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One-year follow-up of the immune profile in serum and selected sites of generalized and localized aggressive periodontitis

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ABSTRACT

Background: The local and systemic immunological profiles of important inflammatory mediators in the localized (LAgP) and generalized (GAgP) forms of aggressive periodontitis are still unknown, as well as the effect of periodontal therapy on these parameters. The aim of this prospective study was to evaluate clinical and immune responses of patients with AgP undergoing nonsurgical treatment.

Material and methods: Eighteen patients with GAgP, 10 with LAgP and 10 healthy participants were included in this study. AgP participants were submitted to scaling and root planing plus systemic antibiotics (amoxicillin and metronidazole). At baseline and 1-year follow-up were measured clinical parameters, such as probing depth [PD] and clinical attachment loss [CAL], and the levels of 10 immunological mediators (GM-CSF, M-CSF, MCP-1, ICAM-1, CXCL8, IL-1β, TNF-α, IL-17, IL-4, and IL-10) in the gingival crevicular fluid (GCF) of selected sites [AgP forms: PD ≥ 6 mm or the deepest, bleeding on probing (BoP) and bone loss measured by periapical radiography; healthy individuals: PD ≤ 3 mm, no BoP, no bone loss] and serum.

Results: After periodontal treatment both forms of AgP presented a significant reduction of PD and CAL, an increase of GM-CSF, ICAM-1, MCP-1, TNF-α, IL-17, IL-4, and IL-10 in the GCF, as well as of GM-CSF and IL-4 in the serum, and a reduction in the serum concentration of IL-1β. Serum levels of M-CSF, ICAM-1, and MCP-1 remained significantly below those found in healthy individuals in both forms of AgP even after therapy. An increase in the systemic or local levels of MCP-1, ICAM-1 and the anti-inflammatory profile (IL-4, IL-10) was correlated with an improvement in clinical parameters of LAgP patients. Also, a local reduction of IL-1β levels in both forms of AgP was correlated with an increase in the clinical attachment gain.

Conclusion: Nonsurgical periodontal therapy was successful in improving clinical parameters and modulating the immune response in both forms of AgP. However, this therapeutic approach does not seem to affect the deficient level of important serum mediators involved in mechanisms of cell transmigration.

1. Introduction

Since 1999 the International Workshop for a Classification of Periodontal Diseases and Conditions has defined aggressive periodontitis (AgP) as a periodontal disease that occurs in systemically healthy individuals presenting rapid clinical attachment loss and alveolar bone resorption with familial aggregation of cases [1]. Besides conceptualization, this disease was also classified in localized (LAgP) and generalized (GAgP) forms according to its extent. In this sense, LAgP has been described as the form involving no more than two teeth besides the first molars and incisors, presenting a circumpubertal onset and a robust serum antibody response against periodontopathogens. Meanwhile, GAgP usually affects subjects under 30 years of age presenting generalized interproximal attachment loss and a poor serum response [1,2].

Systematic reviews have highlighted that scaling and root planing (SRP) associated with systemic antibiotic therapy with amoxicillin and metronidazole would be effective in treating biofilm-induced chronic inflammatory diseases, such as AgP forms [3–5], but little evidence has considered whether both LAgP and GAgP subtypes have similar response to this therapy. In fact, the response to periodontal treatment in the AgP is much less understood when compared to chronic

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periodontitis, despite the fact that this disease affects 2.4–6.1% of people between 14 and 29 years in the South America [6].

Furthermore, sparse data indicate that both AgP forms are distinguished entities by the immune response [2,7–9], although a definitive conclusion is still lacking. Also, some studies have pointed out that patients with LAgP may present a hyper-responsive phenotype that would impair clinical outcomes [10,11], differently of the generalized form in which the main factor responsible to impact on periodontal parameters could be the ability of certain pathogens to induce a deranged immune profile [7,12,13].

In our previous study evaluating the percentage of subgingival levels of periodontopathogens in selected sites (deepest pockets) and the serum IgG antibody levels against them, we reported that a difference in the host immune defense between the two forms of AgP may exist [14]. However, we hold that is necessary to analyze the inflammatory profile in these pockets and in the serum of such individuals, as well as mediators involved in mechanisms of cell transmigration, which appear to be altered in both forms of AgP [15–17].

Therefore, this prospective study was performed to evaluate immunological profiles and periodontal clinical parameters of patients with LAgP and GAgP undergoing SRP plus antibiotic therapy.

2. Material and methods

2.1. Study population

AgP participants were recruited from the population referred to the Periodontal Clinic at School of Dentistry - University of São Paulo (FOUSP, São Paulo, Brazil), between August 2010 and November 2011. All eligible participants were thoroughly informed of nature, potential risks and benefits of their participation in the study and signed a Term of Informed Consent. This prospective study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000, and its protocol was reviewed and approved by the Research Ethics Committee at FOUSP (Protocols 155/2010 and 350.873/2013).

2.2. Inclusion and exclusion criteria

All AgP participants (18 GAgP; 10 LAgP) were classified according to the clinical criteria suggested by the International Workshop for a Classification of Periodontal Diseases and Conditions [1,2] and previous studies [18,19], as follows:

LAgP: < 35 years of age; interproximal attachment loss (> 4mm) on at least two permanent teeth, one of which is a first molar, and involving no more than two teeth other than incisors and first molars, with alveolar bone loss confirmed by periapical radiography; familial aggregation (at least 1 other member of the family presenting or with a history of periodontitis); and systemically healthy individuals.

GAgP: < 35 years of age; generalized interproximal attachment loss (> 4mm) affecting at least three permanent teeth other than the first molars and incisors, with alveolar bone loss confirmed by periapical radiography; familial aggregation (at least 1 other member of the family presenting or with a history of periodontitis); and systemically healthy individuals.

Periodontally healthy participants (10 controls) were also recruited from the population referred to the School of Dentistry, University of Sao Paulo, and the inclusion criteria for this group were as follows: (1) lack of sites with probing depth (PD) and clinical attachment loss (CAL) > 3 mm; (2) Bleeding on probing (BoP) < 10%; (3) no caries or extensive restoration; (4) at least 28 permanent teeth.

The exclusion criteria for all groups were as follows: (1) subgingival periodontal therapy or antibiotic treatment in the previous 6 months; (2) systemic diseases that could affect the progression of periodontitis (e.g. diabetes and immunological disorders); (3) pregnant or lactating; (4) smokers.

