https://doi.org/10.54589/aol.36/1/24

Clinical and microbiological assessment in a subpopulation of young Argentine patients with severe periodontitis

Constanza Pontarolo[1], Florencia L Bozza[2,3], Federico G Galli[1], Hernán Bontá[1], Susana L Molgatini[2,3], Facundo Caride[1], Laura A Gliosca[2,3]

1. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Periodoncia, Buenos Aires, Argentina.
2. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Microbiología, Laboratorio de Diagnóstico Microbiológico y Molecular, Buenos Aires, Argentina.
3. Universidad de Buenos Aires, Facultad de Odontología, Instituto de Investigaciones en Salud Pública, Buenos Aires, Argentina.

ABSTRACT

Aggressive periodontitis (AP) is the most serious entity of periodontal disease (stage III/IV, grade C periodontitis according to the latest classification, 2017). ***Aim:*** *to enhance knowledge of periodontal microbiota in AP in native Argentine patients and describe the effect of a combined pharmacological-mechanical periodontal treatment on clinical and microbiological parameters.* ***Materials and Method:*** *The study analyzed 42 periodontal sites in 11 patients diagnosed with AP. Clinical periodontal parameters were recorded at baseline, 45, 90 and 180 days. Microbiological samples were taken before treatment and at 180 days. PCR was used to determine presence of the periodontopathic bacteria Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi) and Fusobacterium nucleatum (Fn). Patients underwent periodontal therapy including antibiotics (Amoxicillin 500mg + Metronidazole 250mg; 8hs/7 days), and were reevaluated at 45, 90 and 180 days.* ***Results:*** *Mean age was 28.4 ± 7.9 years. The initial PCR detected the following frequencies: Aa 14.3%, Pi 61.9%, Pg 71.4%, Tf 81.0%, Fn 95.2% and Td 97.6%. Baseline microbiological samples revealed significantly higher prevalence of Pg over Aa ($p=0.012$). Clinical parameters improved significantly after treatment (73.8% PS<5 mm; PS, NIC, SS $p<0.001$). At 180 days, a significant decrease in microbiological detection rates was observed (Fn, Td, Tf, Pi, Aa $p<0.05$). Aa was no longer detectable while Pg did not decrease significantly ($p=0.052$). Fn was the only study species detected in 100% (n=11:42) of residual pockets (PS≥5 mm) ($p=0.053$).* ***Conclusion:*** *In the initial samples, there was significant prevalence of Pg over Aa. Significant clinical improvement was achieved after the mechanical-pharmacological treatment, with undetectable levels of Aa, while Fn persisted in residual pockets, and Pg was present at most of the treated sites.*

Keywords: *Periodontal disease - aggressive periodontitis - periodontal pathogens - periodontal therapy - molecular detection - PCR.*

To cite:
Pontarolo C, Bozza FL, Galli FG, Bontá H, Molgatini SL, Caride F, Gliosca LA. Clinical and microbiological assessment in a subpopulation of young Argentine patients with severe periodontitis. Acta Odontol Latinoam. 2023 Apr 30;36(1):24-33. https://doi.org/10.54589/aol.36/1/24

Corresponding Author:
Constanza Pontarolo
constanza.pontarolo@odontologia.uba.ar

Received: May 2022.
Accepted: February 2023.

Periodontitis severa en jóvenes argentinos Evaluación clínica y microbiológica en jóvenes argentinos con periodontitis severa

RESUMEN

La periodontitis agresiva (PA) es la entidad más grave de la enfermedad periodontal (clasificación 2017: periodontitis estadio III/IV, grado C). ***Objetivo:*** *mejorar el conocimiento sobre la microbiota periodontal de la PA en sujetos nativos argentinos y describir el efecto de un tratamiento mecánico-farmacológico periodontal sobre los parámetros clínicos y microbiológicos.* ***Materiales y Método:*** *se estudiaron 42 sitios periodontales correspondientes a 11 pacientes con PA. Los parámetros clínicos se registraron a 0, 45, 90 y 180 días. Las tomas microbiológicas se realizaron antes de iniciar el tratamiento y a los 180 días. La determinación de especies periodontopáticas (Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi) y Fusobacterium nucleatum (Fn)) se realizó por PCR. Los pacientes iniciaron terapia básica periodontal junto con antibioticoterapia (Amoxicilina 500 mg + Metronidazol 250 mg; 8 hs/7 días) y fueron evaluados a los 45, 90 y 180 días.* ***Resultados:*** *la edad media fue 28,4 ± 7,9 años. Las detecciones iniciales fueron: Aa 14,3%, Pi 61,9%, Pg 71,4%, Tf 81,0%, Fn 95,2% y Td 97,6%. En las muestras iniciales la prevalencia de Pg sobre Aa fue significativamente superior ($p=0,012$). Los pacientes tuvieron una respuesta clínica favorable al tratamiento (73,8% PS<5 mm; PS, NIC, SS $p<0,001$). A 180 días, se observó una disminución estadísticamente significativa en la detección microbiana (Fn, Td, Tf, Pi, Aa $p<0,05$). En igual plazo, Aa no fue detectado, mientras que Pg mostró una disminución no significativa ($p=0,052$). Fn fue el único detectado en el 100% (n=11:42) de las bolsas periodontales residuales (PS≥5 mm) ($p=0,053$).* ***Conclusión:*** *Las muestras iniciales evidenciaron prevalencia significativa de Pg sobre Aa. El tratamiento logró una significativa mejora clínica con niveles indetectables de Aa. La persistencia de Fn en las bolsas residuales y de Pg en la mayoría de los sitios tratados, caracterizaron la muestra poblacional estudiada.*

Palabras Clave: *Enfermedad periodontal - periodontitis agresiva - patógenos periodontales - terapia periodontal - detección molecular - PCR.*

INTRODUCTION

Periodontitis is a multifactorial inflammatory chronic disease associated with dysbiotic biofilms, characterized by the progressive destruction of the periodontal supporting structures of teeth[1]. Its high prevalence, possible functional and esthetic compromise, and negative impact on general health are sufficient reasons to consider it an important public health issue.

