream RVG 5200, Carestream Dental LLC, Atlanta, GA.

RINN XCp-ds, Dentsply Italia S.r.l., Rome, Italy.

‖ IntraSpin, Intra-Lock System Europa SpA, Salerno, Italy.

** Xpression, Fabrication Kit, Intra-Lock System Europa SpA, Salerno, Italy.

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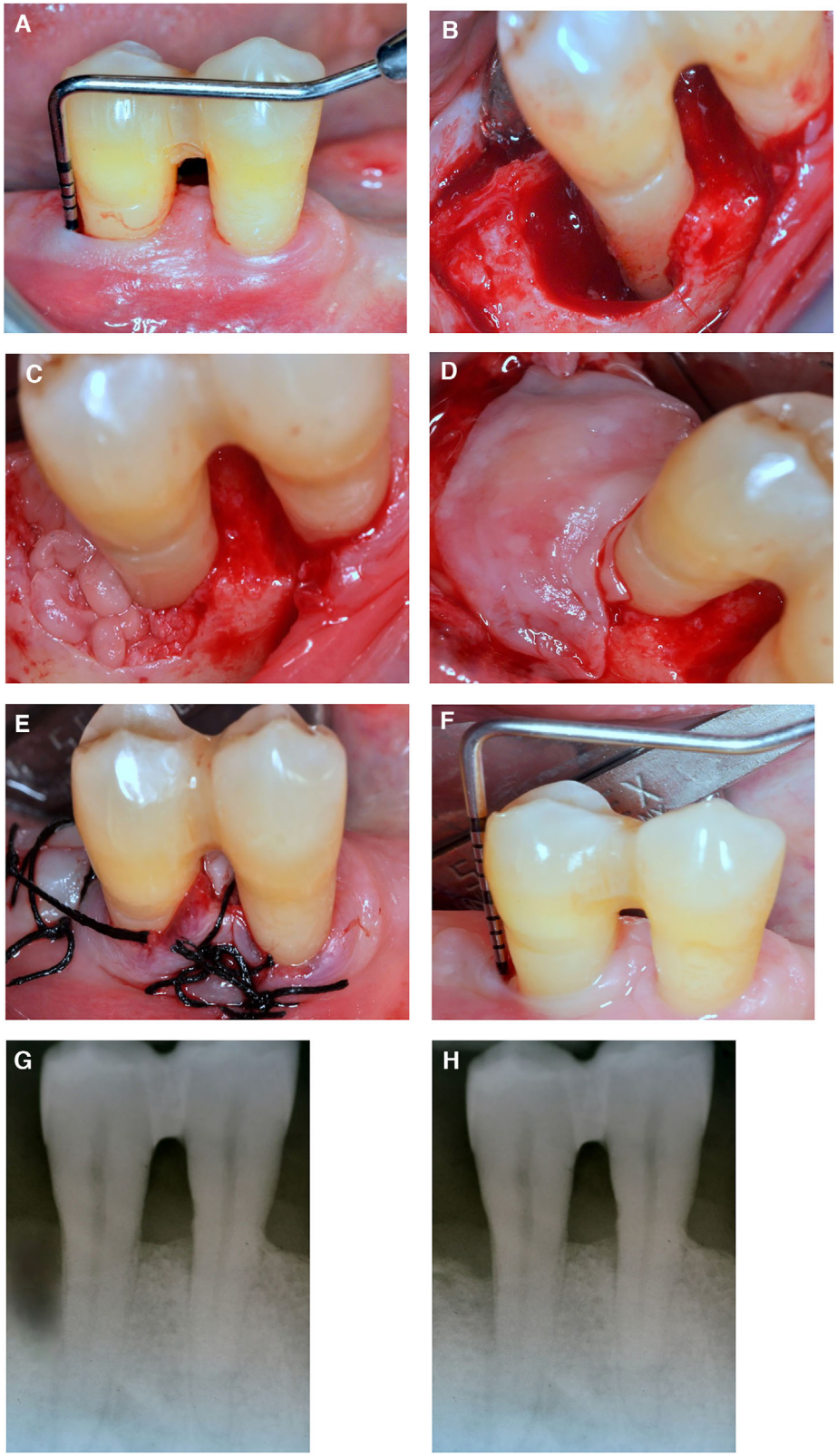