2.3. Clinical measurements

At baseline and 1-year after periodontal treatment, the following clinical parameters were measured: bleeding on probing (BoP) was recorded based on the presence or absence of bleeding up to 30 s after probing; probing depth (PD) was measured as the distance (in millimeters) from the free gingival margin to the bottom of the pocket; gingival recession (GR) was measured as the distance from the cemento-enamel junction to the free gingival margin; clinical attachment loss (CAL) was calculated measured as PD plus GR. All parameters were obtained at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesio-lingual), except for third molars, by using a North Carolina probe (Hu-Friedy®, Chicago, USA). Mean (standard-deviation) for both primary clinical parameters (PD and CAL) was calculated by full-mouth and affected sites. Measurement reproducibility was calculated by intra-class correlation coefficient for PD (ICC = 0.86) and CAL (ICC = 0.85), in two separate examinations.

2.4. Periodontal treatment protocol

All participants with AgP were submitted to the same treatment according to previous studies [5,20]. Briefly, at the baseline appointment, patients received full-mouth supragingival scaling and instructions on proper home-care techniques. One week after this procedure, they were recalled to undergo ultrasonic quadrant debridement + site-specific scaling and root planing with manual curettes (Hu-Friedy®, Chicago, USA). Immediately after treatment, they were prescribed a regimen of 400 mg of metronidazole and 500mg of amoxicillin, 3 times per day for 7 days. All patients received oral hygiene instructions and an accompanying oral care kit consisting of a toothbrush, toothpaste, dental floss, and interproximal brushes as needed. Participants were requested to be present at 3, 6, 9 and 12 months appointments after baseline for re-examination, at which they received additional full mouth supra-subgingival debridement with an ultrasonic device, scaling and root planning of remaining sites with PD > 4 mm, as needed, and oral hygiene instructions. Patients did not receive additional antibiotics at follow-up visits.

2.5. Sample collection

2.5.1. Gingival crevicular fluid (GCF)

GCF was collected after site isolation with cotton rolls and supragingival biofilm removal of selected sites. A periodontally diseased site [PD ≥ 6 mm or the worst PD, with BoP and bone loss measured by periapical radiography] was sampled from each AgP participant [14], and a healthy site [PD ≤ 3 mm, no BoP, no bone loss] was sampled from healthy individuals. The same site was resampled after 1-year follow-up of AgP participants. Filter paper strips (Periopaper®, Interstate Drug Exchange, USA) were inserted 1–2 mm into the sulcus/pocket of selected sites for approximately 30 s. After collection, the strips were placed in empty microcentrifuge tubes and stored at −80 °C.

2.5.2. Serum sampling

Peripheral blood samples (5 mL) were taken from the antecubital vein of all individuals by a standard venipuncture method and collected in vacuum tubes (BD vacutainer®, Becton, Dickson and Company, Brazil). Venous blood samples were centrifuged at 1500*g* for 10 min. Separated serum samples were collected into microcentrifuge tubes and stored at −80 °C.

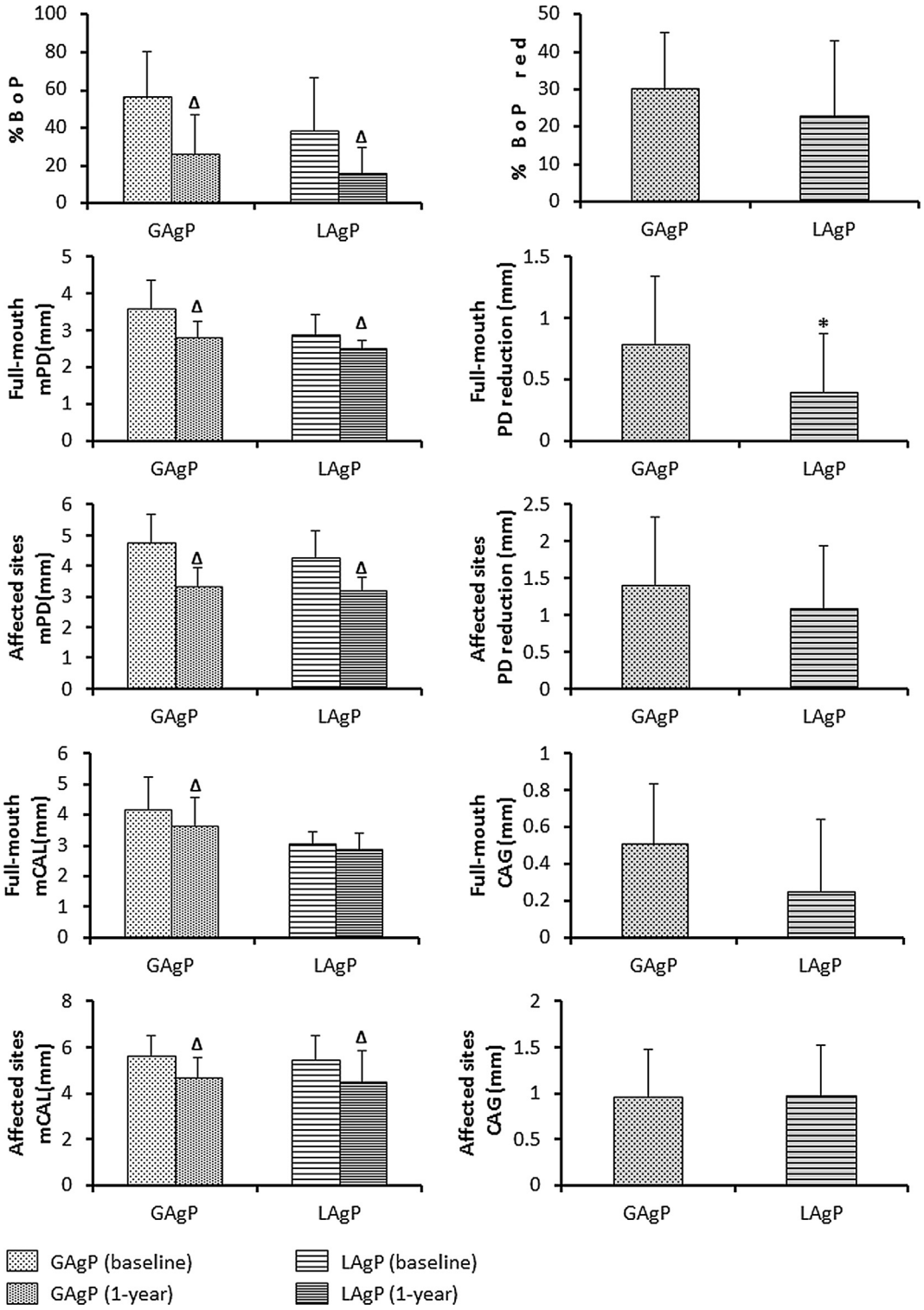

Fig. 1. Clinical parameters of LAgP and GAgP at baseline and 1-year follow-up. %BoP, Mean of bleeding on probing; Affected sites/Full-mouth mPD, mean of probing depth in diseased sites or full-mouth; Affected sites/Full-mouth mCAL, mean of clinical attachment loss in diseased sites or full-mouth; %BoP red, reduction of bleeding on probing ($BoP_{baseline} - BoP_{1year}$); PD reduction, reduction in the mean of PD ($PD_{baseline} - PD_{1year}$); CAG, clinical attachment gain ($CAL_{baseline} - CAL_{1year}$). (Δ) A significant intra-group difference using Wilcoxon signed-rank test; (*) A significant inter-group difference using Mann-Whitney U Test. A significance level of 0.05 was established for all tests.

