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ORIGINAL RESEARCH

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Periodontal and microbiological data in patients with mucous membrane pemphigoid in a French population in 2021–2022: A pilot cross-sectional study

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Abstract

Background and Aims: In the case of mucous membrane pemphigoid with gingival expression (gMMP), the complete healing of the gingiva is generally not achieved despite medical treatment. Therefore, patients' oral comfort is impaired. The dysbiotic periodontal microbiota, generated by a lack of oral hygiene associated with persistent gingival pain, could the immunopathological mechanism to persist. The main objective of this study was to characterize the subgingival microbiota of the gMMP patients, and to highlight a potential link between this microbiological data and the clinical data.

Methods: Subgingival biofilm was collected from 15 gMMP patients, medically treated or not, but not receiving periodontal treatment. The usual clinical periodontal parameters were recorded. The biofilm was analyzed by polymerase chain reaction quantitative. The risk factors of severe erosive gingivitis and severe periodontitis were assessed using Chi-square or Fischer's exact test were used.

Results: Whatever the medical and periodontal conditions of the patients, the results showed the existence of three main communities of periodontopathic, dysbiotic bacteria. The first including *Tannnerella forsythia*, *Peptostreptococcus micros*, *Fusobacterium nucleatum*, and *Campylobacter rectus*, was found in 100% of the patients, the second enriched with *Treponema denticola* in 60% and the third enriched with *Porphyromonas gingivalis* and *Prevotella intermedia* in 26%. Furthermore, there was a significant positive link between the duration of gMMP and the severity of erosive gingivitis ($p = 0.009$), and the loss of deep periodontal tissue ($p = 0.04$).

Conclusion: This pilot study suggests a high periodontal risk in gMMP patients. The pathological processes, autoimmune on the one hand and plaque-induced on the other, may amplify each other. The application of periodontal therapy is therefore necessary in parallel with medical treatment. Nevertheless, further controlled studies are required to validate and complement these preliminary results.

KEYWORDS

autoimmune blistering disease, cicatricial pemphigoid, dentistry, mucous membrane pemphigoid, periodontal dysbiosis, periodontitis, periodontology

1 | INTRODUCTION

Subepidermal autoimmune blistering diseases (AIBD) are generated by autoantibodies directed against the components of dermal-epidermal/chorio-epithelial junction systems.[1] They are characterized by the formation of subepidermal/epithelial blisters that alter the structure and function of the squamous epithelium. Among these diseases, some have mucocutaneous expression involving the oral mucosa.[1] This is the case of mucous membrane pemphigoid (MMP), which is a rare disease (70 new cases per year in France), expressed in 80%–90% of cases by affecting the gums, which may be inaugural, predominant or isolated.[2] The clinical presentation in the acute phase is therefore that of a postbullous erosive gingivitis (EG), called desquamative gingivitis,[3] localized or generalized, algetic, and hemorrhagic, that impedes oral hygiene actions and restricts eating.[4,5] Furthermore, erosive gingival lesions caused by the auto-immune process are almost systematically associated with gingival inflammation caused by dental plaque of variable severity, due to the difficulty patients have in brushing their teeth. Thus, inefficient brushing leads to the formation of dysbiotic biofilms that initially occupy the gingival crevice and induce gingivitis. They can then progress along the surface of the roots and cause periodontal pockets and alveolysis that characterizes periodontitis. Since the periodontal pockets cannot be reached by brushing by the patient, they are colonized by microorganisms that find a favorable environment leading in particular to the development of pathogenic, anaerobic, gram-negative bacteria, with proteolytic activity and a strong invasive capacity.[6] The latter includes certain bacteria qualified as major periodontal pathogens (*Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*) as they are capable of modifying their virulence as a function of their environment, penetrating between tissues from the internal wall of the periodontal pockets and locally breaking down immune tolerance mechanisms, by secreting numerous toxins and enzymes.[7] For example, Arg-specific gingipains from *Pg*, factor H-binding protein from *Td*, and karilysin *from Tf* can alter the efficiency of the complement system to prevent the generation of the membrane attack complex.[8–10] However, in gMMP presenting erosive gingivitis, the amount of dental plaque, gingival inflammation, the depths of periodontal pockets and the clinical attachment losses are greater compared to the same values for control patients.[11,12] This is all the more true since the diagnosis of blistering disease is often delayed.[13] These data explain why some authors consider gMMP as a potential risk factor of periodontal diseases induced by dental plaque and vice-versa.[14] The areas of eroded gums caused by the autoimmune process may favor the virulence of the periodontal pathogens of dental plaque. Indeed, the accessible conjunctive surfaces are rich in nutrients and are propitious to intratissular translocations due to the loss of integrity of the oral gingival epithelium. In addition, the dysbiotic periodontal microbiota may aggravate the gingival lesions of the gMMP, either directly by activating inflammatory routes, or indirectly by degrading cellular and extracellular matrix components.[15,16]

Indeed, in response to bacterial aggression, the host's periodontal tissue cells (epithelial, endothelial, inflammatory, connective) secrete a large quantities of different metalloproteinases, including the metalloproteinases 2 and 9,[17] which, according to Hiroyasu et al. (2019)[18] could be involved in the pathological mechanism that alters the adhesion of the basal keratinocytes with the matrix constituents of the basal membrane. The intensive activation of the interleukin 23/interleukin 17 (IL23/IL17) axis in periodontitis could, in part, explain the increased secretion of these proteases within the inflamed gingival chorion.[19] The main function of IL23, secreted by activated dendritic cells and macrophages, is to regulate the differentiation of native CD4(+) T cells into T helper 17 (Th17) cells. The latter produce tumor necrosis factor alpha (TNF-α) and IL17, which can stimulate fibroblasts, endothelial cells, macrophages and epithelial cells to secrete numerous metalloproteinases and pro-inflammatory cytokines (interleukins 6, 8, 1β).[19] The controlled immunohistochemical study by Matarese et al. (2013)[20] also shed further light. They demonstrated increased expression of the vascular endothelial growth factor (VEGF) alongside decreased expression of the transforming growth factor β (TGFβ) in the gingiva and periodontal ligament of patients with periodontitis. VEGF is an essential factor in the hypervascularization of the inflamed tissues and TGFβ is known for its immunosuppressive activities and its role in protecting keratinocytes against the oxidative stress.[21] According to these authors, the different expression of these two cytokines helps to maintain the inflammatory cascades that lead to the destruction of the periodontal tissues in periodontitis. Moreover, elevated serum levels of C-reactive protein, α1-antitrysin and pro-B-type natriuretic peptide (NT-proBNP) have been shown to correlate with the severity of untreated periodontitis.[22] An overexpression of these pro-inflammatory mediators would increase the chronic risk of systematic inflammation and endothelial cell dysfunction, resulting in increased production of cytokines, chemokines, cell adhesion molecules and upregulation of reactive oxygen species.[23]

This exacerbated pathological mechanism could therefore explain, at least partially, why gingival cicatrization is generally incomplete despite the application of medical treatment based on anti-inflammatory drugs and/or immunosuppressors aimed at stabilizing the gMMP[24] (Figure 1).