FIGURE 2 (**A**) Test site. Deep periodontal pocket associated with an unfavorable intrabony defect. (**B**) The wide, circumferential intrabony defect after debridement. (**C**) The intrabony defect is filled by the L-PRF and autogenous bone composite graft. (**D**) The graft is covered by the L-PRF membrane. (**E**) The surgical site after suture. (**F**) Clinical aspect of the test site 1 year after surgical treatment. (**G**) Periapical radiography of the test site at baseline. (**H**) Periapical radiography of the test site 12 months after surgical treatment. (**I**) Control site. Deep periodontal pocket associated with an unfavorable intrabony defect. (**J**) 1-2 walled intrabony defect at debridement. (**K**) The defect is filled by the EMD-autogenous bone composite graft. (**L**) The surgical site after suture. (**M**) Clinical aspect of the control site 1 year after surgical treatment. (**N**) Periapical radiography of the control site at baseline. (**O**) Periapical radiography of the control site 12 months after surgical treatment

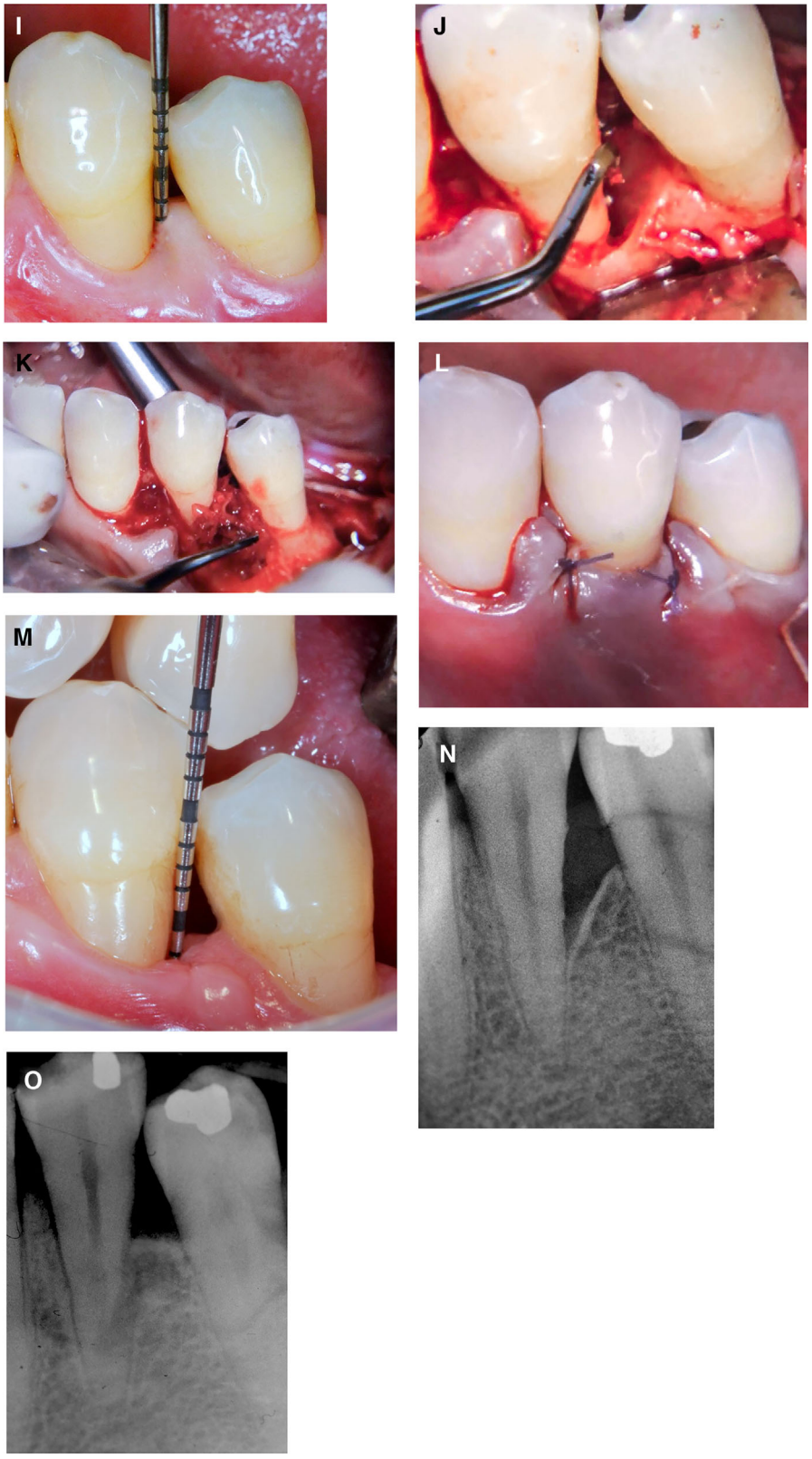

FIGURE 2 Continued

continued intrasulcularly along the buccal aspect of the neighboring teeth and, if necessary, releasing incisions increased accessibility. The palatal incision was intrasulcular and midpalatal. Full-thickness buccal and lingual/palatal flaps were elevated; the granulation tissue was removed and SRP was performed by ultrasonic and hand instruments. Cortical ABG was collected near the experimental teeth using bone scrapers. Then, roots in the CSs were conditioned for 2 minutes with 24% EDTA* and rinsed with saline solution. Internal mattress suture at the defect-associated interdental area was prepared by 4 to 0 silk sutures† and left loose to apply EMD and ABG. EMD was applied according to the "sandwich," technique[20]: a first layer of EMD was put on the root; then, the ABG was placed to fill the IBD. Finally, a second layer of EMD covered the ABG particles and the root coronal to the bone crest. Finally, the flap was repositioned tensioning the internal mattress suture and closing the interdental space with an interrupted suture.

In TSs, after defect debridement, one L-PRF membrane was cut into small pieces and mixed with the ABG to fill the IBD. Two L-PRF membranes were then adapted over the grafted defect without suturing. The flap was sutured similarly to CSs.

2.10 | Postoperative care

All patients were administered 2 g/d amoxicillin-clavulanic acid‡ for 6 days to prevent post-operative infections; pain was controlled by 400 mg oral ibuprofen§ twice daily, if needed. Patients rinsed with 0.12% chlorhexidine¶ twice daily for 3 weeks. Sutures were removed after 14 days. Gentle brushing with a soft toothbrush and interdental brushing were recommended only 2 to 4 weeks respectively after sutures removal: meantime, patients applied a 1% chlorhexidine gel# twice daily. Patients were weekly recalled for 6 weeks, undergoing supragingival tooth cleaning and reinforcement of hygiene instructions.