In 1999, the American Academy of Periodontology (AAP) established the differences between the chronic and aggressive forms of disease, based on clinical parameters, microbiological composition of subgingival biofilm and host immunological aspects[2].

Aggressive periodontitis (AP) affects the minority of patients with periodontal disease. Nevertheless, its relevance lies in the rapid progression that can lead to early edentulism[3]. There has recently been a change in the classification of periodontal and peri-implant diseases, with the weighting focusing on clinical diagnosis, risk factors, and type of injury, according to which the patients in the current study are classified as stage III or IV and grade C periodontitis[4].

The search for the specific etiologic agents associated with periodontal disease was begun several decades ago by Slots J. and Socransky S. At present we know that the interaction among bacteria and the balance between pathogenic and beneficial species of the subgingival biofilm affect the progression of disease and the response to treatment[5,6]. It is essential to understand the relationships among subgingival microbiota in order to comprehend the biology of the subgingival ecosystem and plan strategies to control it.

Moreover, the microbiologic profile varies according to geographic areas, habits, ethnicities, development level and living conditions[7]. There is currently little available information about prevalence of periodontal pathogens in native patients with AP in Argentina.

Aims: To enhance current knowledge on the periodontal microbiota of aggressive periodontitis in a sample of native Argentine patients, thereby enabling estimation of the microbiological traits in the local population, and to describe the effect of a combined pharmacological-mechanical periodontal treatment on the proportion of the subgingival bacteria found and on the clinical results.

MATERIALS AND METHOD

This was an experimental clinical study with prospective longitudinal design and six-month follow-up. The eligible population included native Argentine patients with clinical-radiological diagnosis of AP. The patients were referred to the Department of Periodontology, School of Dentistry, University of Buenos Aires (FOUBA) between October 2017 and September 2019. Due to the design features and the prevalence of the study disease, a sample of 10 cases/year was calculated, with a drop-out rate of 10%. Patients were invited to participate, and after being informed of the protocol, benefits and possible drawbacks related to the periodontal procedures, they confirmed their voluntary participation by signing the informed consent form (FOUBA EC Exp N 006/2017).

Informed consent

Informed consent was established pursuant to the Declaration of Helsinki. The project and informed consent were accepted in a timely manner by the FOUBA Ethics Committee - 006/2017.

Inclusion criteria

Native Argentines, 18-40 years old, with clinical-radiographic signs of AP (stage III or IV and grade C periodontitis) with PD≥5mm, CAL≥5 and BOP(+) in at least 4 sites (1 site per quadrant).

Exclusion criteria

Foreigners, pregnant or breast-feeding women, smokers, patients with systemic disease such as diabetes, immuno-compromise and/or other pathologies affecting periodontal tissues[8] (developed prior to or during the study), patients with history of metronidazole and/or penicillin hypersensitivity, patients treated with selective antimicrobials within 6 months before the beginning of the study.

Diagnosis was based on the information obtained systematically through: 1. Medical history recorded at the FOUBA. 2. Determination of periodontal clinical parameters (6 sites per tooth): probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), mobility, furcation involvement. 3. Routine radiographic assessment using periapical serial radiographs[2]. All patients were evaluated by the same calibrated operator (CP) ($\kappa \geq 0.74$).

Clinical-microbiological procedures

The protocol consisted of an initial interview during which a complete medical history was prepared, collecting patient's personal data, medical history and current medication. On the same day, clinical-radiographic screening was performed. Patients who met the inclusion criteria were invited to participate in the protocol and informed of the benefits and potential discomfort they might experience during the course of the protocol. After granting consent, they were summoned to a visit in 7 days to start the protocol.

The initial sample comprised twenty patients, but only eleven completed the study. (Fig. 1). Clinical parameters were recorded at baseline (T0); 45 days (T1); 90 days (T2) and 180 days (T3) after treatment. Six sites per tooth were evaluated by the same calibrated investigator ($\kappa \geq 0.74$) with appropriate lighting, employing standardized instruments (North Carolina periodontal probe and dental mirror). After initial periodontal recording and radiographic evaluation, 4 sites (1 per quadrant) were selected according to the inclusion criteria (PD≥5 mm, CAL≥5 and BOP) for the microbiological study.

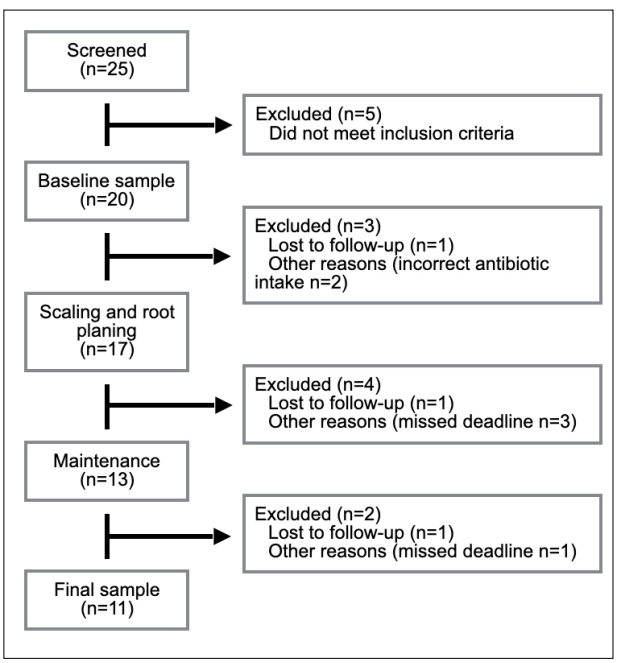

Fig. 1: Conformation of the population sample. Fourteen patients were excluded for any of the following reasons: allergic to penicillin and its derivatives, did not attend maintenance sessions, selected periodontal pockets belonged to hopeless prognosis teeth, mistakes in taking prescribed antibiotics, did not complete periodontal therapy, or did not conform to the established protocol times.

