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HUMAN RANDOMIZED CONTROL TRIAL

Impact of N-terminal pro-B-type natriuretic peptide and related inflammatory biomarkers on periodontal treatment outcomes in patients with periodontitis: An explorative human randomized-controlled clinical trial

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Abstract

Background: N-terminal portion of the B-type natriuretic propeptide (NT-proBNP) has potentially been shown to play an important role in the development of periodontitis and cardiovascular disease (CVD). This study evaluated the efficacy of periodontal treatment on NT-proBNP and related CVD biomarkers and explored whether subjects harboring high NT-proBNP at baseline showed increased clinical benefits with the non-surgical periodontal treatment performed with full-mouth scaling and root planing (FM-SRP) at 6-month follow-up.

Methods: Forty-eight patients with stage III periodontitis were randomized to receive minimal standard oral care (SOC) ($n = 24$) or FM-SRP ($n = 24$) protocol. Clinical periodontal parameters (probing depth, clinical attachment loss, bleeding on probing), serum NT-proBNP, α1-antitrypsin, C-reactive protein (hs-CRP), endothelial cell-specific molecule-1 (ECM-1), and neutrophil gelatinase-associated lipocalin (NGAL) concentrations were assessed at baseline and at 1-, 3-, and 6- month follow-up.

Results: At 6 months, FM-SRP was more effective than SOC in reducing periodontal parameters and mean proportions of NT-proBNP ($p = 0.004$), hs-CRP ($p = 0.003$), α1-antitrypsin ($p = 0.012$), ECM-1 ($p = 0.014$), and NGAL ($p = 0.045$). At 6-month follow-up, the reduced NT-proBNP, α1-antitrypsin, hs-CRP, ECM-1, and NGAL levels were significantly correlated with the extent of periodontitis ($p < 0.05$). Furthermore, the analysis of variance analysis evidenced that, at 6-month follow-up, FM-SRP significantly impacted the reduction of NT-proBNP, hs-CRP, ECM-1, and NGAL. Moreover, high levels of NT-proBNP, hs-CRP, ECM-1, and NGAL at baseline significantly influenced the efficacy of periodontal treatment positively.

Conclusion: In this study, FM-SRP was more effective than SOC in reducing clinical variables and NT-proBNP levels, although subjects who harbored high NT-proBNP concentrations at baseline showed greater clinical benefits of periodontal treatment at 6-month follow-up.

KEYWORDS
C-reactive protein, endothelial cell-specific molecule-1, neutrophil gelatinase-associated lipocalin, periodontal debridement, periodontitis, pro-brain natriuretic peptide

1 | INTRODUCTION

Periodontitis is a chronic, multifactorial inflammatory disease caused by infectious biofilm which could determine, if not properly treated, the destruction of the tooth-supporting tissues and, finally, tooth loss.[1] During the past few decades, several large-cohort studies have acknowledged that the various forms of periodontitis may affect over 50% of the population worldwide and also negatively influence several systemic diseases such as cardiovascular disease (CVD),[2,3] preterm birth,[4] metabolic syndrome,[5] and determines an overall low quality of life.[6] Some evidence has shown that the progression of periodontitis can be associated with the dysregulation of some pro-inflammatory mediators released in the bloodstream, such as metalloproteases, interleukins, prostaglandins, and high-sensitive c-reactive proteins (hs-CRP),[7] which could determine an increased chronic risk of systemic inflammation, endothelial dysfunction, and CVD through a specific oxidative stress pathway.[8]

Among biomarkers of early CVD risk, it has been demonstrated in some studies that the N-terminal pro-B-type natriuretic peptide (NT-proBNP), a fragment secreted by cardiac ventricles myocytes in response to the heart volume expansion or pressure load, is associated in the early diagnosis, risk stratification, and follow-up outcomes of patients with CVD and heart failure.[9,10] It has been indicated that elevated serum NT-proBNP, together with α1-antitrypsin and endothelial cell-specific molecule-1 (ECM-1) levels, were associated with a high risk of ventricular wall stress, a phenomenon that occurs in early heart failure in weakly symptomatic patients and in the patient's risk stratification in acute coronary syndrome and CVD.[10,11] Specifically, NT-proBNP and $\alpha-1$ antitrypsin have been used as inclusion criteria for several large-population trials such as a surrogate metric to assess the relationship between CVD treatment effect and heart failure overall outcomes.[12,13]

The significant association between periodontitis, heart failure, and CVD has also been proven by some large cohort clinical trials, such as the Periodontitis and Its Relation to Coronary Artery Disease (PAROKRANK) study, in which periodontitis patients were more frequently associated with an upregulation of CVD biomarkers correlated with early myocardial infarction risk in comparisons with matched controls.[14] Another preliminary evidence reported that, in comparison with healthy subjects, periodontal inflammation could increase CVD risk through the main role exerted by the upregulation of inflammatory mediators such as hs-CRP and NT-proBNP[15] and that periodontal treatment could determine positive effects on systemic inflammatory CVD markers such as hs-CRP,[16] ECM-1,[17] or neutrophil gelatinase-associated lipocalin (NGAL),[18] even through with variability in the reported outcomes among trials.[19] Specifically, in patients with periodontitis, it has been reported that, among others, periodontal treatment performed through a full-mouth scaling and root planing (FM-SRP) approach was demonstrated to be efficacious in reducing several clinical outcomes and serum markers of systemic inflammatory risk.[20–23]

There is growing interest in evaluating the impact of periodontal treatment on early biomarkers related to early CVD risk and evaluating the effects of biomarker levels on the long-term efficacy of periodontal therapy. For the above-mentioned reasons, the aim of the present study was to assess the impact of two non-surgical periodontal treatment protocols on serum NT-proBNP and related CVD biomarkers in patients with periodontitis. It was also investigated if high baseline NT-proBNP, hs-CRP, ECM-1, and NGAL levels influenced the efficacy of periodontal treatment at 6-month follow-up. The null hypothesis to be invalidated was that periodontal treatment protocols did not affect any analyzed biomarkers and vice versa.