2.11 | Data processing

Statistical software‖ was used to perform the data analyses.

The null hypothesis H0 stated that CAL gain μC from the gold standard treatment (EMD+ABG) was greater by at least one Non-Inferiority Δ (ΔNI) than PRF+ABG, μE with ΔNI = 1mm.

$$H0 : \mu\_C - \mu\_E \geq \Delta NI$$

The alternative hypothesis was:

$$H1 : \mu\_C - \mu\_E < \Delta NI$$

Because trial's goal was the direct comparisons of single outcomes, multiple univariate analyses were chosen, to which a multivariate one (with CAL gain, PPD reduction and DBL gain as responses, to avoid singularity) was added for completeness.

The primary outcome of the study was CAL gain at 12 months. Changes in PPD, GR, and DBL were secondary outcomes.

Relying on a theoretical correlation between baseline- and gain-score of 0.71,[21] an ANCOVA was envisaged because, by virtue of the expected correlation, sample size could be consistently reduced. As a reference, the ANOVA was performed as well.

All data collected, the ANCOVA model was definitively chosen after seeing NS treatment-by-covariate interaction whereas an observed $r_{pre.gain} = 0.73$ confirmed that 42 patients were enough to detect an $M_2 = 0.5$ mm margin.

Non-inferiority of the main outcome's gain-scores difference between the two treatments was assessed by mean of confidence intervals. For the non-inferiority to be established, the CI upper bound should not attain the margin. Secondary outcomes were analyzed similarly.

3 | RESULTS

3.1 | Study population

All 44 enrolled patients completed the study with full compliance of specifications. Therefore, although the conservative evaluation criterion intended for the study was a per-protocol analysis, an intention-to-treat analysis was performed as well.

3.2 | Clinical and radiographic outcomes

As confirmed by intra-surgical inspection, all experimental defects met the anatomical inclusion criteria. The anatomical features of experimental defects are reported in Table 1. The surgeon met with no defect with inadequate amount of donor autogenous graft.

At surgery, no experimental site showed plaque and marginal gingival inflammation (redness/edema/swelling). After 12 months, none of the experimental sites showed visual signs of inflammation, bleeding on probing or plaque. Accordingly, the FMPS, and FMBS remained <20%

* Prefgel, Institute Straumann, Basel, Switzerland.

† Ethicon, Johnson & Johnson, Pomezia, Italy.