2.6. Quantification of cytokines/chemokines

Before the assay, GCF samples were eluted from the strip into 300 μL of phosphate buffered saline (PBS). After being centrifuged at 400g for 4 min, the strips were removed and total protein content of the eluate GCF was determined by BCA protein assay. This quantification was also performed with the serum samples.

Multiplex cyto/chemokine detection kit (Millipore, St. Charles, MO, USA) was used according to the manufacturer's instructions for the detection and quantitation of 10 mediators (granulocyte-macrophage

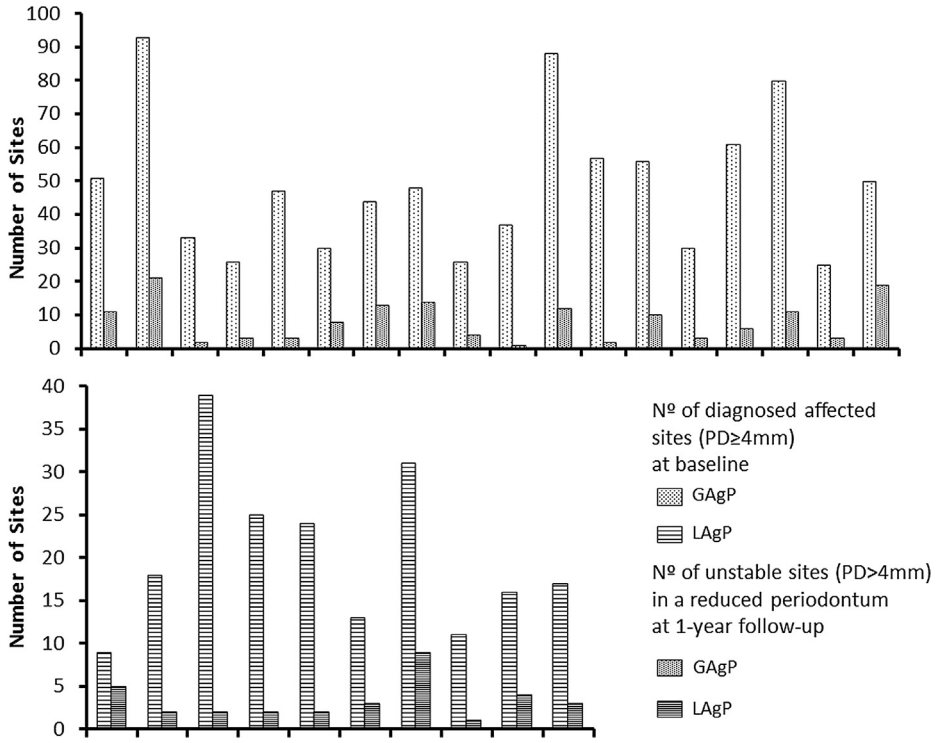

Fig. 2. Number of sites for each GAgP (N = 18) or LAgP (N = 10) patient with PD > 4 mm at baseline (affected sites at diagnosis) and PD ≥ 4 mm after 1-year follow-up (unstable sites in a reduced periodontum after therapy). All PD measurements assuming no pseudo pockets.

colony-stimulating factor [GM-CSF], macrophage colony-stimulating factor [M-CSF], monocyte chemotactic protein-1 [MCP-1], intercellular adhesion molecule-1 [ICAM-1], C-X-C motif chemokine ligand 8 (CXCL8), Tumor Necrosis Factor-α [TNF-α], interleukins [IL-1β, IL-17, IL-4, IL-10]).

Data were acquired using a multiplex assay suspension array instrument (Luminex Bio-plex®200 Suspension Array System, Bio-Rad, USA). The concentrations of the cyto/chemokines in the GCF and serum samples were estimated from a standard curve using a third-order polynomial equation and the GraphPad Prism 5 software and expressed as pg/ml normalized to total protein content from each sample. Samples below the detection limit of the assay were recorded as zero, while samples above the upper limit of quantification of the standard curves were assigned the highest value of the curve.

2.7. Statistical analysis

The sample size calculation was based on data from a pilot study designed to assess whether there was a difference between Control, GAgP and LAgP groups in relation to the level of IL-1β levels in the GCF. Considering a minimum difference of 5 pg/mL to be detected between groups, a power of 80% to detect this difference, level of significance of 5%, and a standard deviation of 3 pg/mL, a minimum of 9 individuals per group would be necessary.

Min-Max normalization was applied to reduce inter-participant variability in mediator's levels and in order to outline chemokine, pro-inflammatory and anti-inflammatory scores. So, the absolute concentration of each mediator was normalized, such that the highest value from each one was given 1 and the other values were a proportion of it [21]. After normalization, immunological scores were computed: GM-CSF, M-CSF, MCP-1, ICAM-1, and CXCL8 were evaluated together to compute a Chemokine score; IL-1β, TNF-α, and IL-17 computed a pro-inflammatory score; IL-4 and IL-10 were added up to an anti-inflammatory score.

The patient was maintained as the unit of measurement and statistical analyses were performed with GraphPad Prism 5 software. Data of quantitative variables were tested for normality using Kolmogorov–Smirnov test with Lilliefors correction. Mann–Whitney *U* test was employed to evaluate inter-group differences and Wilcoxon signed-rank test was used to assess intra-group differences in relation to the clinical parameters. Kruskal–Wallis test with Dunn's multiple comparisons was used to assess inter-group differences in immunological mediators. A significance level of 0.05 was established for all tests. Correlation analysis between clinical parameters [BoP, PD, and CAG] and immunological levels were examined using Spearman's correlation coefficients [strong correlation ($r > 0.60$, $p < 0.01$), moderate correlation ($0.4 < r < 0.59$, $p < 0.05$)] [21].

3. Results

3.1. Demographic data

All participants selected at baseline completed the study, so there was no withdrawal or participant removal. Ten (9 males/1 female), 18 (15 males/3 females), and 10 (6 males/4 females) participants addressed the Control, GAgP, and LAgP groups, respectively. Participants' mean age (in years) were 22.1 ± 1.7, 28.4 ± 3.9, and 25.6 ± 7.8 for the Control, GAgP, and LAgP, respectively. A significant difference was found only between Control and GAgP in relation to the mean age ($p = 0.023$).