2 | MATERIALS AND METHODS

2.1 | Study design and sample

The study was designed as a single-center, randomized, parallel-group clinical. The study was conducted in agreement with the CONSORT guidelines[24] and followed the Helsinki Declaration on medical research of 2016. Before the study, all enrolled patients signed a written informed

consent which specified the study risks and characteristics. The ethical approval was obtained before the patient enrollment from the International Review Board of the University of Catania, Catania, Italy (n. 125-20/PO).

For the study, enrolled were consecutive patients with a diagnosis of periodontitis[1] recruited at the Unit of Periodontology of the Dental School at the University of Catania, Catania, Italy. In order to obtain an equal gender and age proportion, male and female patients aged between 35 and 70 years were enrolled, ensuring that at least 50% of them were male. The inclusion criteria were (1) having good general health, (2) a minimum of six teeth per quadrant, (3) at least two teeth in each quadrant having a probing depth (PD) ≥5 mm and a clinical attachment level (CAL) ≥4 mm, (4) at least ≥40% of periodontal sites with bleeding on probing (BOP), (5) no involvement of furcation, and (6) at least ≥2 sites with radiographically alveolar bone loss (ABL) verified on periapical radiographs.[25] The exclusion criteria were (1) periodontal therapy in the past 12 months preceding the study, (2) use of antibiotics in the past 6 months preceding the study, (3) status of pregnancy or lactation, (4) presence of systemic condition that could affect the study results, (5) use of mouthwash containing antimicrobials in the previous 3 months prior the study, (6) medication by anti-inflammatory, immunosuppressive, or contraceptive drugs, (7) history of drinking, (8) smoking, and (9) class II and III tooth mobility.

After identification, all eligible participants undergo a medical history and demographic parameters such as age, gender, body mass index (BMI), comorbidities (if present), medications, and educational levels were recorded. BMI (kg/m^2) was calculated by dividing patient's weight and the square of the patient's height. Moreover, the patient's socioeconomic status (SES) was recorded based on previous work experience and economic and social positions. Each patient was classified on high, middle, and low SES after the interview.[3] On the basis of patient's smoking history, participants were categorized as current smokers, ex-smokers (stopped smoking ≥5 years), and non-smokers.

A masked examiner achieved a full dental and periodontal examination on each patient. On six sites per tooth, periodontal charting was obtained using a standardized periodontal probe*, by setting CAL as a primary variable and recording PD, BOP, ABL, and plaque index score (PI).

2.2 | Sample size and reliability analysis

The power sample analysis was calculated using statistical software†. The sample size was obtained by setting serum NT-proBNP as a primary outcome variable[15] and considering two groups of patients, an effect size of 0.30, a 2-sided level of 0.05, a standard deviation of 1.5, and a power level of 80%. Therefore, it was fixed a priori that at least 23 patients per group were needed. However, to avoid potential dropouts during the 6-month follow-up, 25 patients were enrolled, so the primary variable (CAL) achieved a power value of 0.85.

The inter- and intra-examiner reliability analysis was achieved using PD and CAL as reference values for the reliability analysis and using the intraclass correlation coefficient (ICC). The ICC analysis showed good agreement among examiners for both PD (ICC = 0.812) and CAL (ICC = 0.814); the first examiner had good reliability for (PD, ICC = 0.816; CAL, ICC = 0.811), as well as the second examiner (PD, ICC = 0.815; CAL, ICC = 0.814).

2.3 | Randomization

Through a permuted block design, the randomization was performed by a single clinician (G.T.) not involved in the subsequent trial stages, which generated a random assignment of a treatment using a sequence 1:1 ratio by a computer random-number generator.

Each patient was allocated to receive minimal standard oral care (SOC) (control group) or FM-SRP (test group). The allocation concealment to the clinician that performed the treatment through serially numbered sealed envelopes and the details of the sequence that were blinded to all other clinicians. Before each treatment, a clinician not involved in the data processing performed the assignment of the treatment through sealed envelopes marked with the treatment and with the initials of the patient's name and date of birth.

Just before each treatment session, another clinician (S.S.) opened the envelope with the assigned treatment given to the operator who performed the type of treatment. The same clinician with 10 years of experience (G.I.), blinded to previously recorded data to avoid bias in the experimental data evaluation, performed all the procedures.

2.4 | Treatment

Each patient received oral hygiene instructions (OHI) shortly after the baseline assessments.

Patients allocated to the FM-SRP group undergo a full-mouth SRP in one side of the mouth for each session, within 24 h in two separate sessions on 2 consecutive days. Two right quadrants were instrumented during the morning session, whereas the other two were in the afternoon session. Treatments were recorded in minutes

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and performed under local anesthesia only if necessary. SRP was performed using both hand and ultrasonic instrumentation by tips No. 5/6/7[‡], which was used with constant water irrigation with a 20.000 Hz frequency.

The control group received minimal SOC, which included two sessions of supragingival professional mechanical plaque removal prophylaxis aimed at eliminating supragingival plaque and calculus with an ultrasonic scaler without subgingival instrumentation. For the control group, the same periodontal treatment performed in the test group was provided upon completion of the study.

At the end of each type of treatment, all patients were instructed and motivated to perform personal oral hygiene.

2.5 | Outcome measures

The primary outcome was the analysis of serum NT-proBNP expression changes at baseline and at 6 months after treatment between the two groups. Furthermore, ESM-1, α1-antitrypsin, NGAL, fibrinogen, and hs-CRP concentration changes were analyzed before and after 6 months of treatment.

The secondary aim was to analyze the impact and interaction of the periodontal treatment protocol (FM-SRP) and treatment timing on NT-proBNP and hs-CRP changes and if high NT-proBNP, ESM-1, and NGAL levels at baseline influenced the efficacy of periodontal treatment after 6-month follow-up.

2.6 | Sampling

All patients' serum samples were collected at baseline, 1-, 3-, and 6-month follow-up by the same examiner between 8:00 and 10:00 am before any clinical evaluation. Immediately after sampling, serum samples were centrifuged at 4°C (1000× g for 2 min). The levels of serum NT-proBNP were obtained using a specific kit[§] according to the manufacturer's instructions. A nephelometric assay kit obtained the hs-CRP levels, while ECM-1, α−1 antitrypsin, and NGAL were determined using human-specific enzyme-linked immunosorbent assay (ELISA) kits.