‡ Augmentin, SmithKline Beecham, Milan, Italy.

§ Nurofen Express 400 mg, Reckitt Benckiser Group, Slough, Berkshire.

¶ Dentosan 0.12 Trattamento Mese, Johnson & Johnson, Pomezia, Italy.

Corsodyl Dental gel, GlaxoSmithKline Consumer Healthcare S.p.A. - Baranzate, Italy.

‖ SPSS v.13.0, IBM, Chicago, IL.

TABLE 1 Anatomical characteristics of experimental bony defects

Group	Predominantly (>50%) 1 wall defects	Combined 1-2 wall defects	1-2 wall defects with buccal and/or lingual extension	Circumferential defects
EMD + ABG	7	9	3	3
L-PRF + ABG	7	8	2	5

throughout the study without significant differences between the groups at each time point or between the time points within each group (Table 2). No postoperative complications were reported.

Our results are reported in Tables 2 and 3 and in Figures 3 and 4. Clinical and radiographic parameters significantly improved 12 months after surgery in both TSs and CTs (Table 2), without differences inter-groups for each measurement.

As for the analysis, all ANOVA and ANCOVA assumptions held, so no data transformation was needed. The homogeneity of variance Levene's test was not significant, and no outliers were detected by Mardia's test of absolute deviations from median. A further comparison between the full-sample analysis and that after removing two cases whose absolute standardized residual was >2 did not disprove the alternative hypothesis.

Both ANOVA and ANCOVA relative to CAL gain showed the upper bounds of the 95% CI of difference between the means (EMD – L-PRF) standing far below the predefined efficacy margin M_1 of 1 mm (allowing an efficacy loss to 28%).

Figures 3 and 4 show the non-inferiority margin M_2 set at 0.5 mm (shrinking the loss to 14%) being neither reached nor exceeded by any of the 95% confidence intervals of both ANCOVA and ANOVA.

The 95% CI of the secondary outcomes shows a very similar pattern as compared to the CAL gain. For all CAL, PPD, GR, and DBL change-score, the CI upper bounds of the Bonferroni adjusted differences never reached M_2, well remaining within the "L-PRF better," side.

Despite its greater power, the multivariate analysis was not significant also ($P = 0.77$). For all the outcomes, comparison between ANCOVA's and ANOVA's CIs suggested no relevant imbalances were present between groups in the baseline variables.

4 | DISCUSSION

4.1 | Principal findings

The aim of this study was to evaluate the existence of non-inferiority in clinical results between two reconstructive techniques (BA+graft+application method) on the healing of unfavorable IBDs. The application methods chosen for the combined therapy with the two BAs reflect those accepted for this purpose in the literature.[20,21]

To our knowledge, this is the first NIT carried out to compare the clinical effectiveness of L-PRF and EMD, both associated with ABG.

Although confirming that both experimental treatments are clinically and radiographically effective, the results obtained in the present study show that the L-PFR+ABG combination produces non-inferior results in comparison with EMD+ABG for both primary and secondary outcomes.

4.2 | Agreements and disagreements with previous findings

The clinical and radiographic results obtained by both techniques are in good agreement with those reported in the Literature for L-PRF and EMD combination therapies, respectively.[3,4] Conversely, few data exist comparing the effectiveness of EMD with L-PRF: Aydemir Turkal et al.[22] investigated on EMD or EMD+PRF in IBDs; both therapies produced significant improvements without intergroup differences; only in well-contained IBDs the EMD+PRF treatment produced a greater bone gain. Gupta et al.[23] compared these BAs in IBDs without any bone graft concluding that they were similarly effective in CAL and PPD improvement but EMD was superior for bone gain. In this connection it should be noted that bone grafting improved bone regeneration when used in combination with PRF.[21,24] This may explain the non-inferior bone gain in the present research at TSs.

The similarity of clinical results obtained by associating ABG with L-PRF or EMD may be explained by their biological effects, whose common denominator consists of an increase in specific cellular anabolic activities.[25-29]

EMD induces the synthesis of alkaline phosphatase and growth factors by periodontal ligament cells,[25] stimulating the proliferation of periodontal ligament cells and osteoblast precursors.[26] EMD increases collagen and protein production, stimulates mineralization and inhibits epithelial cell proliferation[27]; it also contains further mitogenic factors like TGF-β and BMP-like growth factors.[26]

L-PRF releases polypeptide growth factors, such as platelet-derived growth factors (PDGFs), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and insulin-like

TABLE 2 Clinical and radiographic parameter scoring (mm ± SD)