Seven days after the instrumental diagnosis, microbiological samples of subgingival biofilm were obtained by inserting 4 paper points (No. 30-35) per site, having previously removed the supragingival biofilm with a curette. Paper points were consecutively inserted with an absorption time of 20 seconds each. Samples were transported to the laboratory in accordance with the biosecurity rules of the institution, together with two slides obtained from the periodontal pocket soft wall. Study design is shown in Fig. 2.

Periodontal therapy

All patients were instructed in oral hygiene and received full mouth scaling and root planing, systemic administration of antibiotics and ecologic control through caries inactivation and tooth extraction, if needed. Local anesthesia was used as necessary. Full mouth periodontal therapy was accomplished in no more than 2 appointments within a maximum period of 7 days during antibiotic treatment. Patients were administered 250 mg metronidazole (half a tablet of *Ovufem*® 500 mg, Laboratorios Bernabó, Buenos Aires, Argentina) and 500 mg amoxicillin (1 tablet of *Amixen*® 500 mg, Laboratorios Bernabó, Buenos Aires, Argentina) every 8 hours for 7 days, starting 48 hours before instrumentation.

Molecular procedure

Samples were obtained and processed from the selected sites at baseline (T0), before periodontal therapy, and 180 days (T3), after protocolized periodontal treatment. Samples were homogenized for molecular processing. Genomic DNA was extracted by rupture and purified in affinity columns (Presto™ Mini gDNA Bacteria Kit, Geneaid). The endpoint PCR technique was employed to detect six periodontopathic species. The specific primers for *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Prevotella intermedia* (Pi) and *Fusobacterium nucleatum* (Fn) were synthetized as designed by Ashimoto et al.[9]. Amplified sequences of the uncultivable or special nutritional species *T. denticola* and *T. forsythia* were confirmed by sequencing, while in the other cases, pattern strains of ATCC standard collection were used (*P. gingivalis* ATCC 33277; *F. nucleatum* ATCC 25586; *P. intermedia* ATCC 25611). Products of amplification were evidenced by electrophoresis

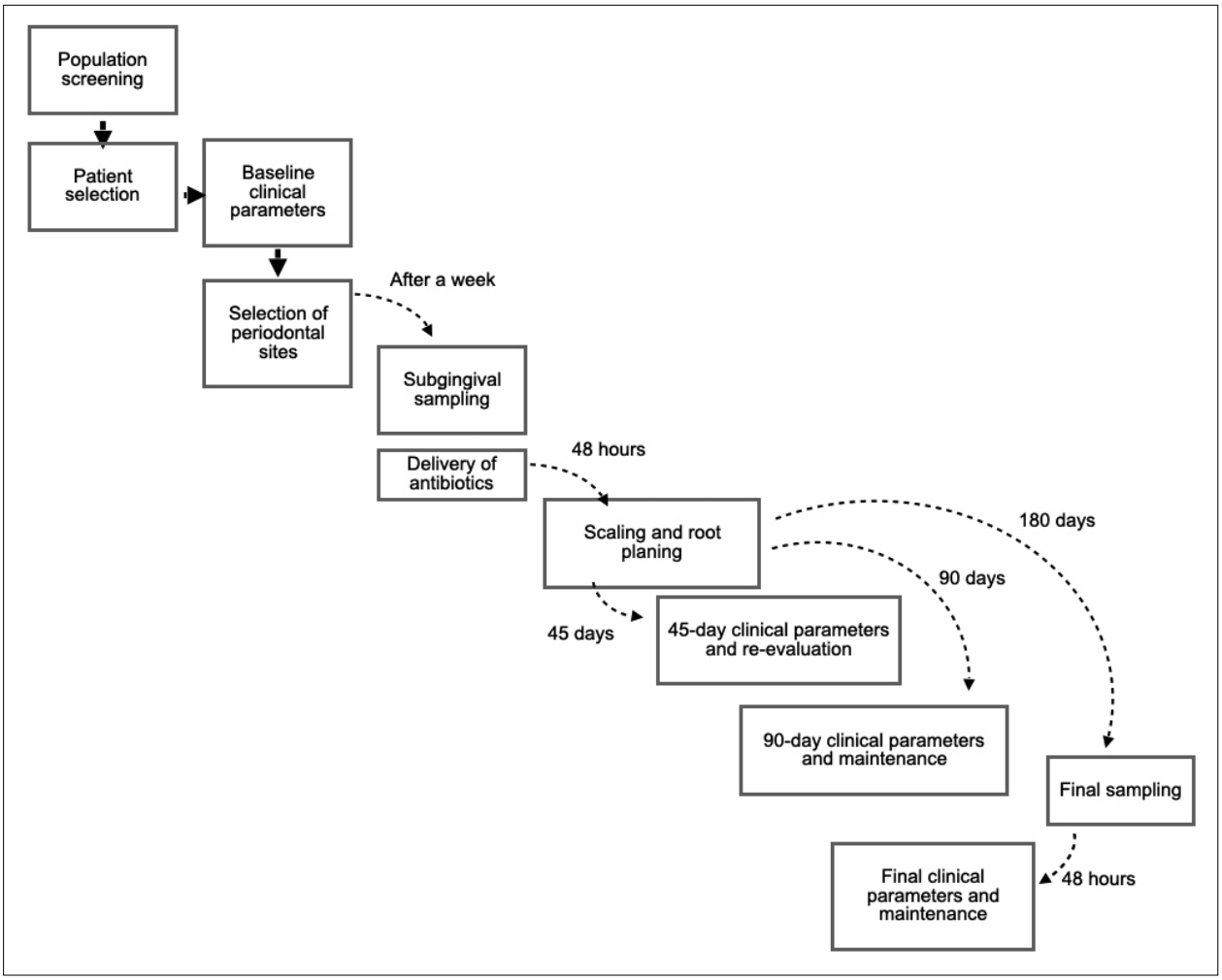

Fig. 2: Study design. Clinical and microbiological procedures.

in 2% agarose gel in TAE buffer, using GelRed® Nucleic Acid Gel Stain as intercalated fluorophore and visualized with The Gel-Doc XR - Gel Imaging System from BIO-RAD®.