Therefore, the characterization of the dysbiotic periodontal microbiota of patients with gMMP could lead to elucidating the etiopathogenic mechanism of this highly specific disease, which to date has still not been elucidated. However, to our knowledge, the data in the literature relating to the bacterial signature of the periodontal microbiota associated with this disease are particularly limited. Only two studies are currently available.[25,26] Consequently, in an initial approach, we wanted to conduct a pilot study to test the hypothesis of an elevated periodontal risk in gMMP patients due to the presence of a dysbiotic periodontal microbiota. We also wanted to verify whether it is relevant to consider gMMP as a risk factor for plaque-induced periodontal diseases, and vice versa.

The main objective of this study was to characterize the subgingival microbiota of gMMP patients, and to highlight a potential link between these microbiological data and the clinical data relating to their bullous disease and periodontal status.

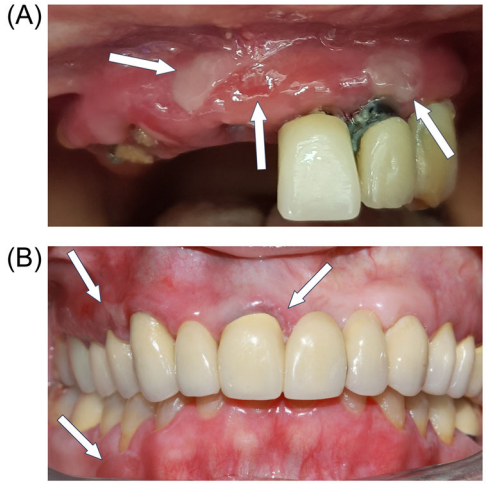

FIGURE 1 Clinical examples of gMMP patients treated solely medically, without associated periodontal treatment. (A) Subject 02.008 treated with dapsone, mycophenolate mofetil, and topical treatment with prednisolone and clobetasol. (B) Subject 01.001 treated with dapsone. In both cases, medical treatment significantly reduced gingival pain, severity, and the extent of erosive gingivitis, but gingival inflammation remained pronounced due to persistent erosion, pseudomembrane, and/or blister (white arrows).

2 | MATERIALS AND METHODS

2.1 | Patient selection

This is a pilot cross-sectional, observational and bicentric study conducted from January 2021 to June 2022. The patients were enrolled in the odontology departments of the university hospital centers of (CHU) Nice (Hôpital Saint Roch) and Créteil (Hôpital Henri Mondor AP/HP). All the patients meeting the selection criteria and accepting inclusion provided a written informed consent form, in conformity with the Helsinki Declaration of the World Medical Association (2002 version). This study was sponsored by the Centre Hospitalier Universitaire de Nice (University Hospital of Nice), subject to regulatory and ethical considerations (registered under number Eudra CT/ID-RCB 2020-A01670-39), and was accredited by the Personal Protection Committee of the Région Sud Méditerranée IV (certification number 20.02499.200909-M02). This test therefore fully complies with the law on Bioethics and the legislative and regulatory provisions of the French Public Health Code. It also obtained the approval of the association of Pemphigus/Pemphigoid Patients of France (Paris, France) and is registered under Clinical Trials number NCT04555681.

The inclusion criteria were the following:

i. adult patients over 18 years of age with acute erosive gMMP in untreated patients, or recurrent or persistent gMMP despite medical treatment;
ii. whatever their state of general health, except patients with ocular or laryngeal pathologies due to the extent of the immunosuppressive treatment implemented in such cases which can influence the microbiological results;
iii. their diagnostic had to be formally established by the attending hospital dermatologist.

The exclusion criteria comprised:

i. the existence of antibiotic or antifungal treatment during the 3 months before the study;
ii. the existence of nonsurgical periodontal treatment in the 3 months before the study;
iii. the patient's refusal to participate in the study and persons subject to legal protection.

2.2 | Calculation of the number of subjects necessary

Since the data in the literature were insufficient for a formal calculation of numbers based on a reliable hypothesis based on figures, we assessed the number of diseased subjects necessary as a function of the active regional files of patients followed-up in the two CHUs concerned by the study. Finally, we estimated this number at 24.

2.3 | Clinical data collection

After the collection of medical data such as age, gender, smoking status, the state of general health, duration of gMMP (definitive diagnostic dating less than or over a year) and the type of topical and/or systemic medical treatment specific to this disease, a full dental-periodontal examination was performed for all the patients by the same experienced periodontist investigator (SMD).

Clinical data associated with gMMP:

- Evaluation of the extent of erosive gingival areas: erosive gingivitis was qualified as generalized (GEG) if it covered more than 30% of the dental sites and localized if otherwise (LEG). For this evaluation, we used the international criteria for assessing the extent of plaque-induced gingivitis, as defined by Chapple et al. (2018).[27]
- Evaluation of type of elementary gingival lesion: erosion, blister, pseudomembrane. We considered the EG as severe if two or three lesions co-existed and not severe if otherwise.

Clinical data associated with the periodontal disease:

- Evaluation of indications of plaque and bleeding: Plaque Control Record (PCR) of O'Leary et al. (1972),[28] Bleeding on Probing (BOP) of Ainamo and Bay (1975).[29] For a denture, the PCR index was used to assess the quantity of supra-gingival dental plaque and the BOP the state of gingival inflammation. A PCR index <20% and a BOP < 10% are clinical criteria associated with periodontal health.