Parameter	Group	1st Visit Mean ± SD (95% CI)	Baseline Mean ± SD (95% CI)	12 Months Mean ± SD (95% CI)	1st Visit – baseline Mean ± SD (95% CI)	Baseline – 12 months Mean ± SD (95% CI)
PPD	PRF + ABG	9.21 ± 1.197(8.75 to 9.68)	7.43 ± 1.425(6.88 to 7.98)	3.21 ± 0.787(2.91 to 3.52)	1.786 ± 1.475(1.214 to 2.358)	4.214 ± 1.100(3.809 to 4.619)
	EMD + ABG	9.29 ± 1.697(8.63 to 9.94)	7.64 ± 1.096(7.22 to 8.07)	3.68 ± 0.670(3.42 to 3.94)	1.643 ± 1.393(1.103 to 2.183)	3.964 ± 1.035(3.559 to 4.369)
	Diff	$P = 0.86$	$P = 0.53$	$P = 0.021$	$P = 0.85$	$P = 0.021$
CAL	PRF + ABG	8.93 ± 1.585(8.31 to 9.54)	8.25 ± 1.110(7.82 to 8.68)	4.82 ± 0.863(4.49 to 5.16)	0.679 ± 1.156(0.230 to 1.127)	3.429 ± 0.741(3.083 to 3.734)
	EMD + ABG	9.18 ± 1.744(8.50 to 9.85)	8.46 ± 1.261(7.98 to 8.95)	5.18 ± 0.772(4.88 to 5.48)	0.714 ± 0.976(0.336 to 1.093)	3.286 ± 0.854(2.983 to 3.589)
	Diff	$P = 0.58$	$P = 0.50$	$P = 0.10$	$P = 0.58$	$P = 0.11$
GR	PRF + ABG	0.18 ± 0.476(−0.00 to 0.36)	0.96 ± 0.576(0.74 to 1.19)	1.61 ± 0.497(1.41 to 1.80)	−0.786 ± 0.630(−1.030 to −0.541)	−0.643 ± 0.492(−0.898 to −0.387)
	EMD + ABG	0.04 ± 0.429(−0.13 to 0.20)	0.82 ± 0.670(0.56 to 1.08)	1.50 ± 0.638(1.25 to 1.75)	−0.786 ± 0.686(−1.052 to −0.520)	−0.679 ± 0.882(−0.934 to −0.423)
	Diff	$P = 0.24$	$P = 0.48$	$P = 0.49$	$P = 0.24$	$P = 0.49$
DBL	PRF + ABG	9.79 ± 1.101(9.35 to 10.21)	9.61 ± 1.066(9.19 to 10.02)	6.68 ± 1.020(6.28 to 7.07)	0.179 ± 0.390(0.027 to 0.330)	2.928 ± 0.716(2.587 to 3.270)
	EMD + ABG	9.82 ± 1.442(9.26 to 10.38)	9.61 ± 1.397(9.07 to 10.15)	6.93 ± 1.052(6.52 to 7.34)	0.214 ± 0.418(0.052 to 0.376)	2.678 ± 1.055(2.337 to 3.020)
	Diff	$P = 0.92$	$P = 0.67$	$P = 0.37$	$P = 0.91$	$P = 0.37$
FMBS	PRF + ABG	0.53 ± 0.191(0.460 to 0.608)	0.13 ± 0.021(0.122 to 0.139)	0.15 ± 0.029(0.135 to 0.157)	0.404 ± 0.193(0.329 to 0.479)	0.02 ± 0.034(0.002 to 0.029)
	EMD + ABG	0.52 ± 0.191(0.450 to 0.597)	0.13 ± 0.020(0.122 to 0.137)	0.14 ± 0.037(0.122 to 0.151)	0.394 ± 0.193(0.319 to 0.469)	0.01 ± 0.031(−0.006 to 0.019)
	Diff	$P = 0.83$	$P = 0.90$	$P = 0.28$	$P = 0.95$	$P = 0.28$
FMPS	PRF + ABG	0.72 ± 0.096(0.68 to 0.76)	0.14 ± 0.019(0.130 to 0.145)	0.15 ± 0.020(0.141 to 0.156)	0.58 ± 0.10(0.541 to 0.619)	0.01 ± 0.029(−0.000 to 0.022)
	EMD + ABG	0.74 ± 0.088(0.702 to 0.77)	0.14 ± 0.016(0.134 to 0.147)	0.14 ±0.0230.131 to 0.149	0.60 ± 0.09(0.560 to 0.630)	−0.00 ± 0.028(−0.011 to 0.011)
	Diff	$P = 0.47$	$P = 0.55$	$P = 0.16$	$P = 0.47$	$P = 0.16$

PPD, pocket probing depth; CAL, clinical attachment level; GR, gingival recession; DBL, defect bone level; FMBS, full mouth bleeding score; FMPS, full mouth plaque score.

