The clinical, microbiological and systemic characteristics of periodontitis and their changes after periodontal therapy

Bizzarro, S.

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CHAPTER 8

General discussion

The aim of this thesis was to explore the systemic, clinical and microbiological characteristics of periodontitis and their changes after periodontal therapy.

Regarding the systemic aspect, this thesis focuses on prothrombotic state, which is a pathological tendency of blood to coagulate. This condition, together with atherosclerosis and endothelial dysfunction, is at the basis of the underlying mechanism of the development of acute cardiovascular events. Under normal conditions, coagulation (a cascade of events which lead to the formation of a blood (micro)thrombus in case of the presence of vessel wall damage) and fibrinolysis (the mechanism which degrades a (micro)thrombus once it has been formed) are in balance. Whenever a disturbance of this balance occurs (either an up regulation of the coagulation and/or a down regulation of fibrinolysis), the result is a prothrombotic state (Winckers et al. 2013). In the perturbation of the coagulation-fibrinolysis system, inflammation plays a central role. Inflammatory mediators including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), are capable to trigger at different levels the hemostatic system: an increase of systemic inflammation leads to endothelial cell dysfunction, activation of platelets and activation of tissue-factor mediated coagulation, impaired function of anticoagulants and suppressed fibrinolytic activity (Margetic 2012, Samad & Ruf 2013).

Since periodontitis has been demonstrated to induce a mild systemic pro-inflammatory state (D'Aiuto et al. 2013, Paraskevas et al. 2008, Teeuw et al. 2014), a case control study, described in chapter 2 and 3, has been undertaken in order to investigate a possible association between periodontitis and markers of prothrombotic state. The following markers of coagulation and fibrinolysis were investigated: prothrombin fragment 1+2 (F1+2), von Willebrand Factor (vWF), Plasminogen activator inhibitor-1 (PAI-1) and D-dimer (Margetic 2012).

In chapter 2, the results of the study showed that PAI-1 activity was elevated, especially in patients with severe periodontitis, also after correction for known confounders. PAI-1 is an acute-phase reactant produced mainly by hepatocytes and endothelial cells but also by adipocytes. Although there is consistent evidence about its role in wound healing in periodontal tissues (Kinnby 2002, Wyganowska-Swiatkowska et al. 2014), the association of periodontitis with serum levels of PAI-1 was hitherto less clear. Based on the current results, it could be speculated that, in this study population, the periodontal inflammation together with a possible role of visceral fat and dyslipidemia, was responsible for the elevation of PAI-

1 activity. This speculation is supported by the finding that body mass index (BMI) and triglycerides, were significant confounding factors in the statistical model.

In chapter 3, in a subset of patients of the same population that participated in the study of chapter 2, it was investigated whether the host response to infection with *Aggregatibacter actinomycetemcomitans* or *Porphyromonas gingivalis* could be partly explanatory for the increased levels of PAI-1. In the periodontitis patients, no association between serum IgG antibody levels against the two periodontal pathogens and PAI-1 was found. However, a correlation was observed between levels of vWf and levels of anti-*A. actinomycetemcomitans* IgG antibodies, but not with anti-*P. gingivalis* IgG antibodies. It could be speculated that, in periodontitis patients, infection with *A. actinomycetemcomitans* may contribute to higher levels of vWF.

Obesity, expressed by BMI and waist circumference, has been strongly associated with prothrombotic state (Blokhin & Lentz 2013). This association was also confirmed by our results presented in chapter 2 and 3, where BMI was consistently associated with the markers of the prothrombotic state. Obesity is also an important risk factor for metabolic syndrome, together with hypertension, dyslipidemia and hyperglycemia. Patients with metabolic syndrome have an increased risk for diabetes mellitus type II and cardiovascular diseases (Alberti et al. 2009, Gami et al. 2007). Inflammation is considered to play an important role for the worsening of the metabolic status since low-grade inflammation can decrease the capacity of adipocytes to store lipids as cholesterol and triglycerides. The result is an increase of the ectopic fat tissue in non-adipose tissues (Liu et al. 2014). In obese individuals, adipocytes are an important source of inflammation that creates a vicious circle that can exacerbate the impairment of metabolic processes, as for instance the function of insulin in the regulation of glucose levels in blood. Furthermore, inflammation from other conditions, for example periodontitis, may also contribute to the impairment of fat storage.