- Evaluation of the number of missing teeth, the depth of periodontal pockets and clinical attachment loss (CAL) using a periodontal probe, PCP UNC 15 (Hu-Friedy®). As a reminder, the depth of a periodontal pocket corresponds to the distance that separates the gingival margin from the bottom of the periodontal pocket and the CAL defines the distance between the cemento-enamel junction and the bottom of the periodontal pocket.[30]
- Evaluation of tooth mobility according to the classification of Lindhe (1998).[31]
- Evaluation of the degree of interdental alveolysis using an orthopantomogram.
- Given the previous clinical criteria, periodontitis was diagnosed in the presence of CAL ≥ 2 mm at the level of two nonadjacent teeth.[32] It was qualified as non-severe if CAL was 3–4 mm (at the most affected site and for at least two nonadjacent teeth) and the interdental alveolysis was ≤ 33%. It was qualified as severe if CAL was ≥5 mm (at the most affected site and for at least two nonadjacent teeth) and the interdental alveolysis was >33% and/or in the presence of a complex severe alveolysis.[32]

2.4 | Sampling dental plaque

For each patient, a sample of subgingival dental plaque was taken by the same investigator (SMD) according to a standardized protocol, only slightly invasive, and widely accepted for microbiological diagnostic tests of periodontal lesions.[33] It was dedicated to the identification by polymerase chain reaction quantitative (q-PCR) of *Candida albicans* and the main bacteria contained in the subgingival plaque belonging to the red, orange, green, yellow, blue, and purple complexes of Socransky[34] (Supporting Information S1: Supplementary Data 1). The chosen protocol was the following: the maximum amount of supra-gingival dental plaque was removed beforehand using a sterile curette, taking care not to injure the gingival margin. Then, using pin tweezers, six points of sterile calibrated paper (Dentsply Sirona®) supplied by the clinical research laboratory for the Institut Clinident SAS (Aix en Provence) were inserted one by one, for 20 s in the three deepest periodontal pockets (two per pocket, per patient). The samples were taken preferentially at the level of pockets located close to the eroded gingival areas (Figure 2). For each patient, the paper points were pooled in a dry, sterile 1.5 mL Eppendorf tube, which was then taken to the clinical research laboratory for the Institut Clinident SAS. Every precaution was taken to avoid contact between the absorbent points, the saliva, and the lingual and cheek mucosa.

2.5 | Identification of microorganisms by q-PCR

The identification protocol was performed by the clinical research laboratory for the Institut Clinident SAS. The total DNA was extracted using the QIAcube® HT Plasticware and Cador® Pathogen 96 QIAcube® HT kits (Qiagen), according to the manufacturer's instructions. The final elution volume was 150 µL. The purity and quantity of DNA extracted was measured with a Nanodrop® spectrometer (Thermo Fisher®). To qualify the total bacterial load (TBL) and that of the target species present in each sample of dental plaque [*Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannnerella forsythia (Tf), Treponema denticola (Td), Fusobacterium nucleatum (Fn), Prevotella intermedia et nigrescens (Pi, Pn), Campylobacter rectus (Cr), Peptostreptococcus micros (Pm), Eikenella corrodens (Ec), Capnocytophaga gingivalis, sputigena, et ochracea (Cg, Cs, Co), Streptococcus mitis (Sm), Streptococcus spp., Actinomyces viscosus et odontolyticus (Av, Ao), Veillonella parvula (Vp), Enterococcus spp., Enterococcus faecalis (Ef), Candida albicans (Ca)*], a quantitative PCR technique was performed using universal primers for the 16S rRNA genes specific to each species. The q-PCR tests were performed in a volume of 10 µL composed of 5 µL SYBR® Premix 2X Ex Taq™ Tli RNaseH Plus (TaKaRa, Shiga, Japon), 2 µL of DNA extract and 0.2 µL of target primer solution. The design of the primers used was derived from sequences of 16S ribosomes and optimized for the q-PCR conditions in real time (Supporting Information S2: Supplementary Data 2). The tests were performed with a Rotor-Gene® Q thermocycler (Qiagen) according to the following program: initial denaturation for 30 s at 95°C (1 cycle), then 40 amplification cycles composed of: step 1 = 10 s at 95°C, step 2 = 10 s at the appropriate hybridization temperature, step 3 = 35 s at 72°C with the fluorescence read at the end of the step in the measurement channel adapted to SYBR Premix 2X Ex Taq™ Tli RNaseH Plus (TaKaRa). Fluorescence was analyzed using Rotor-Gene Q Series software (Qiagen). The specificity of the PCR quantifying the total bacterial load and that of all the species was controlled by analyzing the fusion curve from 70 to 99°C (one cycle) by increments of 1°C with reading of the fluorescence at the end of the step in the measurement channel adapted to SYBR Premix 2×. Serial dilutions of standard bacterial DNA were used for each reaction as the external standard for the absolute quantification of the pathogenic bacteria targeted. The reference bacterial strains whose DNA was used as the standard for the quantitative and

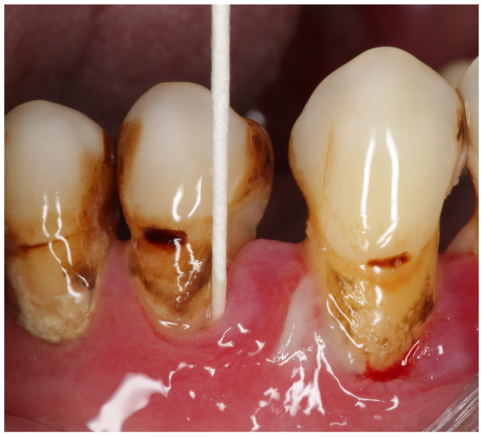

FIGURE 2 Sampling method using a sterile paper point (subject 02.007).

qualitative evaluation of the q-PCR came from microbial collections such as DSMZ, BCMM/LMG and CIP of the Pasteur Institute. The limits of detection (LOD) and quantification (LOQ) were specific to each pathogen (Supporting Information S3: Supplementary Data 2). For each sample and for each microorganism, the results were indicated in absolute quantity and in relative quantity in relation to the total bacterial load present in the sample of dental plaque taken. Then, the association of bacteria whose relative quantity was > at 0.01% was analyzed.

2.6 | Statistical analysis

First, the statistical analysis comprised a flat sort, that is to say a descriptive analysis of the source population and the parameters studied, with an evaluation of the absolute and relative frequencies and their confidence intervals at 95% for the categorial variables, and an evaluation of means and standard deviations, medians and interquartiles for the quantitative variables. We then chose two variables of interest: severe erosive gingivitis and severe periodontitis were crossed one after the other with the other categorial variables using two-sided Chi-square tests when applicable. Failing this, Fischer's exact test was used. The significance threshold was set at 0.05. The software used was SPSS 18.0.

3 | RESULTS

The 24 patients were initially selected between January 2021 and June 2022, but one patient had to be excluded, *a posteriori*, since she had forgotten to mention having taken antibiotic therapy during her inclusion visit. The final sample of patients therefore comprised 23 subjects.

3.1 | General clinical characteristics

Among the 23 patients included, 17 were women and 7 men, aged from 50 to 89 years old (average age 67.4+/−10). Only three patients presented an gMMP in the initial phase. For the 20 other patients, the blistering disease was diagnosed by an attending dermatologist at least 3 months previously and at most 15 years before. Five subjects presented an extra-oral lesion (cutaneous or genital), seven subjects did not present any other disease apart from AIBD, and 16 subjects benefited from medical treatment specific to gMMP (topical and/or general). Moreover, no patient was a smoker (Table 1).