2.7 | Statistical analysis

Numerical data were expressed by mean ± standard deviation (SD), while categorical variables were reported as numbers and percentages. A non-parametric approach was applied because most of the analyzed variables were not normally distributed, as verified by the Kolmogorov-Smirnov test. The Mann-Whitney test was used for the numerical data comparison between groups, while the chi-squared test was used for the comparisons between categorical variables. The single patient was set as a test unit.

For intragroup comparisons, the Friedman test was used in order to compare the numerical variables among four-time points (baseline, 1, 3, and 6 months), while the two-by-two comparisons between dependent groups were calculated using the Wilcoxon test. Bonferroni's correction was used for the multiple comparisons, and the alpha level of 0.050 was divided by the number of possible comparisons (baseline, 1, 3, and 6 months) so that the adjusted significance level was equal to 0.012 (0.050/4). The Spearman's correlation test was used to evaluate a possible significant interdependence between NT-proBNP, α−1 antitrypsin, ESM-1, NGAL, fibrinogen, and hs-CRP and all analyzed variables at 6 months of treatment.

To analyze the impact of the treatment protocol on NT-proBNP and on α−1 antitrypsin, ESM-1, NGAL, fibrinogen, and hs-CRP concentration changes (as continuous variables), after checking the conditions for its applicability, a two-way analysis of variance (ANOVA) was used to estimate whether the mean of the quantitative variable (NT-proBNP-1, α-antitrypsin, ESM-1, NGAL, fibrinogen, and hs-CRP) changes based on the levels of two categorical variables, treatment and duration of treatment. Specifically, it was evaluated how the two independent variables (treatment protocols and duration), alone and in combination, influenced serum NT-proBNP concentration changes. The same models were applied for the secondary outcomes, α-antitrypsin, ESM-1, NGAL, fibrinogen, and hs-CRP changes. Statistical analyses were performed using IBM SPSS version 22 Statistical software for Windows[**]. A significant p-value was set as < 0.05.

3 | RESULTS

3.1 | Patient characteristics

At baseline, 143 patients with periodontitis were first screened. Following the first patient selection, a total of 93 patients were finally excluded because they did not fully meet the study criteria ($n = 62$), refused to participate in the study ($n = 23$), or were absent at the periodontal examination ($n = 8$). Based on their periodontal characteristics, patients were categorized as having stage III, grade B periodontitis. Two patients were lost during the follow-up

[‡] Satelec Ultrasonics, Acteon, Varese, Italy
[§] Elecsys ProBNP; Roche, Basel, Swiss
[**] IBM Corporation, Armonk, NY

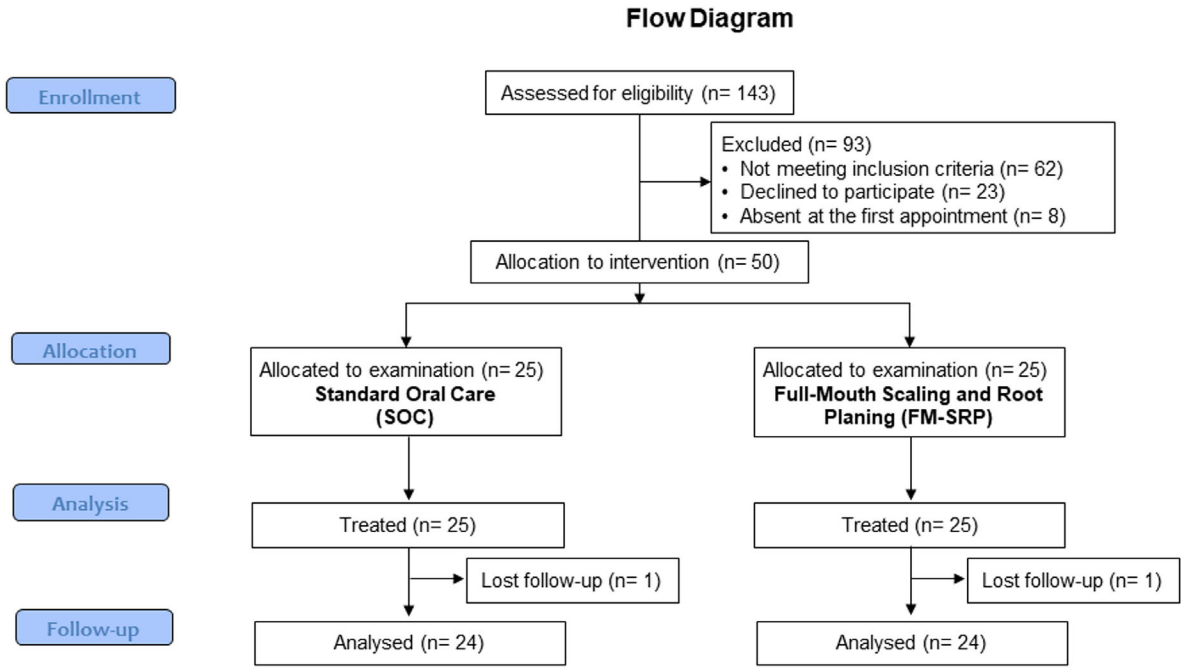

FIGURE 1 Workflow of the study.

TABLE 1 Characteristics of the study sample at baseline.

Characteristics	Control ($n = 24$)	Test ($n = 24$)	p-Value
Male/female, n°	13/11	11/13	0.551
Age, median (IQR)	53 (50.4-56.5)	52 (49.9-55.2)	0.368
Race			0.956
Caucasians, n. (%)	23 (95.8)	23 (95.8)	
Black, n. (%)	1 (4.2)	1 (4.2)	
Education level			0.284
Primary School, n. (%)	13 (54.2)	14 (58.4)	
High School, n. (%)	6 (25)	5 (20.8)	
University, n. (%)	5 (20.8)	5 (20.8)	
BMI (kg/m^2), median, (IQR)	21.4 (18.6-23.5)	21.1 (17.6-22.2)	0.331
Smoking			0.419
Current smokers, n. (%)	1 (4.2)	2 (8.3)	
Former smokers, n. (%)	1 (4.2)	1 (4.2)	
Non-smokers, n. (%)	22 (91.6)	21 (87.5)	
Teeth at baseline median, (IQR)	22 (17.6-23.9)	23 (18.4-24.2)	0.479

Note: Values are reported as frequency, median and IQR, and interquartile range (1st;3rd).
Abbreviations: BMI, body mass index, IQR, interquartile range.

sessions (1 patient per group), and 48 patients were finally analyzed (Figure 1). The patients lost during the follow-up sessions were recalled for periodontal treatment but were excluded from the study.