Statistical analysis

Quantitative variables were described by mean, standard deviation, median, minimal and maximal confidence interval. Nonparametric Friedman's ANOVA $p<0.01$, Bonferroni post-hoc was used for multiple comparisons, giving significantly different probing depth and clinical attachment loss measurements at baseline (T0) compared to the other evaluation times (T1, T2, T3).

To compare periodontal pathogens PCR results between baseline (T0) and final time (T3), the McNemar test was applied. The difference was considered significant when $p<0.05$. All statistical analyses were performed using the SPSS software (version 28), MS Excel and Epidata 4.0.

RESULTS

Initially, 20 patients (80 periodontal sites) were included in the study. The analyzed sample consisted of eleven native Argentine patients, 9 women and 2 men, with clinical-radiological diagnosis of AP, and mean age 28.4 ± 7.9 years (16-39). Forty-two periodontal sites were studied clinically, microbiologically and molecularly (Fig.1).

Microbiological parameters

The baseline (T0) detection frequencies were the following: *A. actinomycetemcomitans* 14.3%, *P. intermedia* 61.9%, *P. gingivalis* 71.4%, *T. forsythia* 81.0%, *F. nucleatum* 95.2% and *T. denticola* 97.6%. In the initial samples, the prevalence of *P. gingivalis*

Table 1. Molecular parameters

	Count	%	Ci 95.0% LL	Ci 95.0% UL	p value
PCR Pg inicial	30	71.4%	56.7%	83.3%	0.052
PCR Pg final	20	47.6%	33.1%	62.5%	
PCR Fn inicial	40	95.2%	85.6%	99.0%	0.039*
PCR Fn final	32	76.2%	61.9%	87.1%	
PCR Td inicial	41	97.6%	89.4%	99.7%	0.001**
PCR Td final	16	38.1%	24.6%	53.2%	
PCR Tf inicial	34	81.0%	67.3%	90.6%	0.004*
PCR Tf final	22	52.4%	37.5%	66.9%	
PCR Pi inicial	26	61.9%	46.8%	75.4%	0.001**
PCR Pi final	5	11.0%	4.7%	24.1%	
PCR Aa inicial	6	14.3%	6.2%	27.1%	0.031*
PCR Aa final	0	0.0%			

PCR results. Initial and final time samples. p(Fisher) *: p < 0.05; **: p ≤ 0.001. Pg: *Porphyromona gingivalis*; Fn: *Fusobacterium nucleatum*; Td: *Treponema denticola*; Tf: *Tannerella Forsythia*; Pi: *Prevotella intermedia*; Aa: *Aggregatibacter actinomycetemcomitans*

over *A. actinomycetemcomitans* was significant (p=0.012). At 180 days after the end of the treatment protocol (T3), *A. actinomycetemcomitans* could no longer be detected by this methodology at any of the study sites. *T. denticola, F. nucleatum, T. forsythia* and *P. intermedia* decreased significantly during the same interval. *F. nucleatum* remained detectable in all the residual periodontal pockets with PD≥ 5mm (p=0.053). *P. gingivalis* was the only species that showed no statistically significant difference between evaluation times (p=0.052) (Table 1; Fig. 3).

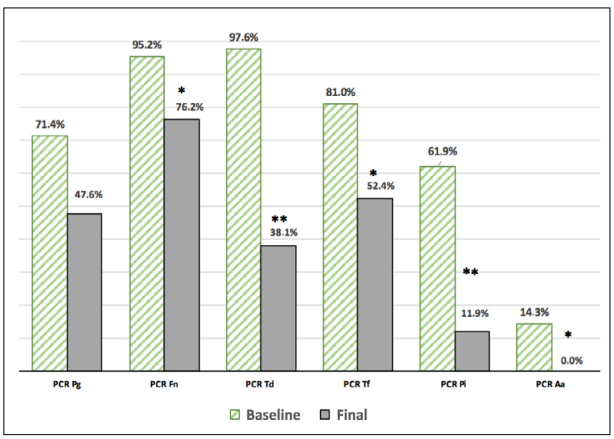

*Fig. 3: Molecular parameters. Baseline and final time samples. McNemar Test *: p<0.05; **: p≤0.001. Pg: Porphyromona gingivalis; Fn: Fusobacterium nucleatum; Td: Treponema denticola; Tf: Tannerella Forsythia; Pi: Prevotella intermedia; Aa: Aggregatibacter actinomycetemcomitans.*

Clinical parameters

Bleeding on probing (BOP): Initially, BOP was present at 100% of the 42 sites. It declined to 19.5% at 45 days, 11.9% at 90 days, and 9.5% at 180 days. Reduction of BOP was statistically significant (p<0.001) (Fig. 4).

Probing depth (PD): Median PD at the selected sites was initially 8 mm (5-12mm). Final median PD was 3 mm (2-10 mm). The difference between the initial value and each instance of re-evaluation was statistically significant (p<0.001) (Fig. 5 A; Table 2).