3.2 | General dental-periodontal characteristics

(Table 2) Fourteen patients presented an LEG and nine a GEG. For all the patients, the plaque index (index PCR) and bleeding on probing (BOP) were particularly high, varying from 48% to 100% (mean > 83%) and from 20% to 98% (mean > 54%) respectively. Eleven patients presented severe periodontitis, 11 nonsevere periodontitis and one, the youngest, severe gingivitis induced by plaque. The results showed a significant positive link between the duration of the gMMP (diagnostic ≥ 1 year) and the severity of the EG on the one hand ($p = 0.009$ Pearson's chi-square test) and the severity of the periodontitis on the other ($p = 0.04$, Fischer's exact test). However, we did not find a significant relation between the severity of the EG and that of the periodontitis or between these two variables and the other medical data: age of patients, existence or not of a treatment specific to gMMP (topical or general), the existence or not of an extra-oral lesion or a concomitant general pathology, or the extent of the EG.

3.3 | Bacterial identification by q-PCR

Our study was performed during the COVID 19 health crisis. Due to administrative reasons, only the samples of the subgingival biofilm of 15 patients could be analyzed by the clinical research laboratory for the Institut Clinident. For 14 of these patients, the absolute quantity of subgingival dental plaque was particularly abundant, $\geq 10^{10}$, whatever the characteristics: (1) of the MBAI (old or not, treated or not); (2) of the EG; or (3) of the associated periodontitis. Only one patient, with an initial gMMP and a nonsevere periodontitis, presented a slightly lower quantity ($9.60.10^{9}$).

Regarding the periodontal pathogenic bacterial species (Socransky red and orange complexes), the results showed that the latter were clearly present in the subgingival plaque of the patients (Figure 3).

Overall, for red complex bacteria (*Tf*, *Td*, *Pg*), which are particularly periodontal-pathogenic, the relative quantities were ≥0.01%, for at least 1 bacterium for 15 patients, 2 bacteria for 11 patients/15 and 3 bacteria for 4 patients/15. For orange complex bacteria *(Pi*, *Pm*, *Fn*, *Cr*, *Pn*), less periodontal-pathogenic, the relative quantities were ≥ 0.01% for 3 bacteria for the 15 patients and 4 bacteria for 12 patients/15 (Supporting Information S3: Supplementary Data 3). *Pn* was not detected in any patient and *Aa* was detected in only one patient.

Regarding bacterial associations, our results showed the existence of three main consortiums:

- consortium 1 comprised *Tf*, *Pm*, *Fn*, *Cr*: 100% of patients were positive for this group,
- consortium 2 comprised *Tf*, *Td*, *Pm*, *Fn*, *Cr*: 60% of patients were positive for this group,
- consortium 3 comprised *Pg*, *Tf*, *Td*, *Pi*, *Pm*, *Fn*, *Cr*: 26% of patients were positive for this group.

Not all of these results were influenced by medical data such as duration of gMMP, the absence of, or the type of, medical treatment specific to this disease, the existence of concomitant general

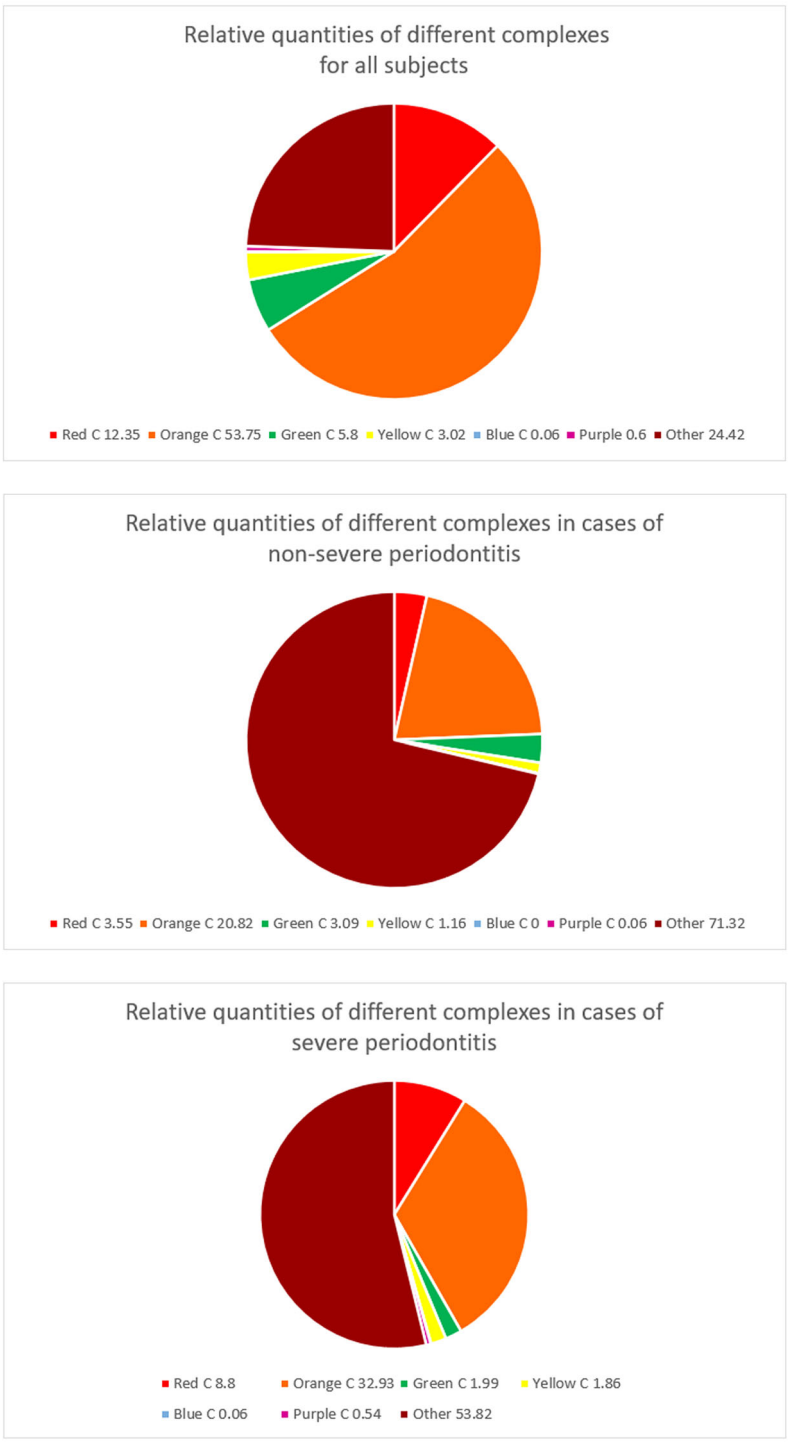

FIGURE 3 Relative quantities of different complexes. (A) For all subjects. (B) In cases of nonsevere periodontitis. (C) In cases of severe periodontitis. Red and orange complexes are particularly prevalent in cases of severe periodontitis, regardless of whether patients are receiving medical treatment.

pathologies and their associated medications. When periodontal criteria were considered, we were unable to demonstrate any association between severe periodontitis and the consortium 3 ($p = 0.05$).