3.2. Clinical parameters

There was a significant reduction ($p < 0.001$) in the mean BoP, mean PD (for full-mouth and affected sites) and mean CAL for affected sites in both LAgP and GAgP groups from baseline to 1-year follow-up (Fig. 1). A greater reduction in PD was observed in participants with GAgP ($p = 0.033$) when the mean full-mouth was used, but this difference between GAgP and LAgP was not observed when only affected sites were measured ($p = 0.125$). Possibly this difference was related to a data dilution (mean of PD reduction) when a full-mouth analysis is used for a localized periodontal disease. In addition, no differences

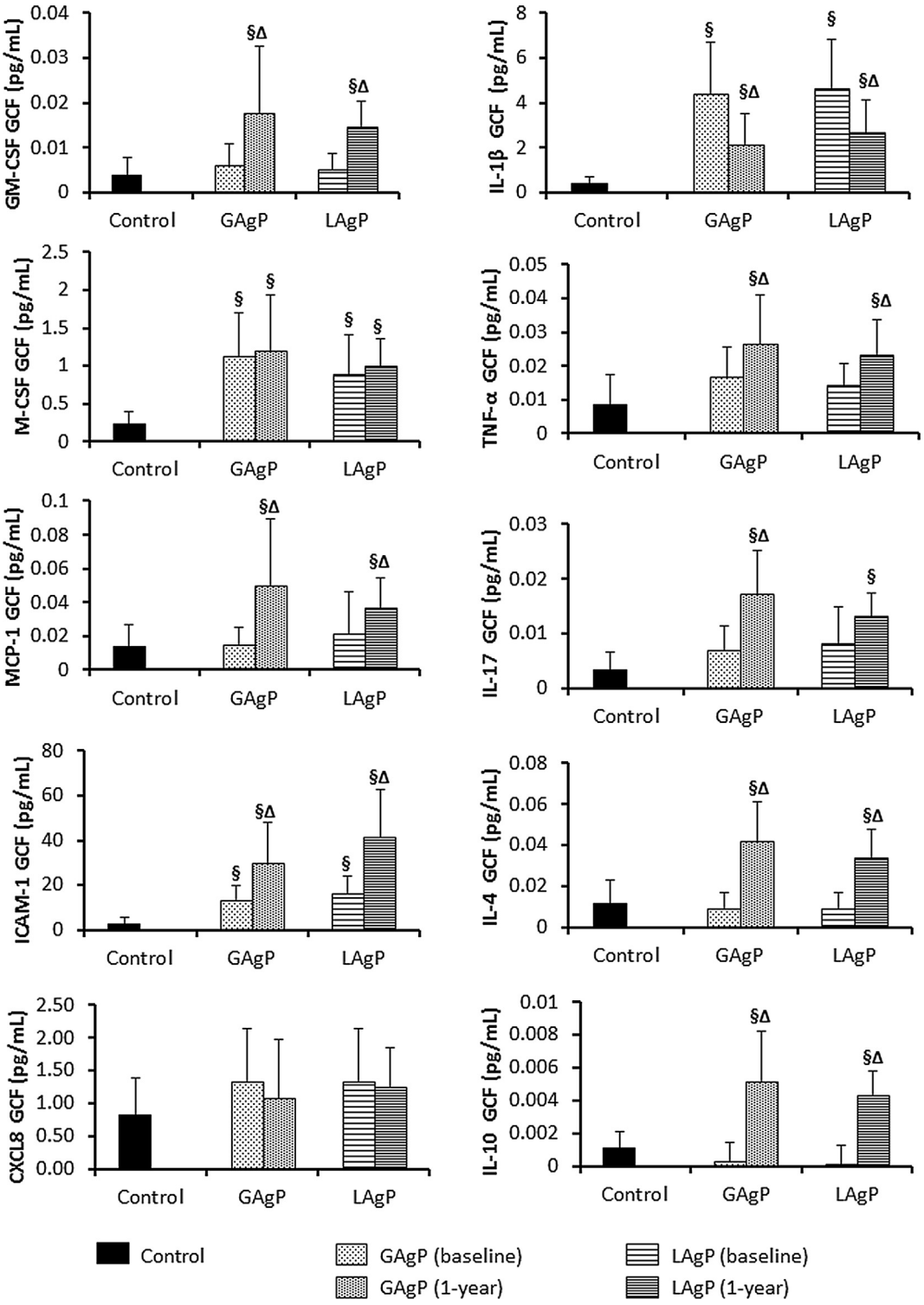

Fig. 3. Chemo/Cytokine profiles in the GCF of selected sites [AgP forms: PD ≥ 6 mm or the worst PD, BoP and bone loss measured by periapical radiography; Healthy individuals: PD ≤ 3 mm, no BoP, no bone loss] in controls and patients with both forms of AgP, at baseline and after periodontal treatment (SRP plus systemic antibiotics) with 1-year follow-up. (Δ) A significant intra-group difference using Wilcoxon signed-rank test; (§) A significant difference in relation to healthy controls using Kruskal-Wallis test with Dunn's multiple comparison. A significance level of 0.05 was established for all tests.

were observed between patients with GAgP and LAgP in relation to the mean of clinical attachment gain (CAG) and percentage of reduction of BoP ($p > 0.05$) (Fig. 1).

When assessing the relation of the sites affected at the baseline (PD ≥ 4 mm) and those that still remain unstable and with risk of progression in a reduced periodontium after treatment (PD > 4 mm) [22], we observed that only 16.2% (± 10) remained unstable in patients with GAgP and 19.4% (± 16) in patients with LAgP, with no significant difference in the therapeutic effectiveness between both forms of AgP. The number of sites affected (baseline) and unstable (1-year follow-up) for each patient were listed in Fig. 2.