Therefore, one of the aims of this thesis was also to study whether the reduction of periodontal inflammation could contribute to an improvement of the metabolic status. Up to now, the majority of the investigations used basic periodontal therapy without any concomitant adjunctive therapy. For this reason, a randomized controlled trial was designed to test the antimicrobial support to the basic periodontal therapy on parameters of periodontitis and metabolic syndrome, during a follow-up period of one year (Chapter 4 and 5). The adjunctive therapies were local disinfection with NaOCl and/or systemic antibiotics. Next to

the traditional clinical parameters of periodontitis, the Periodontal Inflamed Surface Area (PISA) was also included, as a marker of periodontal inflammation (Nesse et al. 2008).

The clinical results in chapter 4 showed that the local disinfection with NaOCl did not show any significant adjunctive effect compared to the basic periodontal therapy alone. The adjunct use of antibiotics showed a mild but significant better reduction of the PISA and of the number of residual deep pockets throughout the total follow-up period.

In chapter 5 it is reported that after basic periodontal therapy with or without adjunctive therapies, there was an improvement of some parameters of metabolic syndrome. However, although the clinical results in chapter 4 showed that the use of antibiotics lead to a significant additional reduction of periodontal inflammation in comparison to the basic periodontal therapy alone, the adjunct of systemic antibiotics failed to demonstrate any additional effect to the parameters of metabolic syndrome. For the whole study population, a significant decrease in systolic blood pressure and levels of triglycerides was found through the whole one-year follow-up. Furthermore, from 30 patients, who were diagnosed with metabolic syndrome at baseline, 14 (43%) were no longer diagnosed with this condition through the end of the study follow-up. Thus, for these patients, a considerable improvement of their metabolic status was seen. On the basis of the results of the present trial, it is not possible to establish that this improvement of metabolic status is solely related to periodontal therapy; it cannot be excluded that some patients have changed their lifestyle, for example, after having received information about risk of overweight, diet and about the negative effects of smoking. Indeed, the study protocol did not included any control on change in physical activity or diet, which are factors that may influence the level of markers of metabolic syndrome as well. However, only 3 patients, out of the 110, stopped smoking shortly before the start of the treatment and one patient stopped smoking 8 months after therapy. Furthermore, the waist circumference did not significantly decrease between baseline and 12 months after therapy and therefore we speculate that the improvements in the markers of metabolic syndrome are mainly due to periodontal treatment.

A limitation of this trial is the absence of a control group composed of patients with periodontitis, but left periodontally untreated for the whole follow-up, which would have been good to compare. However, as already discussed in chapter 5, the inclusion of such a control group is unethical, and further on, such a control group has shown to be susceptible to a high drop-out rate by patients who seek periodontal therapy elsewhere (Couper et al. 2008). It

remains therefore difficult to assess a role of true causality of periodontal inflammation on systemic conditions, by measuring the change of surrogate markers, since these markers are influenced by multiple factors that in long follow-up studies are difficult to control.