Both groups of patients were well matched for age ($p = 0.368$), gender ($p = 0.551$), and number of smokers ($p = 0.419$) (Table 1). Regarding the periodontal treatment time, there were no differences among the control (37.4 ± 4.3 min) and test (37.5 ± 2.7 min) groups.

The periodontal characteristics of the study sample are represented in Table 2. In comparison with the control group, the treatment with FM-SRP (test) yielded a significant reduction of mean PD at 3 months (control: 4.22 ± 0.4 mm; test: 3.65 ± 0.5 mm, $p = 0.005$) and at 6 months (control: 3.59 ± 0.4 mm; test: 2.71 ± 0.4 mm, $p < 0.001$) after therapy, and in the percentage of pocket (PD) ≥4 mm (control: 26.2 ± 11.3%; test 17.3 ± 15.6%, $p < 0.001$) at 6 months after therapy. Moreover, FM-SRP

TABLE 2 Periodontal characteristics of the sample at baseline and at each follow-up session.

Variable	Control ($n = 24$)	Test ($n = 24$)	p-Value
PD, mm			
Baseline	4.95 ± 0.3	4.88 ± 0.4	0.662
1 month	4.22 ± 0.4[a]	3.65 ± 0.5[a]	0.012
3 months	3.79 ± 0.3[b,d]	3.42 ± 0.4[b]	0.005
6 months	3.59 ± 0.5[c,f]	2.71 ± 0.4[c]	<0.001
CAL, mm			
Baseline	5.21 ± 0.3	5.15 ± 0.3	0.558
1 month	4.42 ± 0.3[a]	4.09 ± 0.4[a]	0.002
3 months	4.19 ± 0.3[b,d]	3.55 ± 0.3[b]	<0.001
6 months	3.87 ± 0.5[c,f]	2.75 ± 0.3[c]	0.005
% sites with PD ≥4 mm			
Baseline	36.4 ± 13.5	35.8 ± 16.5	0.205
1 month	32.1 ± 17.1[a]	28.4 ± 14.3	0.012
3 months	29.6 ± 13.4[b]	22.9 ± 11.4[b]	0.003
6 months	26.2 ± 11.3[c]	17.3 ± 11.6[c]	<0.001
BOP, %			
Baseline	48.4 ± 15.1	47.2 ± 16.5	0.289
1 month	35.1 ± 16.5[a]	31.6 ± 15.6[a]	0.016
3 months	27.5 ± 18.1[b,d]	22.2 ± 16.1[b,d]	0.002
6 months	24.3 ± 17.1[c]	18.1 ± 15.6[c,e]	<0.001
PI, %			
Baseline	38.6 ± 12.2	39.1 ± 15.4	0.441
1 month	31.2 ± 14.3[a]	23.6 ± 11.2[a]	0.012
3 months	24.7 ± 16.5[b,d]	21.4 ± 10.3[b,d]	0.006
6 months	21.2 ± 15.6[c,f]	17.3 ± 14.2[c,f]	0.011

Note: Values are reported as mean ± standard deviation (SD).
Abbreviations: BOP, bleeding on probing; CAL, clinical attachment loss; PD, probing depth; PI, plaque index.
[a]Significance between baseline and 1 month.
[b]Significance between baseline and 3 months.
[c]Significance between baseline and 6 months.
[d]Significance between 1 month and 3 months.
[e]Significance between 1 month and 6 months.
[f]Significance between 3 months and 6 months.
p-Value significant < 0.008 (Bonferroni's correction).

TABLE 3 Comparisons between analyzed variables at baseline and at each follow-up session.

Variable	Control ($n = 24$)	Test ($n = 24$)	p-Value
NT-proBNP pg/ml			
Baseline	139 ± 0.2	141 ± 0.5	0.069
1 month	132 ± 0.4[a]	121 ± 0.4[a]	0.016
3 months	129 ± 0.5[b,d]	119 ± 0.5[b,d]	0.001
6 months	127 ± 0.4[a]	102 ± 0.4[c,e]	0.004
hs-CRP mg/L			
Baseline	3.69 ± 0.4	3.73 ± 0.5	0.408
1 month	3.59 ± 0.5	3.18 ± 0.3[a]	0.047
3 months	3.45 ± 0.3[b,d]	2.79 ± 0.4[b,d]	0.059
6 months	3.41 ± 0.2[c,e]	2.55 ± 0.5[c,e]	0.003
α−1 antitrypsin mg/dL			
Baseline	143.2 ± 0.15	145.6 ± 0.15	0.228
1 month	140.5 ± 0.2[a]	134.5 ± 0.8[a]	0.003
3 months	138.6 ± 0.3[d]	132.2 ± 0.12[b,d]	0.048
6 months	137.9 ± 0.4[c,f]	128.6 ± 0.7[c,e,f]	0.012
Fibrinogen mg/dL			
Baseline	325.4 ± 11.5	331.5 ± 9.8	0.443
1 month	327.6 ± 10.6[a]	319.5 ± 9.4[a]	0.041
3 months	323.3 ± 12.4[d]	318.2 ± 8.12[a,b]	0.053
6 months	318.4 ± 13.1[c]	315.5 ± 0.5[c,e,f]	0.065
ECM-1 mg/dL			
Baseline	319.5 ± 5.6	317.8 ± 6.2	0.578
1 month	313.5 ± 6.2[a]	306.4 ± 5.8[a]	0.048
3 months	314.2 ± 5.8	301.5 ± 6.1[b]	0.052
6 months	309.4 ± 6.7[c]	292.1 ± 4.9[e,f]	0.014
NGAL (pg/L)			
Baseline	479.6 ± 16.2	477.6 ± 13.5	0.105
1 month	473.5 ± 15.3[a]	467.6 ± 11.2[b]	0.087
3 months	471.5 ± 14.3	461.3 ± 10.2[d]	0.047
6 months	468.6 ± 12.5[c,e]	452.6 ± 11.4[d,e,f]	0.045