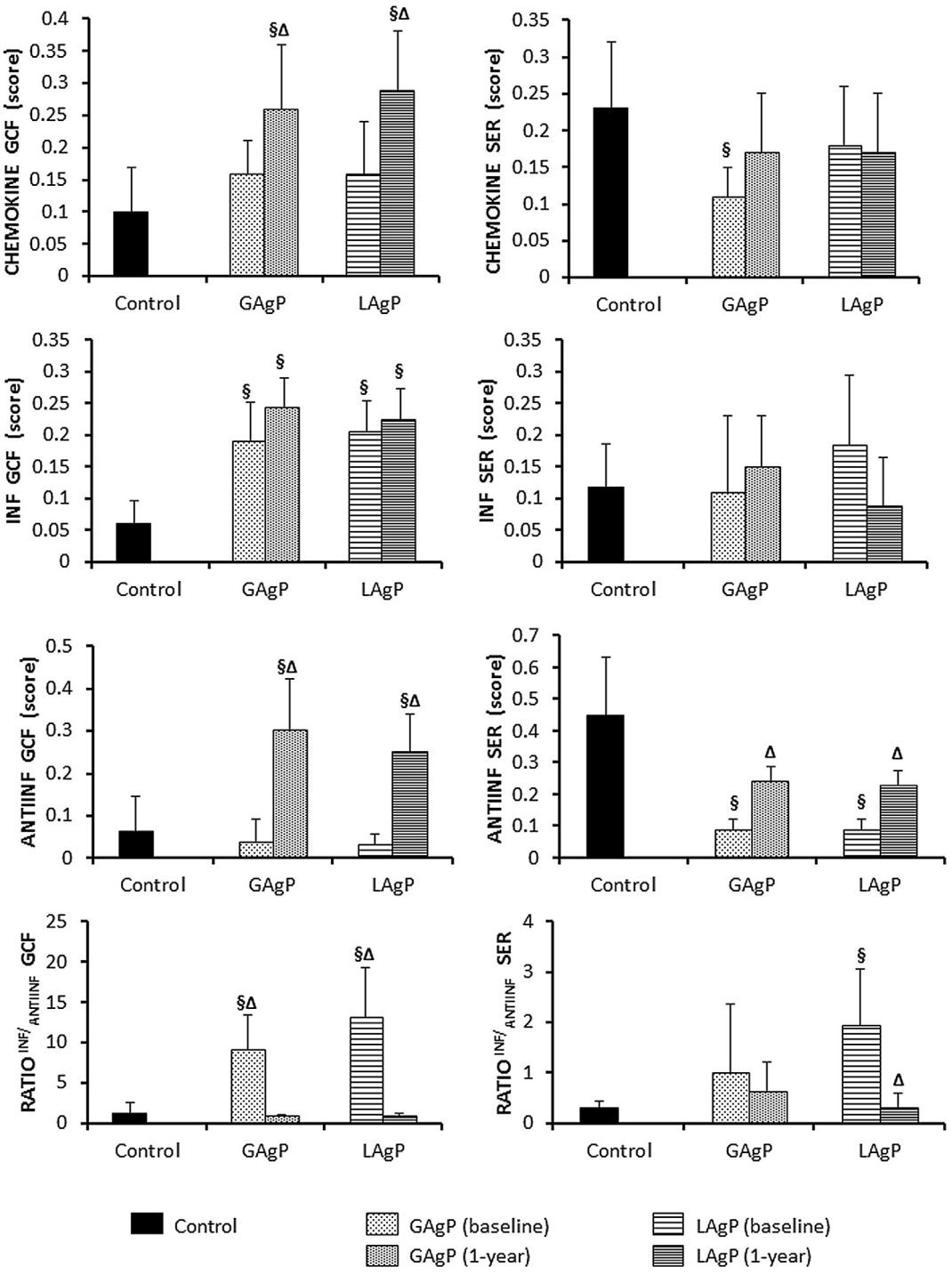

Fig. 4. Immunological profiles in the GCF of selected sites and serum (SER) of controls and AgP patients at baseline and 1-year follow-up. Min-max normalization was performed for each mediator to establish each profile. CHEMOKINE score computed GM-CSF, M-CSF, MCP-1, ICAM-1, and CXCL8 levels; INF score, inflammatory score added IL-1β, TNF-α, and IL-17 levels. ANTIINF score, anti-inflammatory score computed IL-4 and IL-10 levels. (Δ) A significant intra-group difference using Wilcoxon signed-rank test; (§) A significant difference in relation to healthy controls using Kruskal-Wallis test with Dunn's multiple comparison. A significance level of 0.05 was established for all tests.

3.3. Profile of immune mediators in the GCF

At baseline, increased levels of M-CSF, ICAM-1, and IL-1β were observed in both forms of AgP when compared to healthy controls ($p < 0.001$), which was also observed even after periodontal treatment and 1-year follow-up (Fig. 3). Subsequently, ICAM-1 levels increased significantly in relation to baseline in both groups with AgP ($p = 0.0012$), whereas the amount of IL-1β was reduced ($p < 0.01$). Conversely, high levels of GM-CSF, MCP-1, TNF-α, IL-17, IL-4, and IL-10 were observed in GAgP and LAgP only after treatment.

Considering the overall effect on mediator levels, in both AgP forms was observed that GCF of selected pockets changed from a baseline chemokine score (GM-CSF, M-CSF, MCP-1, ICAM-1, CXCL8) similar to healthy controls ($p = 0.102$) to significantly higher levels after 1-year follow-up ($p = 0.0001$). On the other hand, there was also a shift from a high pro-inflammatory cytokine ratio (IL-1β, TNF-α, IL-17: IL-4, IL-10)

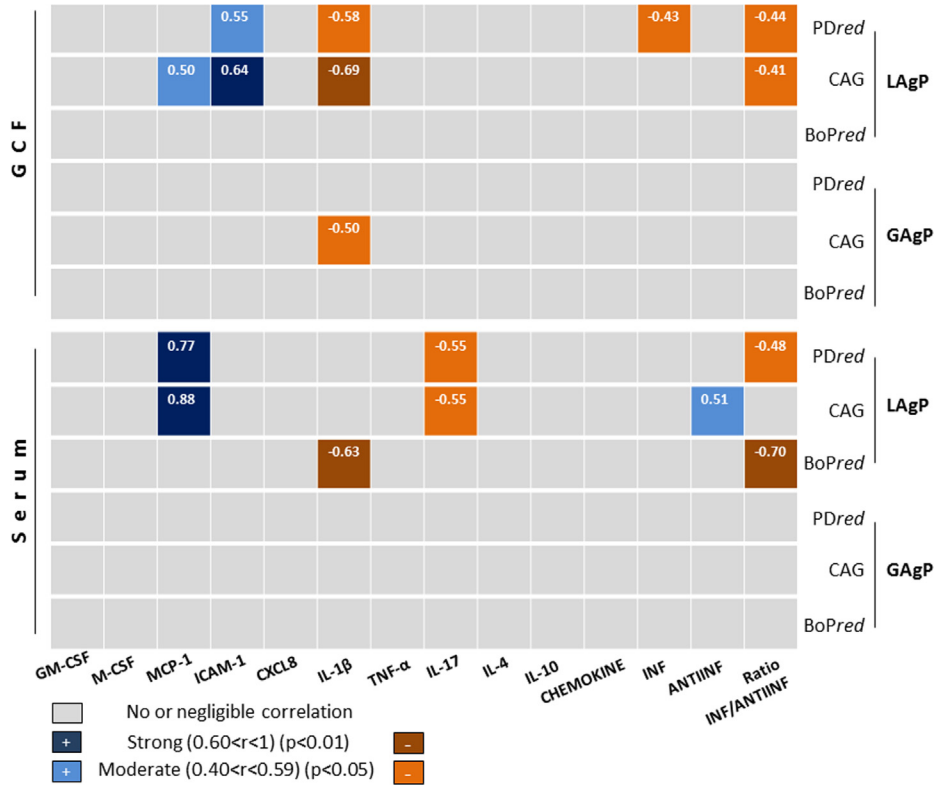

Fig. 5. Grid-plot of the Spearman rank correlation coefficients among the mean of the clinical parameters [PD reduction (PD*red*), CAG, and BoP reduction (BoP*red*)] and changes in immunological profiles. The color code indicates the correlation level.

at baseline ($p = 0.0002$) for a level similar ($p = 0.327$) to healthy controls after treatment (Fig. 4).