The chapters 4, 6 and 7 of this thesis focused on the microbiological characteristics of periodontitis. The study described in Chapter 4 investigated the change in the prevalence and proportions of the traditional bacterial species associated with periodontitis. Based on results of culturing, no significant superior effect of the adjunctive therapies, both local disinfection and systemic antibiotics, on the proportions of these species was found. More specifically, there was no significant decrease of prevalence of *A. actinomycetemcomitans,* which is traditionally the main bacterial target of this antibiotic therapy, in patients treated with the combination of amoxicillin and metronidazole, (Pavicic et al. 1994, van Winkelhoff et al. 1989). This latter result may be due to the recolonization of the subgingival environment by this microorganism. Alternatively, it may suggest that, in The Netherlands, there may be an increase in bacterial resistance against this combination of antibiotics and that a higher dosage of these antibiotics may be necessary. However, clinical trials should be designed to investigate whether higher dosage of antibiotics would lead to a more favorable ratio clinical benefits/adverse events.

There is still an unresolved debate about the validity of bacterial diagnosis for the routine clinical indication of the use of systemic antibiotics (Cionca et al. 2010, Guerrero et al. 2014). At the basis of the difficulty to solve this debate is the possible limited information provided by targeted techniques of bacterial detection. The aim of the study in chapter 6 was to explore the differences in the information given by traditional techniques (as anaerobic culturing and polymerase chain reaction [PCR]) and a high output open-ended next generation DNA sequencing technique (454-pyrosequencing). This investigation was performed in a subset of patients included in the intervention trial at baseline. The results of the 454-pyrosequencing revealed that patients sharing a similar disease status show a high variability of the subgingival microbiome. Even among smoker patients with periodontitis, a high variability in the subgingival microbiome was noted. For a number of patients, the traditional periodontal taxa were dominant for the major part of the subgingival microbiome. However, a group of patients was identified, who showed a distinct taxonomical composition with a predominance of genera that are not traditionally associated with periodontitis, the most important one

Pseudomonodaceae. Furthermore, the results of this study showed that a lower taxonomical diversity was associated with more severe periodontal breakdown, especially in smokers.

The study in chapter 7, with the same open-ended technique for microbiological detection, further investigated in depth the changes in the composition of the subgingival microbiome after periodontal treatment, with or without the use of systemic antibiotics. Thereafter, it was investigated whether specific characteristics of the subgingival microbiome could be related to the treatment outcomes (Chapter 7). For this aim, a subsample of the population of the clinical trial presented in chapter 4 was used. Subgingival plaque samples of 37 consecutive patients, who received basic periodontal therapy alone or in combination with systemic antibiotics were analyzed with 454-pyrosequencing. The findings were that, in periodontitis, the impact of antibiotics on the subgingival microbiome in comparison to SRP was mostly evident at three months, but already disappeared after 6 months. Furthermore, relationships between the microbiological profile at baseline, and clinical outcomes at 12 months after treatment, were found. The results revealed that the characteristics of the microbiome at baseline and not the use of antibiotics, was predictive for the treatment outcome. A co-occurrence method, which investigates the relations between different microbial communities, showed that patients were associated with a poorer treatment response if they harbor biofilms with high abundance of species that are typically resistant to amoxicillin and metronidazole, i.e. *Pseudomonodaceae*, or a group of disease-associated bacteria organized in a highly well correlated network within the biofilm. These findings suggest that it may be possible to characterize, based on microbiological profiles, subgroups of patients sharing similar clinical characteristics. This study on the subgingival microbiome, before and after treatment, should be replicated in larger scale and with a design with sufficient power in order to be able to take into account the role of confounders such as smoking, race or diet, in the interactions between host and microorganisms for the development and for the resolution/persistence of the disease.

The local treatment of periodontitis has not changed significantly since the '70s and still it is mainly based on the non-specific disruption of the supra- and subgingival biofilm in the attempt to eradicate as much as possible "pathogenic" species. The new technologies are revealing the great complexity of the interplay between microbial, host and lifestyle factors in the pathogenesis of periodontitis. To improve the quality of the treatment of periodontitis, there is a need to improve the diagnostic tools in order to give more exhaustive information

about the specific patients' profiles. In the future we may expect to be able to treat periodontitis patients on a case-specific basis, not only using clinician's expertise, but also basing therapist's decisions on objective and more comprehensive diagnostic methods.

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