Regarding the microorganisms of the green, yellow, blue and purple complexes, our results showed (Supporting Information S4: Supplementary Data 4):

- that 100% of patients (15/15) and 87% of patients (13/15) presented relative quantities ≥0.01% for all the species of the streptococcus genus (yellow complex) and for *Ec* (green complex), respectively,
- that 67% of patients (10/15) and 60% of patients (9/15) presented relative quantities ≥0.01% for Cs and Co (green complex), respectively.

Lastly, regarding *Ca*, this fungus was detected in 5 patients/15, all treated for their gMMP. However, the relative quantities were only >0.01% in two patients.

4 | DISCUSSION

The main objective of this study was to characterize the subgingival microbiota of the gMMP patients, given the lack of scientific data on this subject. Our results showed that the patients included had a deteriorated periodontal condition, whether or not they were medically treated, which was linked to the existence of a dysbiotic periodontal microbiota. In addition, there was a significant positive association between the duration of gMMP, the severity of erosive gingivitis and the loss of deep periodontal tissue.

On the periodontal level, our study showed that all our gMMP patients presented considerable subgingival plaque (from $9.6.10^9$ to $3.4.10^{10}$). By way of comparison, using a comparable sampling technique, Nonnenmacher et al. (2005)[35] and Field et al. (2012)[36] found at least 100-fold lower absolute quantities of subgingival dental plaque in the case of moderate to severe periodontitis in adults in good general health. This situation can be explained by poor tooth brushing performed by patients, since subgingival dental plaque stems from supra-gingival dental plaque. Most of the time, these patients justify their difficulty due to the gingival pain they feel, or their fear of aggravating gingival lesions and/or bleeding gums. Nonetheless, the quantitative bacterial dimension cannot explain, alone, the periodontal characteristics of these subjects. A specific bacterial profile may provide a second explanation. Indeed, the increase of bacterial mass may promote the development of a dysbiotic film and in particular lead to the colonization of the gums by several periodontal pathogens. Owing to the lack of information on this subject, we felt it advisable to seek in the subgingival dental plaque the presence of 21 species of bacterial complexes according to Socransky,[34] by above all considering their relative quantities. Indeed, the absolute quantity of a microorganism above all allows specifying whether it is present in a sample of dental plaque. Its relative quantity facilitates appreciating its proportion in comparison to the other microorganisms present in the same sample of plaque. In addition, in our study, only relative quantities ≥ 0.01% were taken into account. We opted for this low threshold value because certain periodontal diseases can express their virulence characteristics as from this limit, in other words even if their relative quantity is low in comparison to the global biomass.[37,38]

Regarding the species of the Socransky complexes, they can be detected in a symbiotic biofilm compatible with periodontal health,[39] but when the physiochemical properties of their environment become favorable to their growth, several of them maintain synergetic cooperations allowing them to develop virulence factors and become predominant within the biomass.[40] Among these species, those of the orange and red complexes are involved in the etiopathogenesis of periodontitis.[34,41–43] Thus, our results showed that the bacteria of these two complexes were largely represented in relation to species of other complexes in the subgingival biofilm of 15 gMMP patients, whatever the characteristics of their blistering disease and those of the associated erosive gingivitis. Our study also showed the existence of three main dysbiotic, periodontal, pathogenic bacterial consortiums.

Consortium 1, comprising *Tf*, *Pm*, *Fn*, *Cr*, was present in 100% of patients, whatever their state of health, whether or not they were treated medically, and whatever the extent and severity of the EG and the severity of the associated periodontitis. *Tf*, which belongs to the red complex, secretes numerous proteases capable of degrading the extracellular matrix of the gums and possesses several virulence factors, including lipoproteins, that stimulate the production of pro-inflammatory cytokines by fibroblasts such as interleukin 6 (IL6) and tumor necrosis factor alpha (TNF-α).[7,44] IL-6 can modulate the immune response because it is involved in the differentiation of cytotoxic T lymphocytes and B lymphocytes. The other bacteria belong to the orange complex. *Fn* plays a key role in bacterial co-aggregations[45] and has numerous virulence factors capable of activating inflammatory response and bone resorption.[46] Furthermore, this bacterium can maintain a mutualistic relationship with *Tf*.[47] *Pm* is capable of adhering to epithelial cells and can also bind to *Fn*.[48,49] Lastly, although the periodontal activity of *Cr* has not been fully elucidated, the predominance of this bacterium in relation to other species of the Campylobacter genus is linked to the progression of periodontitis.[50]

Consortium 2, enriched with *Td*, was present in 60% of patients. Td belongs to the red complex. This microorganism has numerous virulence factors that cause the degradation of epithelial junctions, fibrinogen, and matrix macromolecules. Moreover, these factors regulate the migration and function of neutrophiles and facilitate bacterial co-aggregations and the penetration into the epithelial layers of other periodontal pathogenic bacteria with which they cohabit.[51,52]

Consortium 3, detected in 26% of the patients, is composed of 3 bacteria of the red complex (*Tf*, *Td*, *Pg*) and the main ones of the orange complex (*Pi*, *Pm*, *Fn*, *Cr*). It is mainly found in the presence of severe periodontitis. *Pg* is most often identified in this consortium. Its virulence is determined by its capacity to invade many types of cells, including the keratinocytes, and its highly efficient spectrum of enzymatic activities in an inflammatory environment.[53] According to Takeuchi et al. (2022),[54] the gingipains of Pg are capable of degrading epithelial adhesion molecules (JAM1 and CXADR) and therefore damage the permeability of mucosal epithelial cells. This mechanism facilitates the diffusion of the bacterium's enzymatic material to deep epithelial layers, as well as that of other microorganisms with which it is associated. In addition, in an animal model, Gasiorek et al. (2021)[55] showed that the proteolytic activity of the gingipains of *Pg* causes the degradation of Monocyte Chemotactic Protein-Induced Protein 1 (MCPIP-1), expressed constitutively by gingival keratinocytes to prevent the hyperactivity of the gums in response to bacterial aggression. The degradation of MCPIP-1 causes increased sensitivity to bacterial endotoxins and thus excessive inflammatory response favoring the growth of inflammophilic bacteria and thus the deterioration of the tissues supporting the teeth. Therefore, all these data may explain why the association of *Pg* with the two other red complex bacteria is considered a marker of the progression of periodontitis.[7,56]