We observed different correlation profiles in both forms of AgP. Whereas patients with LAgP presented several patterns of immuno-clinical correlation, the same was not observed in GAgP. The clinical attachment gain was strongly correlated ($p < 0.01$) with an increase in local ICAM-1 levels ($r = 0.64$) and a reduction of IL-1β ($r = -0.69$) in LAgP patients. Also, in this AgP form was observed a moderate correlation ($p < 0.05$) between MCP-1 and CAG ($r = 0.5$), ICAM-1 and PDred ($r = 0.55$), IL-1β and PDred (-0.58), INF score and PDred ($r = -0.43$), Ratio INF/ANTIINF and PDred ($r = -0.44$), Ratio INF/ANTIINF and CAG ($r = -0.41$). In GAgP patients, however, only a moderate correlation was observed between an increase in the CAG and a reduction in local levels of IL-1β ($r = -0.50$) (Fig. 5).

3.4. Profile of immune mediators in the serum

At baseline, serum levels of transmigration/chemotactic factors (M-CSF, MCP-1, and ICAM-1), and anti-inflammatory mediators (IL-4 and IL-10) in both forms of AgP were below those found in healthy individuals ($p < 0.0001$) (Fig. 6). Among these mediators, only IL-4 responded to periodontal treatment, resulting in similar levels to those of healthy individuals after 1-year follow-up ($p > 0.05$).

Basal GM-CSF, IL-1β, TNF-α and IL-17 serum levels were similar to controls ($p > 0.05$) in both forms of AgP (Fig. 6). However, after treatment, GM-CSF levels became higher than those of healthy individuals ($p < 0.001$), whereas IL-1β concentration decreased to lower values ($p = 0.005$). Interestingly, although serum levels of IL-17 increased after treatment in both forms of AgP, only in GAgP such levels became significantly higher than in controls ($p = 0.014$). Moreover, baseline levels of CXCL8 were higher in the LAgP form when compared to healthy controls and GAgP ($p < 0.001$). This difference, however, was no longer observed after treatment ($p = 0.488$).

Considering the overall effect on chemokines, GAgP subjects exhibited a deficient systemic chemokine score (GM-CSF, M-CSF, MCP-1, ICAM-1, CXCL8) at baseline ($p = 0.01$), but after treatment this discrepancy was no longer observed. Furthermore, our data indicated in both AgP forms a shift from a high pro-inflammatory ratio (IL-1β, TNF-α, IL-17: IL-4, IL-10) at baseline ($p < 0.0001$) to a level similar ($p > 0.05$) to controls after treatment (Fig. 4).

A strong positive correlation ($p < 0.01$) in patients with LAgP was observed between an increase in serum levels of MCP-1 and periodontal outcomes [PD reduction ($r = 0.77$) and CAG ($r = 0.88$)]. The mean of BoP reduction of these same patients was strongly correlated with IL-1β ($r = -0.63$) and the ratio INF/ANTI-INF ($r = -0.70$). Moderate correlations were evidenced between IL and 17 and PDred ($r = -0.55$), IL-17 and CAG ($r = -0.55$), Ratio INF/ANTI-INF and PDred (-0.48), ANTIINF score and CAG (0.51), all of them in LAgP patients (Fig. 5).

4. Discussion

The main findings of the present prospective study indicate that both AgP forms have similar clinical (BoP, PD, and CAL reductions), local (GCF) and systemic (serum) immunological responses. Regarding the diagnostic criteria established in the current work, some points deserve to be highlighted. The literature has been imprecise regarding the proper diagnosis of aggressive periodontitis [23], and the current knowledge suggests that there are no differences between the chronic and aggressive generalized forms in terms of immunological parameters [24] and response to periodontal treatment [25], although the molar-incisive pattern destruction affecting young patients still needs to be better elucidated.

It could be seen as a limitation of this study that a longer follow-up could provide a more reasonable view of the actual effect of non-surgical periodontal treatment on both forms of AgP, as well as the evaluation of a greater panel of chemo/cytokines. It should also be noted that there was a difference between the mean age of patients with GAgP and controls, which can be understood by the difference in the age