Note: Because significant results in the between-groups comparisons at Baseline are suggestive that equality assumption was not holding, no *P* value was adjusted for multiplicity.

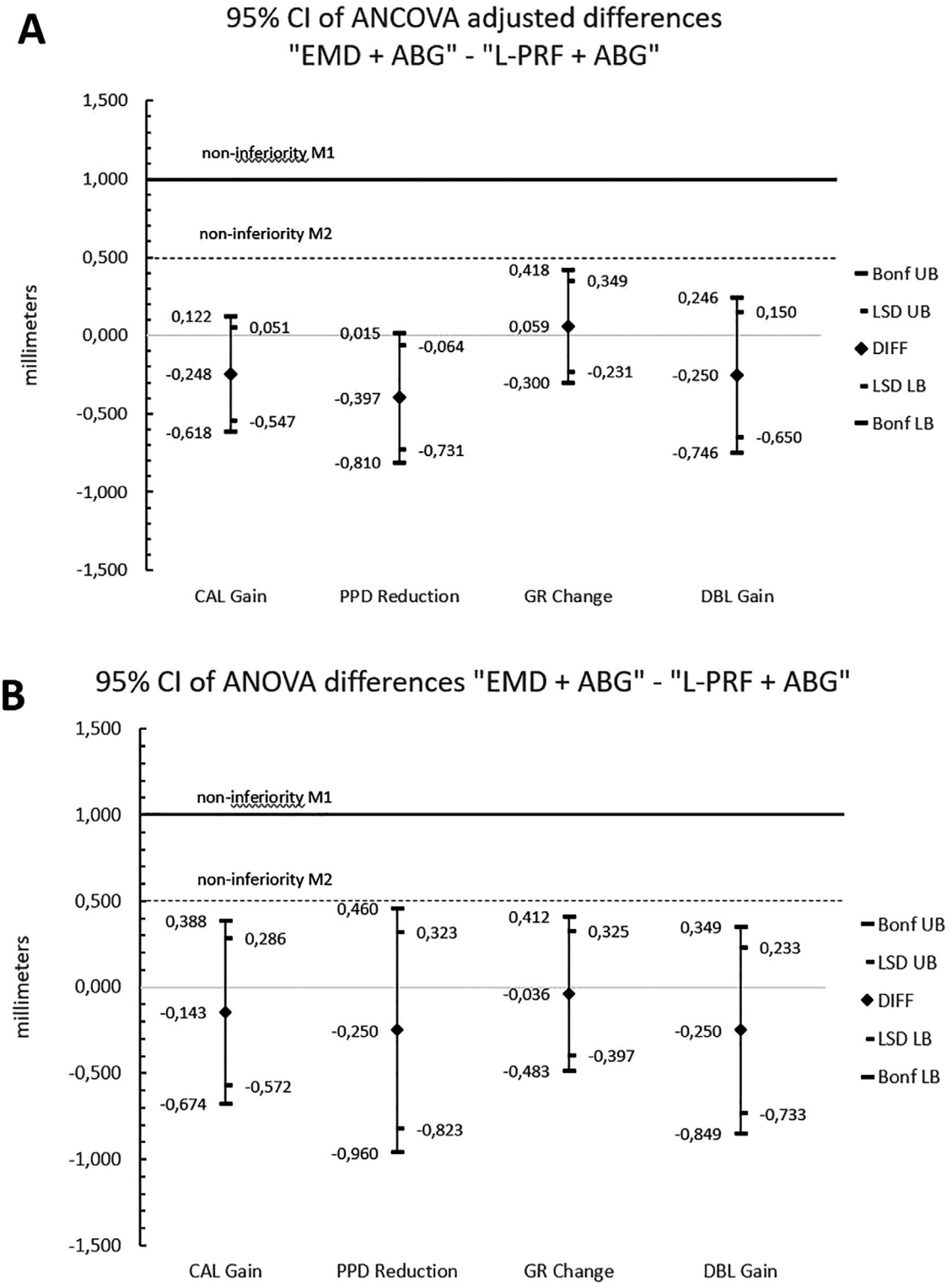

FIGURE 3 A total of 95% confidence intervals of ANCOVA (**A**) and ANOVA (**B**) adjusted differences between test and control groups. PPD, pocket probing depth; CAL, clinical attachment level; GR, gingival recession; DBL, defect bone level; Bonf UB, Bonferroni adjusted upper bound; LSD UB, least significant difference upper bound; DIFF, difference; LSD LB, least significant difference lower bound; Bonf LB, Bonferroni adjusted lower bound