Clinical attachment level (CAL): Median CAL was 7 mm (5-12 mm) at baseline, and 4 mm (1–10 mm) at 180 days. The difference between the baseline value

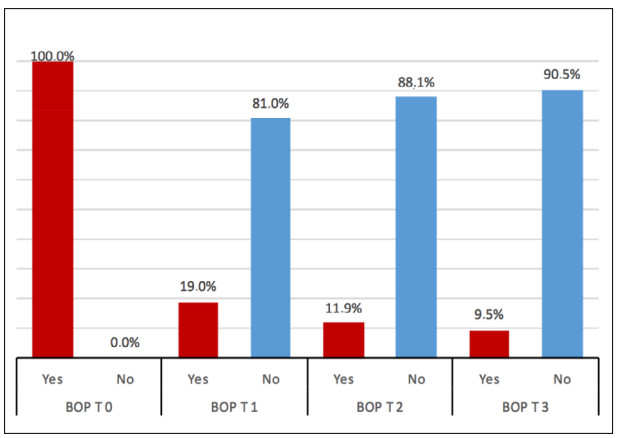

Fig. 4: Bleeding on probing. BOP 0 baseline, BOP 1 45 days, BOP 2 90 days, BOP 3 180 days. McNemar Test.

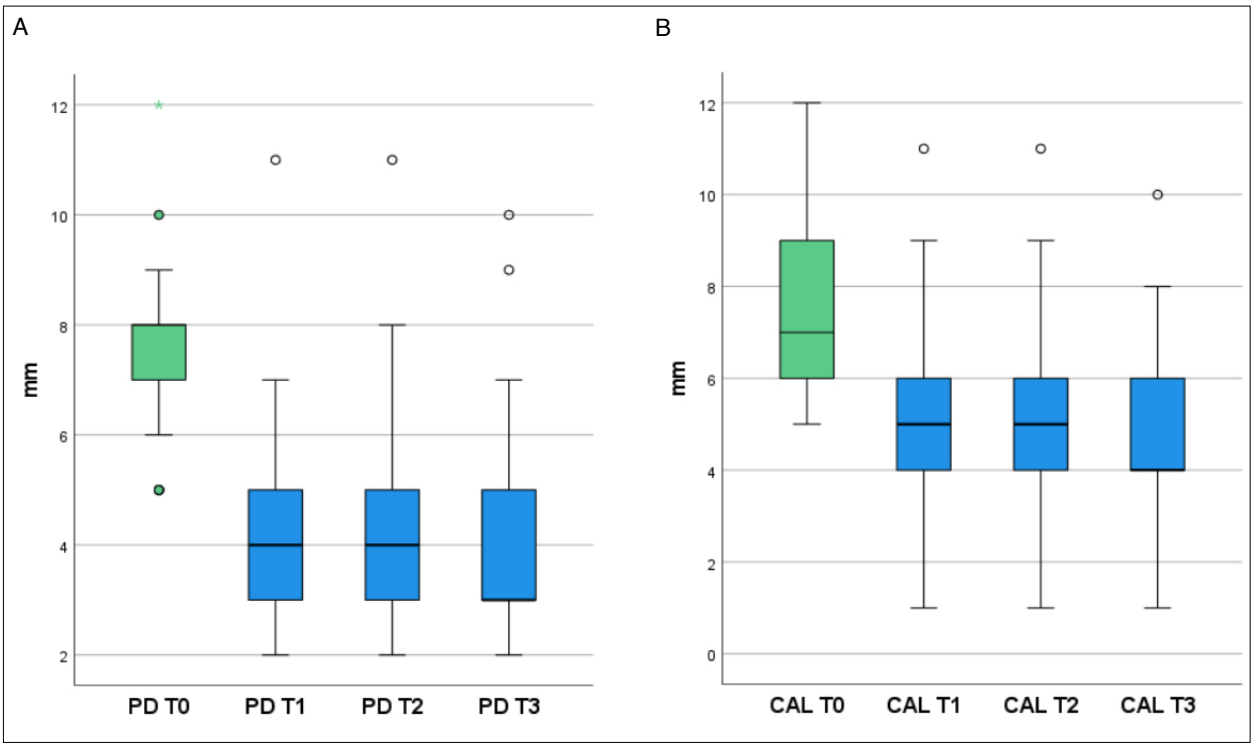

Fig. 5: Probing depth and clinical attachment level. A. Probing depth: PD 0 baseline, PD 1 45 days, PD 2 90 days, PD 3 180 days. B. Clinical attachment level: CAL 0 baseline, CAL1 45 days, CAL2, 90 days, CAL3 180 days.
Nonparametric Friedman's ANOVA p<0.01, Bonferroni post-hoc was used for multiple comparisons, giving significantly different measurements at baseline (0) compared to the other evaluation times (1,2,3).

Table 2. Probing depth and clinical attachment loss

	Mean	Standard Deviation	CI 95.0% LL	CI 95.0% UL	Percentile 25	Median	Percentile 75	p value
Probing Depth								
PD T0	7.57	1.4	7.14	8.01	7	8	8	<0.001**
PD T1	4.33	1.65	3.82	4.85	3	4	5	
PD T2	4.31	1.7	3.78	4.84	3	4	5	
PD T3	4	1.74	3.46	4.54	3	3	5	
Clinical Attachment loss								
CAL T0	7.29	1.93	6.69	7.89	6	7	9	<0.001**
CAL T1	5.21	2.03	4.58	5.85	4	5	6	
CAL T2	5.1	1.82	4.53	5.66	4	5	6	
CAL T3	4.71	1.76	4.17	5.26	4	4	6	

Probing depth and clinical attachment loss. p(Fisher) between T0 and T1,T2 and T3. **: p ≤ 0.001. Probing depth: PD T0: initial; PD T1: 45-day; PD T2: 90-day; PD T3: 180-day. Clinical attachment loss: CAL T0: initial; CAL T1: 45-day; CAL T2: 90-day; CAL T3: 180-day.

and each instance of re-evaluation was statistically significant (p<0.001) (Fig. 5 B; Table 2).