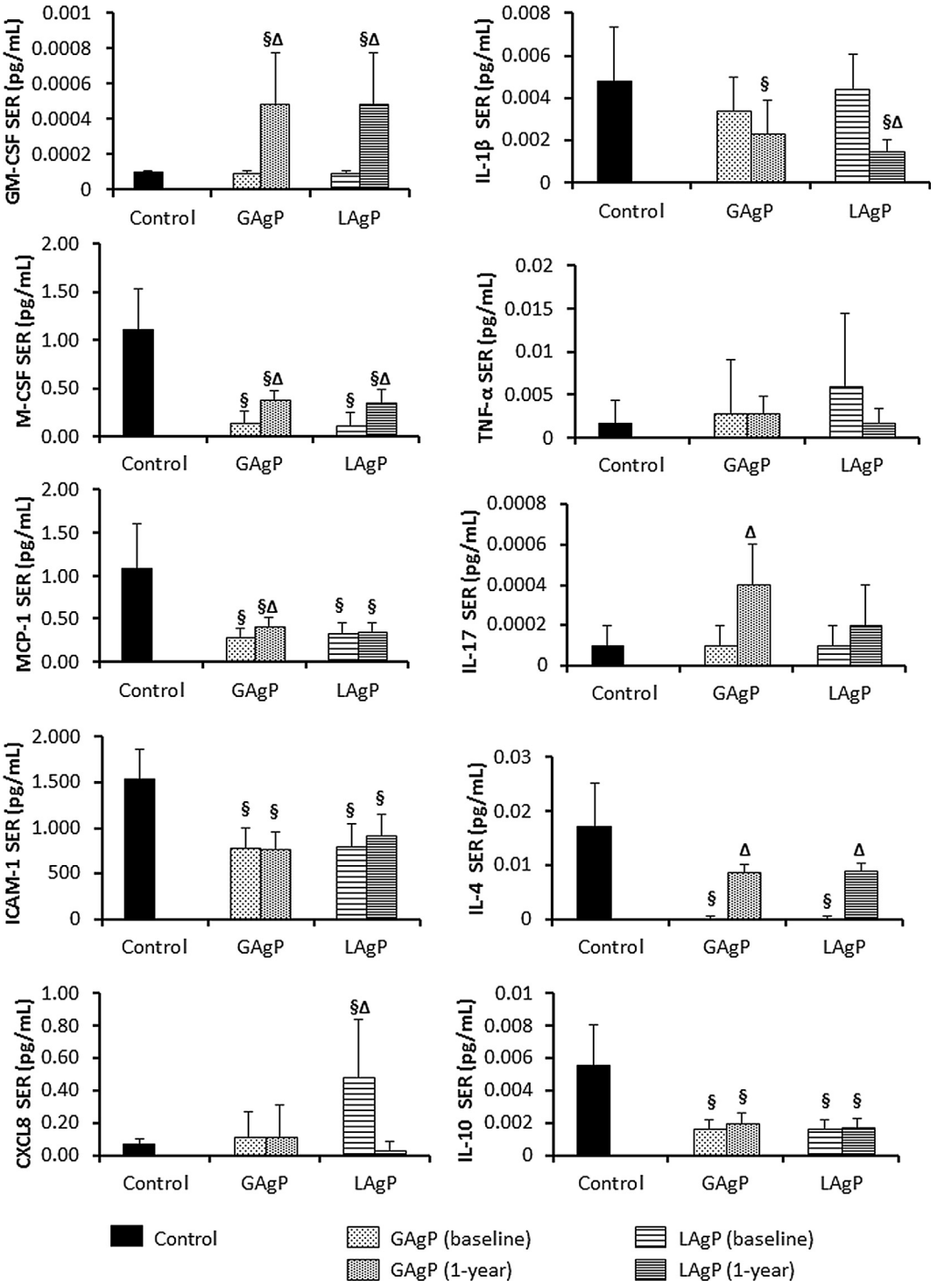

Fig. 6. Chemo/Cytokine profiles in the serum (SER) of controls and patients with both forms of AgP, at baseline and after periodontal treatment (SRP plus systemic antibiotics) with 1-year follow-up. (Δ) A significant intra-group difference using Wilcoxon signed-rank test; (§) A significant difference in relation to healthy controls using Kruskal-Wallis test with Dunn's multiple comparison. A significance level of 0.05 was established for all tests.

profile of both forms of AgP, which prevented an adequate age-pairing of the three groups. In addition, only deep pockets were evaluated, since we used a study design previously elaborated by our group [14], and accordingly, other trials can be conducted using a greater number of affected sites as well as healthy periodontal sites.

However, our data indicated that GAgP and LAgP subjects showed low baseline serum levels of transmigration/chemotactic factors (M-CSF, MCP-1, and ICAM-1). Although the periodontal treatment has increased the levels of some of these mediators, they still remained lower than those compatible with health. On the other hand, at the 1-year follow-up of both forms of AgP, the periodontal treatment provided a shift, locally and systemically, from a high initial pro-inflammatory ratio (IL-1β/TNF-α/IL-17: IL-4/IL-10) to levels similar to those of healthy individuals. This finding points out the systemic impact of aggressive periodontitis, but, more interestingly, the beneficial effect of its treatment.

Some studies have pointed out a deficiency of chemoattractant mediators in the ethiopathogensis of AgP forms, which could generate

in these individuals an inability to eradicate periodontopathogens present in the subgingival biofilm associated to the destruction of the periodontal tissues. In this line, polymorphonuclear neutrophils from LAgP patients show decreased transmigration across the vascular endothelial barrier, and therefore exhibit diminished chemotaxis [7,16,26–28]. Alongside this, an active CXCL8 concentration in the GCF of these patients was shown to be similar to healthy subjects, in spite of the dense Gram negative bacterial stimulation in LAgP [29], which was also corroborated by our results. Thus, in AgP forms, an increase in the chemokine synthesis could represent an increase in the phagocytic response against pathogenic microorganisms with concomitant benefits to clinical response. Not surprisingly, the increase in MCP-1 and ICAM-1 were correlated with an improvement in clinical outcomes such as clinical attachment gain and reduction of probing depth in LAgP patients. Moreover, the overall chemokine score (GM-CSF, M-CSF, MCP-1, ICAM-1, and CXCL8) in the GCF increased significantly in both forms of AgP between baseline and 1-year follow-up, which was followed by an improvement in periodontal parameters.

GM-CSF and M-CSF are important growth factors that regulate the maturation of myeloid cells following their proliferation and differentiation. M-CSF circulates at detectable levels in the steady state, and it is constitutively produced by several cell types, including fibroblasts, endothelial cells, stromal cells, and macrophages. In contrast, GM-CSF regulates maturation and activation of neutrophils and macrophages, and its synthesis usually requires an additional stimulus, such as an infection, to be detected *in vivo* [30,31]. Its role is essential in the immune response since the antimicrobial immunity is compromised by GM-CSF depletion in mice or by the loss of this functional CSF in humans [30]. Therefore, under conditions of microbial challenge, as occurs in periodontitis, the synthesis of these CSFs should be essential for an adequate host response, which does not appear to occur in untreated AgP subjects.

Thus, it seems that the periodontal treatment may have a beneficial effect by increasing the serum and local levels of GM-CSF, especially because under higher local infectious conditions [14] such levels in patients with both forms of AgP are similar to healthy controls. In fact, GM-CSF can present a dual role, increasing the production of pro-inflammatory cytokines by LPS-induced macrophages [32] but also acting as a tolerogenic or immunossupressive mediator and as a stimulus to wound repair [32,33], which suggest a beneficial effect of GM-CSF on AgP.

In a related context, the low baseline levels of ICAM-1 in the serum and its rise in the GCF after treatment also indicate that this chemotactic factor should be involved in AgP. ICAM-1 is an endothelial- and leukocyte-associated transmembrane protein long known for its importance in stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration when binding to the lymphocyte function-associated antigen (LFA-1) [34]. Its increased expression along the junctional epithelium barrier has been characterized as a site for the cellular entry of periodontopathogens [35], and polymorphisms of gene rs5498 ICAM-1 have already been associated with severe forms of chronic periodontitis [17]. Serum levels of ICAM-1 and M-CSF in AgP patients, both constitutively and systemically expressed [30,34], were detected at baseline and after treatment at values lower than in the healthy group. In another study conducted with AgP patients, but without differentiating both forms nor assessing controls, the same periodontal treatment did not alter serum levels of ICAM-1 [36]. However, it would be conceivable to raise the argument that an increase in local levels of ICAM-1 in AgP forms after periodontal therapy could result in an improvement of the local defense due to an enhancing in cell influx (e.g. neutrophils, macrophages), which was associated with an improvement of clinical parameters. Such interpretation needs further studies since the role of transmembrane proteins and chemokines upon the local response to periodontal treatment in AgP forms is still little known.