Note: Results are expressed as mean and SD (standard deviation).
Abbreviations: ECM-1, endothelial cell-specific molecule-1; NGAL, neutrophil gelatinase-associated lipocalin.
[a]Significance between baseline and 1 month.
[b]Significance between baseline and 3 months.
[c]Significance between baseline and 6 months.
[d]Significance between 1 month and 3 months.
[e]Significance between 1 month and 6 months.
[f]Significance between 3 months and 6 months.
p-Value significant <0.008 (Bonferroni corrections).

significantly reduced CAL ($p = 0.005$), BOP ($p < 0.001$) and PI ($p < 0.001$) at 6 months after therapy (Table 2).

3.2 | Primary outcome

In comparison with the control group, FM-SRP produced, at −3 ($p < 0.001$) and 6 months ($p = 0.004$) after therapy, a significant reduction of the serum NT-proBNP levels (Table 3). Furthermore, the Friedman test evidenced that, in comparison with the control group, the FM-SRP treatment determined a significant reduction, after 3 months of treatment, in α1-antitrypsin ($p = 0.048$) and NGAL ($p = 0.047$) and, after 6 months, in hs-CRP ($p = 0.003$), α1-antitrypsin ($p = 0.012$), ECM-1 ($p = 0.014$), and NGAL ($p = 0.045$) levels (Table 3).

The correlation analysis evidenced that, at 6-month follow-up, there was a positive correlation between serum NT-proBNP and smoking ($p = 0.044$), number of pockets

TABLE 4 Correlation analysis among hs-CRP, NT-proBNP, α−1 antitrypsin, fibrinogen, α−1 antitrypsin, NGAL and the analyzed variables at 6-months of treatment.

	hs-CRP		**NT-proBNP**		**α−1 antitrypsin**	
Variable	***Rs coeff.***	***p-Value***	***Rs coeff.***	***p-Value***	***Rs coeff.***	***p-value***
Age	0.331	0.058	0.058	0.548	0.658	0.528
Sex	0.285	0.225	-0.478	0.317	0.551	0.047
Smoking	0.425	0.057	0.333	0.044	-0.257	0.129
Education	0.332	0.658	-0.258	0.095	0.332	0.256
BMI	-0.158	0.254	0.208	0.158	0.387	0.698
Pocket PD ≥4 mm	0.105	0.046	0.298	0.022	0.147	0.079
No. of teeth	0.208	0.106	0.118	0.031	-0.331	0.098
CAL	0.202	0.055	0.321	0.039	0.287	0.023
BOP	0.306	0.041	0.157	0.258	0.418	0.041
PI	0.155	0.089	-0.357	0.332	-0.277	0.365
	Fibrinogen		**ECM-1**		**NGAL**	
	Rs coeff.	***p*-Value**	**Rs coeff.**	***p*-Value**	**Rs coeff.**	***p-value***
Age	-0.228	0.058	0.114	0.105	-0.236	0.471
Sex	0.158	0.331	0.258	0.022	-0.551	0.265
Smoking	-0.136	0.059	-0.249	0.568	-0.257	0.489
Education	0.228	0.417	-0.258	0.226	-0.251	0.544
BMI	-0.204	0.105	0.196	0.332	-0.176	0.205
Pocket PD ≥4 mm	0.205	0.089	-0.205	0.046	0.368	0.169
No. of teeth	0.332	0.045	0.201	0.031	0.284	0.238
CAL	0.179	0.028	0.442	0.055	0.115	<0.001
BOP	-0.248	0.051	-0.389	<0.001	0.484	0.036
PI	0.236	0.089	0.117	0.065	0.387	0.079

Note: For sex, males served as a reference.

Abbreviations: ECM-1, endothelial cell-specific molecule-1; NGAL, neutrophil gelatinase-associated lipocalin.

with PD ≥4 mm ($p = 0.022$), number of teeth ($p = 0.031$), and BOP ($p = 0.039$); between serum hs-CRP and number of pockets with PD ≥4 mm ($p = 0.046$) and BOP ($p = 0.041$); between serum α−1 antitrypsin and male sex ($p = 0.047$), CAL ($p = 0.023$), and BOP ($p = 0.041$); between fibrinogen and number of teeth ($p = 0.045$) and CAL ($p = 0.028$); between ECM-1 and male sex ($p = 0.022$), PD ≥4 mm ($p = 0.046$), BOP ($p < 0.001$), and number of teeth ($p = 0.031$); between NGAL and CAL ($p < 0.001$) and BOP ($p = 0.036$). The other analyzed variables were not statistically significant (Table 4).

3.3 | Secondary outcome

Finally, the estimation of models with a two-way ANOVA, aimed at identifying the impact of FM-SRP and treatment duration on NT-proBNP, hs-CRP, α−1 antitrypsin, fibrinogen, ECM-1, and NGAL concentration changes after 6 months of treatment, evidence that FM-SRP determined a significant impact on the reduction of serum NT-proBNP ($p < 0.001$), hs-CRP ($p < 0.001$), ECM-1 ($p < 0.001$), and NGAL ($p < 0.001$) together with the timing of treatment for NT-proBNP ($p < 0.001$), hs-CRP ($p < 0.001$), α−1 antitrypsin ($p = 0.015$), ECM-1 ($p = 0.006$), NGAL ($p = 0.021$) concentrations (Table 5).