TABLE 3 Differences between treatments in clinical and radiographic parameter changes (mm – SE) from baseline to 12 months

Parameter	Treatment Group	ANCOVA Estimated Mean ± SE	ANCOVA 95% CI	ANOVA Observed Mean ± SE	ANOVA 95% CI
CAL Gain	PRF + ABG	3.481 ± 0.105	3.270 to 3.692	3.429 ± 0.151	3.125 to 3.732
	EMD + ABG	3.233 ± 0.105	3.022 to 3.444	3.286 ± 0.151	2.983 to 3.589
	Group Difference				
CAL Gain	"EMD + ABG" - "PRF + ABG"	−0.248 ± 0.149	−0.547 to 0.051	−0.143 ± 0.214	−0.572 to 0.286
PPD Reduction	EMD + ABG" - "PRF + ABG"	−0.397 ± 0.166	−0.731 to −0.064	−0.250 ± 0.286	−0.823 to 0.323
GR Change	"EMD + ABG" - "PRF + ABG"	0.059 ± 0.144	−0.231 to 0.349	−0.036 ± 0.180	−0.397 to 0.325
DBL Gain	"EMD + ABG" - "PRF + ABG"	−0.250 ± 0.200	−0.650 to 0.150	−0.250 ± 0.241	−0.733 to 0.233

Baseline$_{CAL}$ = 8.36, Baseline$_{PD}$ = 7.53, Baseline$_{GR}$ = 0.89. Baseline$_{BBD}$ = 9.61; PPD, pocket probing depth; CAL, clinical attachment level; GR, gingival recession; DBL, defect bone level

Note: The estimated means are evaluated at the following average values of covariates.

growth factor -1(IGF-1)[28] for at least 7 to 28 days.[29] PDGF has mitogenic effects on stem cells and osteoblasts, stimulates cell replication of endothelial cells promoting angiogenesis whereas TGF-β activate fibroblasts, cementoblasts, and osteoprogenitor cells; endothelial cells are also activate to produce new capillaries.[28] IGF-1 exerts chemotactic effects towards human osteoblasts, regulating cell migration, proliferation, differentiation, and matrix synthesis.[29] Finally, both EMD and L-PRF have antibacterial properties: EMD reduces the vitality of dental plaque,[30] specifically inhibiting the growth of periodontal pathogens.[31] Leukocytes trapped in L-PRF mesh have anti-infectious effects; PRF fibrin network stimulate the immune response through fibrinogen degradation products enhancing neutrophils migration and phagocytosis.[28] All the above described biological activities of both BMs, stimulating the biological functions of specific cells playing a pivotal role in periodontal regeneration, in association with their specific application methods, can explain our results.

4.3 | Study design

This is a NIT with the researchers' scope limited to ascertain if the new therapy has, at best, the same effectiveness of the standard treatment, but showing some advantages for safety, convenience or cost. NITs require the enrollment of fewer patients than superiority ones; consequently, they are less expensive and of shorter duration.

We used two application methods validated by the literature,[20,21] yet not identical; to guarantee a fair controlled comparison of BAs, a simple way would be standardizing the application-methods. However, because of the materials' different physical consistency, the methods could never be identical, unless sacrificing virtually useful properties (i.e., membrane effect). Although to investigate the specific BAs' biological properties could be the major aim of the researcher, the clinician's primary interest is to be able to rely on a substantial overall effect of the treatment. In this perspective, the trial was designed to detect the overall effect of the BA confounded with its application-method, not to spot any differences exclusively related to the BAs' biological properties.

To refer the whole effects to BAs could seem reasonable, yet some hypotheses suggest that the application of an L-PRF membrane onto the graft in the TSs could have contributed to the graft and blood clot stabilization, retaining them within the walls of the IBD[32] in the early phases of the healing process and favoring the defect's healing also through a mechanism independent from the BAs' biological properties. However, it is true that in meta-analyses[9,33] EMD showed not to take advantage for IBDs healing from the simultaneous membrane application. Still less conceivable is the existence of an efficacious barrier effect by L-PRF membrane, considering its short reabsorption time (1 to 2 weeks).[34]

Notice that the presence of confounding does not imply that the detected non-inferiority in the treatments' effect is not-true; it only somehow distributes the casual effect responsibility between BA and application-method, if any.