Regarding the other microorganisms belonging to the yellow, green, blue, and purple complexes, they are not considered to be

directly periodontally pathogenic. Whatever the case, their quantification appears necessary since, as they are early colonizers of dental surfaces, they initiate gingival inflammation and participate in interbacterial cooperations, leading to the breakdown of the symbiotic equilibrium. Among all these bacterial species, our results show that those belonging to the yellow and green complexes were the most abundant. The bacteria of the yellow complex are involved in the occurrence of caries. However, we cannot hypothesize on the existence of a risk of caries in our patients since these cariogenic species are above all present in the supra-gingival plaque whose quality was not assessed in our study. Regarding the green complex bacteria, *Ec* was that represented most. Its role remains to be specified. However, its pathogenic potential is not insignificant, as in vitro, this bacteria is able of stimulating the secretion of MMP2 by gingival fibroblasts,[57] which could promote the penetration of major periodontopathogenic bacteria into the inflamed gingiva.

In parallel, we thought it interesting to seek the presence of *Ca* in the subgingival plaque of the patients. This microorganism belongs to the symbiotic microbiota; however, in the presence of soft inflamed tissue or if the oral ecosystem is disturbed by a corticoid-based drug, this fungus can acquire virulence characteristics that allow it to colonize the epithelia and form perfectly viable biofilms in the periodontal pockets.[58] Furthermore, recent studies have shown that the pseudo-filaments of *Ca* serve as attachment sites for *Fn*, itself involved in heterotypical bacterial aggregations.[59] Our results showed that this microorganism was detected in 5 patients/15, who were treated medically and presented a particularly degraded periodontal state. Nonetheless, we cannot hypothesize on a specific fungal infection in the case of gMMP since we did not verify under an optical microscope whether *Ca* was present in the subgingival dental plaque in pathogenic form (pseudo-filament). However, this risk should not be underestimated in the case of a topical corticoid-based treatment.

Taken altogether, our data make it possible to put forward two working hypotheses for further research.

First, the degradation of gingival tissue initiated by the autoimmune process may aggravate periodontal conditions before the onset of desquamative gingivitis, by generating erosive gingival areas providing sources of nutrients and propitious for the translocation of periodontal pathogenic bacteria in the chorion membrane. It is possible to envisage that the modification of the environment of bacterial biofilms can impact the expression of the virulence genes of bacteria, thereby influencing their metabolic activity, their competitiveness and, finally, their respective quantities. The quality of the host response would determine the result of the pathological process.

Second, the degradations of tissues caused by periodontal pathogenic bacteria may aggravate the local immune response. Feedback loops progressively create a vicious circle to the detriment of the gingival tissue targeted by the autoimmune system. This second hypothesis explains the link found between the duration of the MMP and the severity of the EG or periodontitis.

Our results confirm those presented by Lo Russo et al. (2014)[25] in their cross-sectional study of 12 patients with desquamative gingivitis including 8 in the framework of gingival lichen planus and 4 in the framework of MMP. These authors showed, using q-PCR, the presence of a large quantity of *Pg*, *Tf*, *Td*, *Fn*, and *Pi* in the subgingival plaque of diseased patients, whatever the general pathology and whether the gingival sites were erosive or not. The controlled study of Arduino et al. (2017)[26] presented other results. This study included 33 patients with desquamative gingivitis including 19 in the framework of gingival lichen planus and 14 in the framework of MMP. These authors were able to compare, through a nonquantitative PCR analysis, the rates of 11 periodontal pathogenic bacteria of the subgingival plaque between the erosive and non-erosive sites of diseased subjects versus control sites of subjects in good general health with gingivitis caused by dental plaque. Regarding the diseased sites, they showed clearly higher rates of *Aa*, *Fn*, *Fusobacteium periodonticum* and *Ec*. However, a statistically positive association was found only between desquamative gingivitis and *Ec* (OR: 12.78). In addition, the authors recorded comparable rates for the three red complex bacteria whatever the sampling site. However, it is important to point out that the periodontal conditions were not specified as a function of general ill health and that the evaluation of the microbiological results was only semi-quantitative. Furthermore, for these two studies, the microbiological results were provided only as a function of the nature of the sites sampled.

Our bicentric study nonetheless presents certain limitations. There was no control group and the number of subjects included was low since gMMP is a rare disease. Moreover, the bacterial evaluation could only be carried out for 15 patients out of a total of 23. It concerned only several species and therefore did not reflect the richness of the microorganisms living in the periodontal microbiota.

However, our study raises the possibility of a high periodontal risk in gMMP patients, notably in the presence of a concomitant periodontitis. This possibility is fully compatible with the latest scientific data that show that there is not only one model of dysbiosis but several according to the individuals concerned. Each model reflects the quality of the host's immune response. Thus, the diversity of these models mirrors the clinical heterogeneity of periodontal diseases.[60] Based on the results of this study, microbiological analyzes using a high-throughput metagenomic approach should be carried out in gMMP patients in the future.

Initially, these should enable us to better characterize the periodontal dysbiosis of these subjects. Indeed, it is not the presence of a few micro-organisms in particular in dental plaque that causes the symbiotic balance to break down, but rather the existence of numerous inter-species cooperations whose synergistic activities encourage the growth of virulent pathogenic bacteria that eventually become predominant.[61] Research into the existence of a link between periodontitis and other autoimmune inflammatory diseases makes this approach relevant. For example, in rheumatoid arthritis, clinical studies have shown that the oral microbiota of patients, compared with controls, was enriched in periodontopathogenic pathogens, including *Porphyromonas gingivalis*.[62] This bacterium is thought to be able of inducing joint changes during the preclinical inflammatory phase via one of its virulence factors, peptidylarginine

desiminase (PAD), which converts the arginine associated with a peptide into peptidylcitrulline. The pathological mechanism behind a breakdown in immune tolerance is thought to be based on the hypercitrullination of numerous bacterial and host proteins within periodontal pockets. The presentation of these citrullinated proteins to lymphocytes by antigen-presenting cells then leads to the production of anti-citrullinated peptide antibodies. Finally, the vascular diffusion of antigens, antibodies and immune complexes to the joints is thought to promote local activation of osteoclasts and low-noise inflammation.[62]