At 180 days after treatment, pocket closure (PD≤4mm) was achieved in 73.8% of the study sites (Fig. 6). Eleven sites from 6 patients remained with PD≥5 mm, 8 of which were in premolar or molar areas. All these residual pockets showed presence of *F. nucleatum* (p=0.033), and in 10 sites, at least 2 of the study species were found. *P. gingivalis* was detected in 6 residual pockets from 3 patients. 3:11 sites with final PD≥5 mm showed BOP, and *P. gingivalis* was detectable in 2 of these 3 sites.

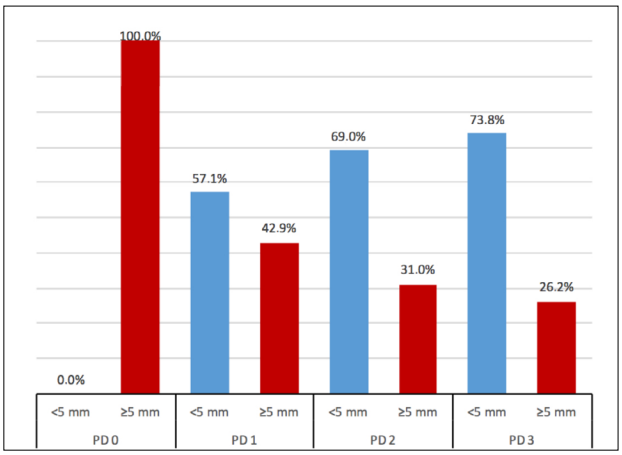

Fig. 6: Proportion of sites with PD≥5 mm vs. sites with PD<5mm at different intervals. PD 0: baseline; PD 1: 45 days; PD 2: 90 days; PD 3: 180 days. McNemar Test.

DISCUSSION

Most of the information on the prevalent microorganisms in AP comes from other regions. However, the microbiologic profile of periodontitis in different populations seems to differ in frequency and composition.

In Argentina, little is known about the microbiological composition or relative abundance in subgingival biofilm of native patients with this infrequent disease. According to data collected in the department of Periodontology at the FOUBA between 1999 and 2005, only 7% (n=365) of the treated diseases were diagnosed as AP[10]. Two local reports evaluated the prevalence of periodontal pathogens in chronic periodontitis (CP)[11,12]. A pilot study was recently conducted on patients with AP[13] at the Fundación Independencia, Universidad Nacional de Cuyo, which analyzed 30 sites in 5 patients, finding significant prevalence of *P. gingivalis, T. forsythia,* and *T. denticola*, over *P. intermedia* and *A. actinomycetemcomitans*. Even though the results of the present study were similar, the sample showed a 61.9% prevalence for *P. intermedia* and 14.3% for *A. actinomycetemcomitans,* slightly higher levels than reported by Usin et al.[13]. Another study on a 2011-2015 cohort at FOUBA[14] considered 18 patients with clinical-radiological diagnosis of AP, and 32 with CP. Prevalence of *P. gingivalis* in subgingival biofilm was 50% in patients with AP and 54.2% in patients with CP, while prevalence of *A. actinomycetemcomitans* was 32.8% and 19.7%, respectively. Although *P. gingivalis* was 1.6 and 2.7 times higher than *A. actinomycetemcomitans,* the latter was significantly associated to AP (p=0.039) in relation to CP[14].

Despite the small sample size, the results of the current study suggest that *P. gingivalis* is the prevalent keystone pathogen, and *A. actinomycetemcomitans* is a marker of this particularly aggressive disease. This local information agrees with the results reported by other research groups such as Kamma et al.[15], Mombelli et al.[16], Dahlen et al.[17], Mullally et al.[18], Mayorga-Fayad et al.[19], Cortelli et al.[20] and Gajardo et al.[21] Nevertheless, other scientific evidence reports *A. actinomycetemcomitans* as the most prevalent pathogen in in the etiopathogenesis of rapidly progressive severe periodontal disease[22].

Recent studies on subgingival microbiome outline new bacterial combinations with periodontopathogenic profile, such as *Cryptobacterium curtum, Dialister pneumosintes, Filifactor alocis, Mitsuokella dentalis, Slackia exigua, Selenomonas sputigena, Solobacterium moorei, Treponema lecithinolyticum* and *Synergistes* sp.[22,23].

Mechanical instrumentation by means of scaling and root planing remains the basis of non-surgical periodontal therapy. However, results are not always predictable and depend on multiple factors, which is why the use of systemic antibiotics as an adjuvant to mechanical therapy has been studied for many years[24]. In a systematic review, the use of systemic antibiotic therapy together with scaling and root planing reduced the risk of progression of loss of attachment and showed statistically significant differences in the gain of clinical attachment level in deep pockets over mechanical therapy alone[25]. These results agree with a meta-analysis by Rabelo et al.[26].

Within possible antibiotic regimens, the combination of amoxicillin and metronidazole has been indicated in the treatment of AP. This strategy assumes that multiple species can be eliminated or inhibited simultaneously during periodontal treatment, achieving reestablishment of the commensal microbiota and a healing response from the host[27]. A recent systematic review and meta-analysis by Teughels et al.[28] assessed the effect of the adjunctive use of different systemic antimicrobial schemes in the active phase of periodontal treatment. The best outcomes were observed with amoxicillin plus metronidazole, especially in AP, showing statistically significant benefits in all the clinical parameters studied[28].