4 | DISCUSSION

The purpose of the present study was to examine the effect of periodontal therapy on NT-proBNP, hs-CRP, α−1 antitrypsin, fibrinogen, ECM-1, and NGAL and to determine, at 6 months, the impact of periodontal treatment performed either with FM-SRP or OHI, on NT-proBNP, hs-CRP, α−1 antitrypsin, fibrinogen, ECM-1, and NGAL concentration changes. It was also investigated whether analyzed baseline biomarkers concentration levels impacted the long-term efficacy of periodontal treatment. The results of the study evidenced that periodontal treatment performed through FM-SRP determined, at 6-month follow-up, a positive impact on the reduction of the analyzed biomarkers. In the present study, compared to baseline, both treatments determined positive effects in serum NT-proBNP as

TABLE 5 Results of two-way ANOVA for the dependent variable NT-proBNP, hs-CRP, α−1 antitrypsin.

	NT-proBNP		
Source of variation	**MS**	**F**	***p*-Value**
Group	1612.25	275.24	<0.001
Timing	3442.25	541.22	<0.001
Group*Timing	27.89	4.28	0.009
Within	5.41		
	hs-CRP		
Source of variation	**MS**	**F**	***p*-Value**
Group	184.15	141.28	<0.001
Timing	157.15	120.21	<0.001
Group*Timing	6.89	5.41	0.002
Within	1.41		
	α−1 antitrypsin		
Source of variation	**MS**	**F**	***p*-Value**
Group	422.36	148.65	0.106
Timing	161.28	133.36	0.015
Group*Timing	7.28	4.48	0.106
Within	1.66		
	Fibrinogen		
Source of variation	**MS**	**F**	***p*-value**
Group	384.58	189.68	0.236
Timing	166.79	220.21	0.114
Group*Timing	4.77	3.12	0.066
Within	1.12		
	ECM-1		
Source of variation	**MS**	**F**	***p*-Value**
Group	266.28	141.28	<0.001
Timing	147.79	120.21	0.006
Group*Timing	3.66	5.41	0.012
Within	1.79		
	NGAL		
Source of variation	**MS**	**F**	***p*-Value**
Group	384.15	141.28	<0.001
Timing	187.44	442.36	0.021
Group*Timing	14.89	3.36	0.017
Within	4.41		

Abbreviations: F, Fisher test; Group*Timing, interaction term; MS, mean of square.

well as hs-CRP, α−1 antitrypsin, fibrinogen, ECM-1, and NGAL levels. In this context, a number of studies indicate that unbalanced serum NT-proBNP levels might alter the innate host defences against periodontal pathogens during periodontitis.[26] It has been found that bacterial lipopolysaccharide (LPS), during the active stages of periodontitis, can cause overexpression of serum and salivary NT-proBNP levels through a mechanism involving proteolysis product formation.[27] Another evidence showed that patients with periodontitis exhibited elevated amounts of serum NT-proBNP levels, whose expression was strictly associated with the periodontal inflamed surface area of gingival tissues.[15]

In this regard, the statistical difference between treatments of about 1 mm mean of PD or 10% more PD closed pockets are not well appreciated in terms of its clinical importance. A recent meta-analysis comparing the effectiveness of adjuvant systemic antimicrobials to subgingival scaling in RCTs revealed an extra PD decrease of 0.48 mm at a 6-month follow-up following periodontal therapy.[28] The clinical importance question is unanswered when considering pocket closure as the most significant periodontal variable. A recent European Federation of Periodontology workshop considered pocket closure a more relevant clinical result than mean probing depth since shallow sites (4 mm) without >30% hemorrhage are one of the greatest predictors of periodontal stability.[29] This roughly 8% increased percentage of pocket closure does not approach the 14.4% pocket closure that may be attained with greater antibiotic administration.[28] In this regard, some recent studies and meta-analyses,[21,30] which evaluated the different non-surgical approaches, confirmed the previous findings[31] that periodontal treatment positively affects clinical parameters and that there is no great clinical difference between different SRP protocols. Our study may have produced statistically significant results that were more favorable than those of the trials included in these meta-analyses due, in part, to the inclusion of a different number of patients if compared with each other.[21] Suppose a baseline indicates a tiny baseline PD; in that case, the difference between baseline and post-treatment PD is usually equally minimal, even if the probing error may be independent of the degree of the PD.[32] Regarding the elevated serum NT-proBNP and α1-antitrypsin levels found in the present study, the upregulation of these biomarkers has also been showed to be connected with the activation of the tumor necrosis factor-alpha pathway, being overexpressed in PD patients compared to healthy controls.[26] Therefore, the observed decrease in serum NT-proBNP and α1-antitrypsin, ECM-1, and NGAL levels 6 months after FM-SRP suggests that this approach successfully determines a more efficacious degradation of the bacterial biofilm and a related better host response.

Several pieces of evidence reported that both NT-proBNP,[33] α1-antitrypsin,[34] ECM-1,[35] and NGAL[18] upregulated levels were involved in controlling the inflammatory process and alveolar bone homeostasis and resorption and that the expression of both ECM-1 and NGAL has been linked to changes in bone homeostasis and osteoclast differentiation.[36] During CVD, the overexpression of serum NT-proBNP, hs-CRP, and ECM-1 levels have been associated with the reduction of endothelial dysfunction and high risk of CVD, corroborating the findings

of the present investigation.[37] The NT-proBNP and hs-CRP levels, often used as systemic inflammatory markers, have also been shown to act as a regulator in the early immune response during the early stages of coronary artery disease[38] and periodontitis.[39]

The results of the present study revealed that FM-SRP substantially influenced serum NT-proBNP, hs-CRP, and CVD-related biomarkers at 6 months of treatment. Furthermore, both ANOVA and Spearman correlation analyses highlighted that there was a significant association between the reduction of periodontal parameters and the reduction of biomarkers analyzed by CVD and that the high baseline levels of NT-proBNP and hs-CRP, ECM-1, and NGAL positively influenced the effect of periodontal treatment at 6-month follow-up. From this analysis, it can be assumed that the reduction of NT-proBNP, hs-CRP, ECM-1, and NGAL at 6-month follow-up was influenced by the periodontal treatment and positively influenced the efficacy of periodontal treatment. In agreement, it has been previously shown that, in patients with periodontitis, the periodontal treatment performed through FM-SRP triggers a short-term inflammatory response, leading to a progressive and consistent reduction in systemic inflammation and an improvement in endothelial functions.[40] The present study findings also agree with a trial on 120 patients with severe periodontitis that showed how causative periodontal therapy prompted a short-term acute systemic inflammation mediator such as hs-CRP, interleukin-6, and E-selectin, with a final positive impact on the overall endothelial function.[41] In some preclinical trials, the significant reduction of NT-proBNP,[42] ECM-1,[35] and NGAL[43] was associated at the end of periodontal treatment with improvement in cardiac and diastolic function in patients with periodontitis through a mechanism strictly involved with oxidative stress through a pathway involving nitric oxide.