Second, these metagenomic studies could eventually reveal a relationship between the oral and intestinal microbiota of gMMP patients, as observed by Imai et al. (2021)[63] for Crohn's disease. The prospective, controlled study they conducted showed that patients had oral dysbiosis in addition to intestinal dysbiosis, with significant quantitative and qualitative differences in their microbiota compared with control subjects. In addition, the authors were able to observe that, unlike in control subjects, the two types of microbiota were very similar in diseased patients, and that the presence of periodontitis altered their composition at 12 months, with a concomitant worsening of digestive inflammatory conditions. For these authors, the components of the oral and/or intestinal microbiota could negatively influence host immunoregulation, in particular the differentiation of effector T cells, with a probable impact on susceptibility to oral and extra-oral inflammatory diseases. Although there is no proof of cause to date, this new microbiological concept could be considered in gMMP patients, as erosive gingival areas and periodontal pockets represent two ecological niches that are favorable to the extension of microbial reservoirs. Indeed, from these two tissue entry points, periodontopathogenic bacteria or their secretion products can remotely induce metastatic infections via the hematogenous route or by invading host cells.[64] The controlled study by Kawamoto et al. (2021)[65] reinforces this hypothesis. These authors found that in patients in good general health but suffering from severe periodontitis, there was concomitant oral dysbiosis and intestinal dysbiosis. However, these authors were unable to correlate these two dysbioses directly. They could very well be linked to a particular susceptibility of the host or be triggered by a translocation of oral pathobionts to the intestine and vice versa.

This future metagenomic research could also help to improve the clinical, preventive and curative management of gMMP in cases of associated periodontitis, by assessing the bacterial changes generated by conventional and complementary periodontal treatments, which are thought to modulate the oral microbiota, and a fortiori the periodontal microbiota.

The randomized controlled study by Scribante et al. (2023)[66] showed, for example, that daily subgingival application of an ozone gel for a fortnight in the context of severe periodontitis, in addition to conventional nonsurgical periodontal therapy, improves the main clinical parameters (reduction in periodontal pocket depths and attachment gain) at 6 months. According to these authors, ozone-based antiseptic products enhance the healing of mucosal wounds by reducing the oxidative activity of inflammatory cells and the spread of bacterial biofilms within periodontal pockets, which are particularly anaerobic. Similarly, the use of photobiomodulation (PBM) could be considered for MMP patients, to reduce oxidative stress and potentiate oxygen supply to gingival tissues targeted by the autoimmune process. The randomized controlled trial conducted by Mohamed et al. (2024)[67] in patients with erosive oral lichen planus is a step in this direction. These authors were able to show that the use of PBM using a 980 nm diode laser (300 mW, 1.2 J) produced beneficial effects comparable to those obtained after topical application to erosive mucosa of a gel containing 0.1% triamcinolone acetonide. At 12 weeks, both treatments significantly reduced pain, the extent of erosive mucosal areas and the salivary levels of malondialdehyde (MDA), considered to be a marker of oxidative stress. Ultimately, these beneficial effects would enable patients to perform their hygiene maneuvers more effectively and thus indirectly reduce the overall bacterial load.

In addition, the consumption of probiotics by gMMP patients could help to balance the bacterial populations of their periodontal microbiota. Invernici et al. (2018)[68] showed that twice-daily oral intake of a probiotic consisting of Bifidobacterium animalis subsp. lactis HN019, for 30 days, by patients with severe periodontitis who were also receiving professional subgingival debridement, reduced the quantities of the main bacteria in Socransky's orange and red complexes and significantly increased the crevicular level of anti-inflammatory IL10, at 1 month compared with controls. At the same time, this probiotic treatment promotes the expression by gingival epithelial cells and fibroblasts of β-defensins (BD)-3 and clusters of differentiation 284 (Toll-like receptor 4), 4 (coreceptor for the T-cell receptor) and 57 expressed by Natural Killer Cells.[69]

5 | CONCLUSION

Finally, and to the best of our knowledge, our study is the most in-depth to date regarding the composition of the periodontal microbiota of gMMP patients. It highlights the importance of a global evaluation of periodontal conditions in these subjects to propose early personalized periodontal therapy in parallel with medical treatment (Figure 4). In practice, the odontologists who care for these patients generally formulate their therapy by extrapolating

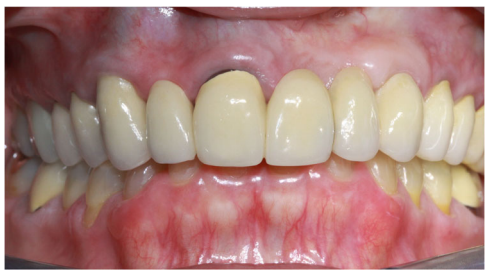

FIGURE 4 Same patient as in Figure 1B, after periodontal treatment had been introduced, gingival healing was much more satisfactory.

TABLE 1 General clinical features of subjects.

			Clinical criteria related to MMP				
Patient	**Gender**	**Age (y)**	**MMP duration**	**Diagnostic delay**	**General treatment Topical treatment**	**Extra-oral involvement**	**Other concomitant medical conditions**
01.001	F	60	5 m	6 m	General (Dapsone) No	No	No
01.002	F	74	4 y	NS	No No	No	Parkinson's disease, Arterial hypertension
01.003	M	66	Initial	NS	No No	No	Gastro-esophageal reflux
01.004	M	63	6 m	3 m	No No	Skin	Arterial hypertension
01.005	F	65	11 m	1 y	General (Dapsone) No	No	No
01.006	F	65	>6 y	1 y	General (Dapsone) No	No	*Hashimoto's* thyroiditis Arterial hypertension
01.007	F	50	9 m	1 y	No No	No	No
01.008	F	58	6 m	NS	No Topical (Prednisolone)	No	Colopathy, Raynaud's disease, Lymphopenia (undetermined origin)
01.009	F	90	6 m	2 y	General (Prednisolone) No	Skin	Venous and aortic insufficiency Hypothyroidism, Arterial hypertension
01.010	F	77	6 m	1 y	No No	Skin	Non-insulin-dependent diabetes
02.001	F	67	11 y	2 y	General (Dapsone) Topical (Clobétasol)	No	Glucose intolerance, Arterial hypertension
02.002	F	73	7 m	NS	General (Dapsone, Mycophenolate Mofetil) Topical (Betamethasone)	No	Arterial hypertension
02.003	F	78	3 m	4 m	General (Dapsone) Topical (Prednisolone)	No	Hairy cell leukemia
02.004	F	62	>3 y	NS	General (Dapsone) No	No	Gilbert's syndrome Vulvar Lichen Planus
02.005	F	70	5 y	NS	No Topical (Betamethasone, Clobetasol)	No	No
02.006	M	59	3 m	> 2 y	No Topical (Prednisolone)	No	Hiatal hernia, Gastro-esophageal reflux
02.007	M	56	Initial	4 m	No No	No	No
02.008	M	89	7 y	NS	General (Dapsone) Topical (Prednisolone, Clobetasol)	Skin	Arterial hypertension, *IgG4-related diseases*, Pernicious anemia
02.009	F	73	> 2 y	6 m	General (Dapsone, Mycophenolate Mofetil) Topical (Prednisolone, Clobetasol)	No	Non-insulin-dependent diabetes, Dyslipidemia, Arterial hypertension
02.010	M	57	8 m	NS	General (Dapsone) Topical (Prednisolone)	No	Chronic migraine