Differing from other chemokines, the GCF levels of CXCL8 in affected periodontal sites were not altered by the periodontal treatment, and did not differ from control healthy sites. On the other hand, elevated levels of CXCL8 in serum were demonstrated at baseline in LAgP, but not in GAgP. The high serum levels of this chemokine in LAgP could be an attempt by the immune system to overcome the deficiency in chemoattractant mediators in the patients with the localized form of AgP. CXCL8 is synthesized to stimulate migration of defense cells [37], and increased level of CXCL8 in serum would help to control the progression of the disease to other sites, keeping the destruction to the initial sites into a localized form. Consistent with this hypothesis, patients with LAgP presented elevated levels of CXCL8 and MCP-1 only in GCF of healthy sites [38]. Nevertheless, as periodontal treatment reduces the bacterial challenge, the levels of CXCL8 in the serum were also reduced to levels compatible with health. In fact, whether this finding may help distinguish LAgP from GAgP is still unclear, but future studies should be conducted to assess why in both forms of AgP appears to be this distinct pattern in the synthesis of CXCL8.

Similarly to a network of signal transduction, interleukins are also crucial for periodontal tissue homeostasis. For instance, IL-17 has synergistic activity with IL-1β and TNF-α, inducing additional production of such pro-inflammatory cytokines by several cell types. These cytokines are known to trigger osteoclastogenesis pathways and modulate the process of cell differentiation of the adaptive immunity into pro-inflammatory subsets, which alongside the innate immune response, act to up-regulate other cyto/chemokines that exacerbate the periodontal tissue breakdown [7,39]. Conversely, IL-4 and IL-10 are interleukins that seem to reverse this process, or at least act to inhibit its progression. It has been reported that the lack of IL-4 may cause increased CD14 expression and a high production of pro-inflammatory mediators such as prostoglandin E2, TNF-α and IL-1β in periodontal tissues [40,41]. Notwithstanding, this anti-inflammatory action depends on functional haplotypes in IL-4 gene [42]. IL-10, in turn, has a critical role upon the regulation of the inflammatory response by directly inhibiting mechanisms of bone resorption [39].

Therefore, on the basis of this cytokine cross-talk paradigm, rather than just analyzing each cytokine separately, these mediators were also evaluated in both pro-inflammatory (IL-1β, TNF-α, and IL-17) and anti-inflammatory (IL-4, IL-10) profiles. A high pro-inflammatory profile (IL-1β, TNF-α, IL-17: IL-4, IL-10) was observed at baseline in GCF of both forms of AgP, which returned to levels similar to healthy controls at the 1-year follow-up visit. Moreover, there was a significant increase in GCF IL-10 levels, as well as both GCF and serum levels of IL-4, which reinforced a post-therapy anti-inflammatory profile increase. These data suggest an anti-inflammatory response after treatment that is responsible for mechanisms of cell clearance during the course of a natural resolution of inflammation [39,43–45].

In addition, serum anti-inflammatory mediators (IL-4 and IL-10) were below those observed in healthy individuals. Indeed, decreased serum levels of IL-10 and other above-mentioned chemokines have already been described in this disease, as well as reduced gene expression of IL-4 and IL-10 [38,40,42,46]. Also, previous data reported that patients with AgP have an anti-inflammatory profile with IL-4, IL-5, IL-10, IL-13 and IL-1ra in the GCF similar to that of healthy controls, but slightly smaller but not significant in the serum. Furthermore, growth factors such as FGF and PDGFβ were not detected in these patients [15]. In fact, mediators such as IL-10 along with TGF-β are signature cytokines of regulatory T-cells, reduce lymphocyte differentiation in Th1 and Th17 proinflammatory phenotypes, and suppress calcium-mediated co-stimulation of receptor activator NF-κB signaling during human osteoclast differentiation [33,47]. Taken all together, these data suggest that a systemic immune dysregulation can be found in AgP patients, characterized by a deficiency in chemoattractant mediators and low levels of regulatory cytokines, which can be partially corrected by the the periodontal treatment of AgP forms. These outcomes have coherent clinical relevance since an increase in the anti-inflammatory profile was correlated with an increase in the clinical attachment gain in patients

with LAgP.

There are currently hypotheses suggesting that the localized form of aggressive periodontitis may precede the generalized disease or be considering both forms as two distinct clinical entities. In our study, we observed similar baseline local and serum immunological profiles in both forms of AgP (except for CXCL8 levels), as well as resembling clinical and immune responses to periodontal treatment (mechanical debridement along with a combination of amoxicillin and metronidazole). Using the same intervention, previous studies investigating only the localized or generalized form have also found an improvement in clinical parameters or even some immunomodulation [3–5]. For instance, the periodontal treatment reduced local levels of metalloproteinases in LAgP [48], and reduced levels of TNF-α and IL-1β in GCF of periodontal pockets of patients with GAgP [49]. Furthermore, the literature points out that there is no difference in the GCF levels of IFN-γ and IL-6 between patients with GAgP and periodontally healthy [50] and although the treatment does not alter IFN-γ levels [50,51], an increase in IL-6 levels in the GCF of these patients can occur after therapy [50].

Our data suggest that although periodontal treatment is able to modulate a pro/anti-inflammatory cytokine ratio, the same does not occur with the systemic levels of mediators involved in leukocyte transendothelial migration (ICAM-1, MCP-1, and M- CSF), which does not seem to have a systemic suitable response. Still, we have also shown that only LAgP patients were characterized by an elevated baseline serum level of CXCL8. Furthermore, GCF and serum levels of MCP-1 and ICAM-1 were correlated with clinical outcomes in LAgP, but not in GAgP. Therefore, these findings seem to indicate that these mediators are involved in the molar-incisor AgP pattern, and could be used as biomarkers for this form of disease.

Despite these encouraging findings, more studies should be carried out in order to evaluate long-term clinical and immunological outcomes of this therapy. Also, cohort studies should be designed to assess possible differences in the progression of the disease in both forms of AgP. Finally, it is necessary to evaluate the long-term effect of this possible poor serum response of chemotactic factors on the susceptibility of such individuals to develop other infectious/inflammatory diseases in the future, which can insert the periodontal treatment in a broader, systemic and multidisciplinary context.

5. Conclusion

In summary, nonsurgical periodontal therapy was successful in improving clinical parameters and modulating the immune response in both forms of AgP, by modulating the pro/anti inflammatory cytokine ratio. However, this therapy does not seem to affect the deficient level of systemic mediators involved in mechanisms of cell transmigration.

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Conflict of interest

The authors have nothing to declare.

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