However, the present study has some limitations, such as the number of included patients. For this analysis, a larger sample would have been useful for better identification of the clinical and serum differences of NT-proBNP and related CVD biomarkers between the different treatment groups. The monocentric design and the ratings for plaque and bleeding at 6 months were still rather high, and a longer monitoring period would be desirable better to determine the differential clinical results for stable periodontal outcomes.

5 | CONCLUSION

The results of the present study indicated that both periodontal treatments were beneficial in all treated patients. However, the periodontal treatment performed with FM-SRP was more efficacious in reducing clinical parameters and serum NT-proBNP and related early CVD risk biomarkers in patients with periodontitis at 6-month follow-up performed with FM-SRP. Subjects who harbored high NT-proBNP concentrations at baseline showed greater clinical benefits of periodontal treatment at 6-month follow-up. The results of this study are still preliminary and demand further studies to better understand the advantages of non-surgical periodontal treatment procedures on serum NT-proBNP and CVD-related biomarker levels.

AUTHOR CONTRIBUTIONS

Gaetano Isola and Vincenzo Iorio-Siciliano planned, and performed the experimental procedures and wrote the manuscript. Gaetano Isola, Gianluca Martino Tartaglia, Alessandro Polizzi, and Simona Santonocito performed the procedure. Ray C. Williams validated the experimental results and revised the manuscript. Gaetano Isola conceived the research and received the funds. This manuscript is dedicated to the lovely memory of Prof. *Ray C. Williams*, who was directly involved in this research and was a guide for all co-authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

1. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(1):S159-S172. Suppl.
2. Isola G, Polizzi A, Alibrandi A, Williams RC, Lo Giudice A. Analysis of galectin-3 levels as a source of coronary heart disease risk during periodontitis. *J Periodontal Res*. 2021;56:597-605.