TABLE 1 (Continued)

Patient	Gender	Age (y)	Clinical criteria related to MMP				Other concomitant medical conditions
			MMP duration	Diagnostic delay	General treatment Topical treatment	Extra-oral involvement	
02.011	F	67	Initial	NS	No No	No	No
02.012	F	74	> 2 y	NS	General (Dapsoone) Topical (Prednisolone, Clobetasol)	No	No
02.013	M	58	15 y	> 1 y	General (Dapsone) No	Genital	Arterial hypertension, Deafness, Osteopenia

Abbreviations: F, female; M, male; m, month; MMP, mucous membrane pemphigoid; NS, not specified; y, year.

TABLE 2 Dento-periodontal conditions of subjects.

Patient	PCR % BOP %	PDCALAverage mm (deepest site mm)	Plaque-induced periodontal disease	Tooth damage	Gingival lesion: E, B, Ps, extent of gingivitisOther oral mucosa affected
01.001	98 40	1.9 (4) 3 (4)	NSP, localized	Two dental decay one endodontic lesion	E + B + Ps, generalized No
01.002	89 60	2.1 (3) 3.9 (6)	SP, generalized	No	E, generalized No
01.003	92 60	2 (3) 2 (3)	NSP, localized	No	E + Ps, localized No
01.004	98 70	2 (4) 3 (4)	NSP, generalized	No	E + B + Ps, generalized No
01.005	82 56	2.1 (4) 2.4 (5)	NSP, generalized	No	E + B; localized No
01.006	64 20	2.2 (5) 3 (5)	SP, generalized	No	E; localized No
01.007	58 20	1.8 (4) 3 (4)	NSP, generalized	No	E; localized No
01.008	60 21	2 (4) 2.1 (4)	NSP, generalized	No	E; localized No
01.009	96 70	1.9 (3) 3.7 (8)	SP, generalized	No	E + B + Ps; generalized Edentulous ridge, alveolar mucosa
01.010	100 80	1.7 (3) 2.4 (5)	NSP, generalized	One dental decay	E + Ps; generalized Cheek
02.001	85 34	2.4 (4) 3.2 (6)	SP, generalized	Two dental decay	E, localized No
02.002	68 20	2.2 (3) 3 (4)	NSP, generalized	No	E, localized No
02.003	100 68	2.6 (5) 3,1 (6)	SP, generalized	No	E + Ps; generalized Palate, tongue
02.004	75 45	2.1 (7) 3.3 (8)	SP, generalized	No	E, localized No

(Continues)

TABLE 2 (Continued)

Patient	PCR % BOP %	PDCALAverage mm (deepest site mm)	Plaque-induced periodontal disease	Tooth damage	Gingival lesion: E, B, Ps, extent of gingivitisOther oral mucosa affected
02.005	100 53	3 (6) 4.9 (9)	SP, generalized	Two dental decay one endodontic lesion	E + Ps, localized No
02.006	62 48	2.5 (6) 3.2 (8)	SP, generalized	No	E + B, localized No
02.007	100 98	3.8 (9) 5.6 (11)	SP, generalized	No	E + Ps, generalized Palate, cheek, edentulous ridge
02.008	100 52	2.3 (3) 4.3 (7)	SP, generalized	Six dental decay six endodontic lesions	E, localized No
02.009	100 70	3.1 (6) 3.4 (10)	SP, generalized	Two endodontic lesions	E, generalized No
02.010	96 85	2.4 (5) 2.7 (5)	NSP, generalized	One dental decay	E, B, localized No
02.011	100 98	2.1 (5) 2.4 (5)	NSP, generalized	One dental decay	E, generalized No
02.012	52 60	2.2 (4) 2.5 (5)	NSP, generalized	No	E, localized No
02.013	48 25	1.7 (3) 1.7 (3)	GIP, generalized	No	E, localized No

Abbreviations: B, blister; BOP, bleeding on probing; CAL, clinical attachment loss; E, erosion; NSP, nonsevere periodontitis; PCR, plaque control record; PD, probing depth; PIG, plaque-induced gingivitis; Ps, pseudomembrane; SP, severe periodontitis.

the bacteriological data of patients affected only by periodontal diseases caused by dental plaque and not by any general disease. Therefore, understanding the impact of the periodontal microbiota in the case of gMMP would make it possible to optimize therapeutic protocols and better program individual periodontal follow-up. Nonetheless, controlled metagenomic studies are required to validate and complete our preliminary results.

AUTHOR CONTRIBUTIONS

Anne-Laure Ejeil: Methodology; supervision; writing—review and editing. **Frédérick Gaultier**: Funding acquisition; investigation; methodology; writing—review and editing. **Bisson Catherine**: Writing—review and editing. **Franck Chaubron**: Methodology; resources; writing—review and editing. **Laurence Lupi**: Methodology; software; writing—review and editing. **Sophie-Myriam Dridi**: Conceptualization; data curation; methodology; project administration; validation; visualization; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

Doctor Frédérick Gaultier and Professor Sophie-Myriam Dridi declare a conflict of interest in relation to this study as they are active members of the association that funded it. However, these authors also certify that the source of support/financial relationships had no impact on the conduct of the study or the interpretation of the data. The other authors declare no conflict of interest in relation to this study or the ethics approval statement.

DATA AVAILABILITY STATEMENT

All authors confirm that all data generated or analyzed during this study are included in this published article (and its supplementary information files).

ETHICS STATEMENT

This work was sponsored by the Centre Hospitalier Universitaire de Nice (University Hospital of Nice), subject to regulatory and ethical considerations (Eudra number CT/ID-RCB 2020-A01670-39), and was accredited by the Personal Protection Committee of the Région Sud Méditérranée IV, France (accreditation number 20.02499.200909-M02).

TRANSPARENCY STATEMENT

The lead author Sophie-Myriam Dridi affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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