4.4 | Clinical implications

In the present study, the BAs+ABG were tested in defects of unfavorable architecture. In this connection it should be reported that the healing potential of IBDs varies according to their architecture. Following conventional surgery Ellegaard and Loe[35] showed a greater bone fill in three-walled compared to two-walled IBDs, whereas in regenerative surgery, it was reported that the wider the radiographic defect angle, the lower the amount of regeneration.[13] When using a GTR technique, the number of residuals bony walls does not influence the tissue gain,[13] if the membrane maintains the space for regeneration. Conversely, in EMD-procedures, the probability of obtaining greater CAL gains is higher in predominantly three-walled defects[36]: with a 2.7 times greater probability of at least 3mm CAL gain compared with one-wall defects. Similarly, a systematic review[37] concluded that in two wall defects treated by GTR, combination therapy provides superior histologic results of bone repair compared to membranes alone. In this study we investigated on the treatment of non-favorable IBDs; in such a scenario, the consistency of L-PRF and EMD alone is not able to guarantee the space-maintaining property that is particularly important for regeneration. When treating non-contained IBDs, the presence of a bone graft provides maintenance of the space for regeneration and further enhances the blood clot stability.[38] In agreement, Matarasso et al.,[3] through a meta-analysis comparing the clinical efficacy of the EMD-bone graft combination with that of EMD used alone in IBDs, showed that the EMD–graft combination produces additional CAL gain. Another meta-analysis on EMD+alloplastic fillers, reported long-term significant advantages for PPD reduction, CAL gain, and defect healing.[39]

The recent L-PRF literature shows similar results: different meta-analysis[5,40,41] concluded that L-PRF+BGs, significantly improves the clinical outcomes. Lekovic et al.,[21] comparing the effectiveness of L-PRF used alone or in association with deproteinized bovine bone, reported that the graft augmented PRF effects in reducing PPD, improving CAL, and promoting defect fill. Chandradas et al.[24] also observed a significant greater defect resolution when demineralized bone matrix was associated to PRF; a similar result was obtained by Pradeep et al.[42] using porous hydroxyapatite.

In the present study we used intra-oral ABGs as grafting material. ABG is considered the "gold standard," graft and there is a large periodontal Literature[43-45] on it, reporting bone gains ranging from 1.2 to 3.8 mm; furthermore, histologic evidence of periodontal regeneration was observed with ABGs in humans.[43,46]

4.5 | Limitations of the study

Some limitations should be highlighted in our research. First, this was not a split-mouth study and we did not use a stent-assisted probing methodology; secondly, the surgery was performed about 3 months after SRP and we did not wait long enough after non-surgical therapy to assess potential radiographic changes in IBDs. Furthermore, we treated heterogeneous IBDs in their architecture by arbitrarily grouping them into a single category of "unfavorable," IBDs. Although all these IBDs presenting anatomical characteristics considered "unfavorable," by the Literature,[13,36] a more rigorous standardization of their anatomy could have guaranteed greater clarity in the results' interpretation. A caveat is that our design detected the overall effect of BAs+graft+application-method, not disentangling the effect of the BA from that of the application method. Finally, the economic advantage offered by the L-PRF+ABG method was not exactly quantified except in a very generic way. This topic needs to be further considered in future researches.

5 | CONCLUSION

Within the limitations of this study, our results suggest that L-PRF+ABG produces non-inferior results for CAL gain, PPD reduction, GR increase, and DBL gain in comparison with EMD+ABG when treating non-contained IBDs. Conversely, L-PRF+ABG combination shows some advantages: first, it is completely autogenous, representing a guarantee from the transmission of known or potentially unknown infectious agents. The clinical safety of EMD was clearly demonstrated[47]; however, some patients prefer to avoid the use of products derived from animals for ethical reasons.

Another factor favoring the L-PRF+ABG combination is its negligible cost, particularly desirable in those countries where a National Health Service is economically responsible for the citizens' periodontal health.

This is the first NIT comparing the clinical effectiveness of L-PRF and EMD, with ABG, to treat non-contained IBDs: further investigations are needed to confirm our results.

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