According to the evidence obtained in the current study, and as Faveri et al.[29] have published recently, full mouth treatment with adjuvant administration of the antibiotic scheme (amoxicillin and metronidazole) may be an effective protocol for the treatment of AP in the young study population. There was a significant improvement of the periodontal parameters in terms of reduction of bleeding on probing, pocket closure and gain on clinical attachment level. It should be highlighted that most of the sites with residual pockets were in molar or premolar areas. Tooth type has been shown to be an important variable that influences the results of periodontal treatment, since non-surgical periodontal therapy is often less effective in deep pockets in multi-rooted teeth[30]. The implementation of the chemical-mechanical protocol produced a shift in the bacterial composition of the subgingival biofilm, with a tendency to restitution of the homeostasis of the subgingival ecosystem. Proof supporting the ecological changes achieved by the combination therapy is provided by the marked decrease in *T. denticola*[31], which is a sensitive marker of periodontal activity.

Within the limitations of this study, the microbiological results showed significant prevalence of *P. gingivalis* over *A. actinomycetemcomitans* in a ratio of up to 5 to 1. The prevalence of *A. actinomycetemcomitans* was lower than reported for other Latin American populations[15-22], and it was the species most susceptible to treatment. Kulik et al.[32] demonstrated the broad susceptibility of *A. actinomycetemcomitans* to Amoxicillin/clavulanic acid and tetracycline and non-susceptibility to clindamycin, metronidazole or phenoxymethylpenicillin. More recent studies[33] showed changes in the susceptibility of *A. actinomycetemcomitans* and *P. gingivalis*, pointing to moxifloxacin as a useful therapeutic option.

Despite the apparent ecological reestablishment after combined pharmacological-mechanical periodontal treatment and standard maintenance appointments, *P. gingivalis* appeared to have a moderate rate of persistence at the end of the study. Although the data from this study do not evaluate recolonization at 45-days, persistence in Pg detection could be more associated with dysbiosis as a result of ecological conditions than resistance to treatment[34]. The persistence of *F. nucleatum* in residual pockets and *P. gingivalis* in most of the treated sites characterized the study sample. Further studies with a larger sample size are needed to enhance local epidemiological data.

ACKNOWLEDGMENTS

We would like to thank Pablo Salgado for his work and assistance with statistical analysis.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

FUNDING SOURCE

This study was supported by a grant from Facultad de Odontología, Universidad de Buenos Aires, stimulus funding to clinical research "Prof. Dr. Rodolfo Erausquin". RES(CD)628/17.

REFERENCES

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018 Jun; 89 Suppl 1: S173– S182. https://doi.org/10.1002/JPER.17-0721
2. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999 Dec;4(1):1-6. https://doi.org/10.1902/annals.1999.4.1.1
3. Griffiths GS, Ayob R, Guerrero A, Nibali L, Suvan J, Moles DR, Tonetti MS. Amoxicillin and metronidazole as an adjunctive treatment in generalized aggressive periodontitis at initial therapy or re-treatment: a randomized controlled clinical trial. J Clin Periodontol. 2011 Jan;38(1):43-9. https://doi.org/10.1111/j.1600-051X.2010.01632.x
4. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornmann KS, Mealey BL, Papapanou PN, Sanz M, Tonetti MS. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Periodontol. 2018 Jun;45 Suppl 20:S1-S8. https://doi.org/10.1002/JPER.18-0157

5. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, McIntosh ML, Alsam a, Kirkwood KL, Lambris JD, Darveau RP, Curtis MA. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. 2011 Nov 17;10(5):497-506. https://doi.org/10.1016/j.chom.2011.10.006
6. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol. 2018 Dec;16(12):745-759. https://doi.org/10.1038/s41579-018-0089-x
7. Nisha S, Samyuktha GS, Shashikumar P, Chandra S. Periodontal disease – Historical and contemporary hypothesis: A review. SRM J Res Dent Sci. 2017 Jul-Sep;8:121-125. https://www.srmjrds.in/text.asp?2017/8/3/121/215013
8. Albandar JM, Susin C, Hughes FJ. Manifestation of systemic diseases and conditions that affect the periodontal attachment apparatus: case definitions and diagnostic considerations. J Periodontol. 2018 Jun;89 Suppl 1:S183-S203. https://doi.org/10.1002/JPER.16-0480
9. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol. 1996 Aug;11:266-273. https://doi.org/10.1111/j.1399-302X.1996.tb00180.x
10. Biagini F, Caride F, Costa OR. Diagnóstico de patologías más prevalentes de pacientes que concurrieron al departamento de posgrado de la Cátedra de Periodoncia de la Facultad de Odontología de la UBA.1999-2005. Rev Fundación Juan José Carraro. 2007;12(25):20-25.
11. Bazzano G, Parodi R, Tabares S, Sembaj A. Evaluación de la terapia mecánica periodontal en bolsas profundas: Respuesta clínica y bacteriológica. Rev Clin Periodoncia Implantol Rehabil Oral. 2012 Dec;5:123-128. http://dx.doi.org/10.4067/S0719-01072012000300004
12. Nogueira MA, Fernandez CL, Furman C, Chiappe V, Marcantoni M, Bianchini H. Clinical and microbiological study of adult periodontal disease. Rev Argent Microbiol 2001 Jul-Sep;33(3):133-140. https://pubmed.ncbi.nlm.nih.gov/11594003/
13. Usin MM, Tabares SM, Menso J, de Albera ER, Sembaj A. Generalized aggressive periodontitis: microbiological composition and clinical parameters in non-surgical therapy. Acta Odontol Latinoam. 2016 Dec;29(3):255-261. https://actaodontologicalat.com/vol-29-%c2%b7-issue-3-december-2016/
14. Gliosca L. Enfermedad periodontal crónica/agresiva: estudio de prevalencia microbiológica y molecular. Tesis Doctoral. 2016. FOUBA.
15. Kamma JJ, Nakou M, Gmür R, Baehnl PC. Microbiological profile of early onset/aggressive periodontitis patients. Oral Microbiol Immunol. 2004 Oct;19:314-21. https://doi.org/10.1111/j.1399-302x.2004.00161.x
16. Mombelli A, Gmür R, Frey J, Meyer J, Zee KY, Tam JOW, Lo ECM, Di Rienzo J, Lang NP, Corbet EF. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in young Chinese adults. Oral Microbiol Immunol. 1998 Aug;13:231-237. https://doi.org/10.1111/j.1399-302X.1998.tb00701.x
17. Dahlen G, Manji F, Baelum V, Fejerskov O. Black pigmented bacteroides species and *A. actinomycetemcomitans* in subgingival plaque of adult Kenyans. J Clin Periodontol. 1989 May;16:305-310. https://doi.org/10.1111/j.1600-051X.1989.tb01660.x
18. Mullally BH, Dace B, Shelburne CE, Wolff LF, Coulter WA. Prevalence of periodontal pathogens in localized and generalized forms of early-onset periodontitis. J Periodontal Res. 2000 Aug;35:232-41. https://doi.org/10.1034/j.1600-0765.2000.035004232.x
19. Mayorga-Fayad I, Lafaurie GI, Contreras A, Castillo DM, Barón A, Aya MDR. Microflora subgingival en periodontitis crónica y agresiva en Bogotá, Colombia: un acercamiento epidemiológico. Biomédica. 2007 Mar;27:21-33. https://doi.org/10.7705/biomedica.v27i1.230
20. Cortelli JR, Cortelli SC, Jordan S, Haraszthy VI, Zambon JJ. Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol. 2005 Aug;32:860-866. https://doi.org/10.1111/j.1600-051X.2005.00777.x
21. Gajardo M, Silva N, Gómez L, León R, Parra B, Contreras A, Gamonal J. Prevalence of periodontopathic bacteria in aggressive periodontitis patients in a Chilean population. J Periodontol. 2005 Feb;76:289-94. https://doi.org/10.1902/jop.2005.76.2.289
22. Marchesan JT, Moss K, Morelli T, Teles FR, Divaris K, Styner M, Ribeiro AA, Webster-Cyriaque J, Beck J. Distinct microbial signatures between periodontal profile classes. J Dent Res. 2021 Nov;100(12):1405-1413. https://doi.org/10.1177/00220345211009767
23. Hiranmayi KV, Sirisha K, Ramoji Rao MV, Sudhakar P. Novel Pathogens in Periodontal Microbiology. J Pharm Bioallied Sci. 2017 Jul-Sept;9:155-163. https://doi.org/10.4103/jpbs.JPBS_288_16
24. Herrera D, Sanz M, Jepsen S, Needleman I, Roldan S. A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. J Clin Periodontol. 2002;29(suppl 3):136-159, discussion 160-162. https://doi.org/10.1034/j.1600-051X.29.s3.8.x
25. Sgolastra F, Petrucci A, Gatto R, Monaco A. Effectiveness of systemic Amoxicillin/Metronidazole as an adjunctive therapy to full-mouth scaling and root planning in the treatment of aggressive periodontitis: a systematic review and meta-analysis. J Periodontol. 2012 Jun; 83(6):731-743. https://doi.org/10.1902/jop.2011.110432
26. Rabelo CC, Feres M, Gonçalves C, Figueiredo LC, Faveri M, Tu YK, hambrone L. Systemic antibiotics in the treatment of aggressive periodontitis. A systematic review and a Bayesian Network meta-analysis. J Clin Periodontol 2015 Jul;42(7):647-657. https://doi.org/10.1111/jcpe.12427
27. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. Ann Periodontol. 2003 Dec;8(1):115-181. https://doi.org/10.1902/annals.2003.8.1.115
28. Teughels W, Feres M, Oud V, Martin C, Matesanz P, Herrera D. Adjunctive effects of systemic antimicrobials in periodontitis therapy: a systematic review and meta-analysis. J Clin Periodontol. 2020 Jul;47 Suppl 22:257-281. https://doi.org/10.1111/jcpe.13264
29. Faveri M, Retamal-Valdes B, Mestnik MJ, de Figueiredo LC, Barão VAR, Silva Souza JG, Mendes Duarte P, Feres M. Microbiological effects of amoxicillin plus metronidazole

in the treatment of young patients with stages III and IV periodontitis: a secondary analysis from a 1-year double-blinded placebo-controlled randomized clinical trial. J Periodontol 2022 Jul;1-11. https://doi.org/10.1002/JPER.21-0171

30. Tomasi C, Leyland AH, Wennström JL. Factors influencing the outcome of non-surgical periodontal treatment: a multilevel approach. Journal of Clinical Periodontology. 2009 Aug;34(8):682-690. https://doi.org/10.1111/j.1600-051X.2007.01111.x
31. Malone ET, Ganther S, Mena N, Radaic A, Shariati K, Kindberg A, Tafolla C, Kamarajan P, Fenno JC, Zhan L, Kapila YL. Treponema denticola-induced RASA4 upregulation mediates cytoskeletal dysfunction and MMP-2 activity in periodontal fibroblasts. Front Cell Infect Microbiol. 2021 May;11:671968. https://doi.org/10.3389/fcimb.2021.671968
32. Kulik EM, Lenkeit K, Chenaux S, Meyer J. Antimicrobial susceptibility of periodontopathogenic bacteria. J Antimicrob Chemother. 2008 May;61(5):1087–1091. https://doi.org/10.1093/jac/dkn079
33. Ardila CM, Bedoya-García JA. Antimicrobial resistance of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* in periodontitis patients. J Glob Antimicrob Resist. 2020 Sep;22:215–218. https://doi.org/10.1016/j.jgar.2020.02.024
34. Conrads G, Klomp T, Deng D, Wenzler JS, Braun A, Abdelbary MMH. The antimicrobial susceptibility of *Porphyromonas gingivalis*: genetic repertoire, global phenotype, and review of the literature. Antibiotics. 2021 Nov;10(12):1438. https://doi.org/10.3390/antibiotics10121438