3. Isola G, Polizzi A, Santonocito S, Alibrandi A, Williams RC. Periodontitis activates the NLRP3 inflammasome in serum and saliva. *J Periodontol*. 2022;93:135-145.
4. Ye C, You M, Huang P, et al. Clinical study showing a lower abundance of Neisseria in the oral microbiome aligns with low birth weight pregnancy outcomes. *Clin Oral Investig*. 2022;26:2465-2478.
5. Li Y, Lu Z, Zhang L, et al. Inhibition of acid sphingomyelinase by imipramine abolishes the synergy between metabolic syndrome and periodontitis on alveolar bone loss. *J Periodontal Res*. 2022;57:173-185.
6. Fileto Mazzonetto AL, Casarin RCV, Santamaria MP, et al. Clinical, radiographic, and patient-centered outcomes after use of enamel matrix proteins for the treatment of intrabony defects in patients with aggressive periodontitis: a 12-month multicenter clinical trial. *J Periodontol*. 2021;92:995-1006.
7. Cosgarea R, Tristiu R, Dumitru RB, et al. Effects of non-surgical periodontal therapy on periodontal laboratory and clinical data as well as on disease activity in patients with rheumatoid arthritis. *Clin Oral Investig*. 2019;23:141-151.
8. Isola G, Santonocito S, Distefano A, Polizzi A, Vaccaro M, Raciti G, Alibrandi A, Li Volti G. Impact of periodontitis on gingival crevicular fluid miRNAs profiles associated witjh cardiovascular disease risk. *J Periodontal Res*. 2023;58:165-174
9. Castiglione V, Aimo A, Vergaro G, Saccaro L, Passino C, Emdin M. Biomarkers for the diagnosis and management of heart failure. *Heart Fail Rev*. 2022;27:625-643.
10. Davis ME, Richards AM, Nicholls MG, Yandle TG, Frampton CM, Troughton RW. Introduction of metoprolol increases plasma B-type cardiac natriuretic peptides in mild, stable heart failure. *Circulation*. 2006;113:977-985.
11. Brazile BL, Butler JR, Patnaik SS, et al. Biomechanical properties of acellular scar ECM during the acute to chronic stages of myocardial infarction. *J Mech Behav Biomed Mater*. 2021;116:104342.
12. Vaduganathan M, Claggett B, Packer M, et al. Natriuretic peptides as biomarkers of treatment response in clinical trials of heart failure. *JACC Heart Fail*. 2018;6:564-569.
13. Staszewsky L, Meessen J, Novelli D, et al. Total NT-proBNP, a novel biomarker related to recurrent atrial fibrillation. *BMC Cardiovasc Disord*. 2021;21:553.
14. Gustafsson N, Ahlqvist J, Naslund U, et al. Associations among periodontitis, calcified carotid artery atheromas, and risk of myocardial infarction. *J Dent Res*. 2020;99:60-68.
15. Leira Y, Blanco J. Brain natriuretic peptide serum levels in periodontitis. *J Periodontal Res*. 2018;53:575-581.
16. Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H. The effect of periodontal treatment on serum leptin, interleukin-6, and C-reactive protein. *J Periodontol*. 2010;81:1118-1123.
17. Tayman MA, Onder C, Kurgan S, Serdar MA, Gunhan M. Endocan (ESM-1) levels in gingival crevicular fluid correlate with ICAM-1 and LFA-1 in periodontitis. *Braz Oral Res*. 2020;35:e005.
18. Tan A, Gurbuz N, Ozbalci FI, Koskan O, Yetkin Ay Z. Increase in serum and salivary neutrophil gelatinase-associated lipocalin levels with increased periodontal inflammation. *J Appl Oral Sci*. 2020;28:e20200276.
19. Lopez NJ, Quintero A, Casanova PA, Ibieta CI, Baelum V, Lopez R. Effects of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: a controlled clinical trial. *J Periodontol*. 2012;83:267-278.
20. Babaloo A, Rahbar M, Babaloo Z, Ghasemi S, Amini A. Evaluation of clinical periodontal indices and serum interleukin-27 by one-stage full-mouth disinfection and quadrant scaling and root planing in periodontitis. *J Contemp Dent Pract*. 2018;19:997-1004.
21. Suvan J, Leira Y, Moreno Sancho FM, Graziani F, Derks J, Tomasi C. Subgingival instrumentation for treatment of periodontitis. A systematic review. *J Clin Periodontol*. 2020;47(22):155-175. Suppl.
22. Quirynen M, Bollen CM, Vandekerckhove BN, Dekeyser C, Papaioannou W, Eyssen H. Full- vs. partial-mouth disinfection in the treatment of periodontal infections: short-term clinical and microbiological observations. *J Dent Res*. 1995;74:1459-1467.
23. Iorio-Siciliano V, Blasi A, Isola G, Sculean A, Salvi GE, Ramaglia L. Resolution of peri-implant mucositis at tissue- and bone-level implants: a 6-month prospective controlled clinical trial. *Clin Oral Implants Res*. 2023;34:450-462.
24. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2012;10:28-55.
25. Isola G, Polizzi A, Alibrandi A, Williams RC, Leonardi R. Independent impact of periodontitis and cardiovascular disease on elevated soluble urokinase-type plasminogen activator receptor (suPAR) levels. *J Periodontol*. 2021;92:896-906.
26. Czerniuk MR, Bartoszewicz Z, Filipiak KJ, Dudzik-Niewiadomska I, Pilecki T, Gorska R. Plasmatic NT-proBNP concentrations in patients with coexistent periodontal disease and congestive heart failure: pilot studies. *Kardiol Pol*. 2017;75:135-142.
27. Sosnin DY, Gileva OS, Mozgovaia LA, et al. The NT-proBNP in saliva and blood serum in norm and under periodontitis. *Klin Lab Diagn*. 2018;63:164-168.
28. Teughels W, Feres M, Oud V, Martin C, Matesanz P, Herrera D. Adjunctive effect of systemic antimicrobials in periodontitis therapy: a systematic review and meta-analysis. *J Clin Periodontol*. 2020;47(22):257-281. Suppl.
29. Loos BG, Needleman I. Endpoints of active periodontal therapy. *J Clin Periodontol*. 2020;47(22):61-71. Suppl.
30. Jervoe-Storm PM, Eberhard J, Needleman I, Worthington HV, Jepsen S. Full-mouth treatment modalities (within 24 hours) for periodontitis in adults. *Cochrane Database Syst Rev*. 2022;6:CD004622.
31. Eberhard J, Jepsen S, Jervoe-Storm PM, Needleman I, Worthington HV. Full-mouth treatment modalities (within 24 hours) for chronic periodontitis in adults. *Cochrane Database Syst Rev*. 2015; CD004622.
32. Grossi SG, Skrepcinski FB, DeCaro T, Zambon JJ, Cummins D, Genco RJ. Response to periodontal therapy in diabetics and smokers. *J Periodontol*. 1996;67:1094-1102.
33. Vedin O, Hagstrom E, Ostlund O, et al. Associations between tooth loss and prognostic biomarkers and the risk for cardiovascular events in patients with stable coronary heart disease. *Int J Cardiol*. 2017;245:271-276.
34. Li L, Wang M, Bao J, et al. Periodontitis may impair the homeostasis of systemic bone through regulation of gut microbiota in ApoE(-/-) mice. *J Clin Periodontol*. 2022;49:1304-1319.
35. Turer CC, Durmus D, Balli U, Guven B. Effect of non-surgical periodontal treatment on gingival crevicular fluid and serum

endocan, vascular endothelial growth factor-A, and tumor necrosis factor-alpha levels. *J Periodontol*. 2017;88:493-501.
36. Kumar G, Ponnaiyan D, Parthasarathy H, Tadepalli A, Veeramani S. Evaluation of endocan and tumor necrosis factor-alpha as inflammatory biomarkers in type 2 diabetes and periodontal disease. *Genet Test Mol Biomarkers*. 2020;24:431-435.
37. Giannoni A, Tani C, Clerico A, et al. When the heart is burning: amino-terminal pro-brain natriuretic peptide as an early marker of cardiac involvement in active autoimmune rheumatic disease. *Int J Cardiol*. 2011;148:161-167.
38. Brozaitiene J, Mickuviene N, Podlipskyte A, Burkauskas J, Bunevicius R. Relationship and prognostic importance of thyroid hormone and N-terminal pro-B-Type natriuretic peptide for patients after acute coronary syndromes: a longitudinal observational study. *BMC Cardiovasc Disord*. 2016;16:45.
39. Loo WY, Yue Y, Fan CB, et al. Comparing serum levels of cardiac biomarkers in cancer patients receiving chemotherapy and subjects with chronic periodontitis. *J Transl Med*. 2012;10(1):S5. Suppl.
40. Graziani F, Gennai S, Marruganti C, et al. Acute-phase response following one-stage full-mouth versus quadrant non-surgical periodontal treatment in subjects with comorbid type 2 diabetes: a randomized clinical trial. *J Clin Periodontol*. 2023; 50:487-499.
41. Tonetti MS, D'Aiuto F, Nibali L, et al. Treatment of periodontitis and endothelial function. *N Engl J Med*. 2007;356:911-920.
42. Wang Y, Liu HN, Zhen Z, et al. A randomized controlled trial of the effects of non-surgical periodontal therapy on cardiac function assessed by echocardiography in type 2 diabetic patients. *J Clin Periodontol*. 2020;47:726-736.
43. Ceylan M, Erbak Yilmaz H, Narin F, Tatakis DN, Saglam M. Gingival crevicular fluid lipocalin-2 and semaphorin3A in stage III periodontitis: non-surgical periodontal treatment effects. *J Periodontal Res*. 2022;57:724-732.

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