
PhD Thesis – SUMMARY

Research on the implied mechanisms and interrelation between periodontal disease and cardiocascular disease.

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INTRODUCTION

Despite the new diagnostic techniques and immediate therapeutic measures, cardiovascular diseases (CVD) remain the main cause of morbidity and mortality worldwide, summing up 1.8 million deaths annually (approximately 31% of all deaths worldwide, respectively 20% of all deaths in Europe). Atherosclerosis, a chronic disease having inflammation as a substrate, is known as the main determinant of CVDs'. On the other hand, periodontal disease (PD) is one of the most frequent oral pathologies, characterized by repeated bacteraemias' which initially lead to a local inflammatory process, afterwards to a systemic one, increasing the cardiovascular risk. However, the association between PD and markers of clinical and subclinical atherosclerosis has not been elucidated so far.

The aim of the present PhD research was to elucidate the relationship between the severity of the PD and coronary atherosclerosis, expressed by total calcium score, subclinical atherosclerosis and plaque vulnerability in patients with acute coronary syndromes who underwent coronary computed tomography angiography. Nonetheless, the PhD thesis aimed to investigate the relationship between the periodontal pathogens and inflammation, in patients with ACS and determine the correlation between periostin, a new inflammatory marker, and systemic inflammation in patients with PD and associated ACS, validating its role as a new cardiovascular risk factor and a predictive one for the vulnerable patient.

METHODOLOGY

The PhD study was a prospective, observational, case-control study, developed between 2018-2019, 52 patients being included in the first study, 17 in the second study and 92 patients in the third study, admitted in the cardiology clinic of the County Emergency Clinical Hospital of Târgu-Mureș, Romania, a level 3 center of acute cardiac care.

RESULTS

Study no. 1: The correlation between periodontal disease, the atherosclerotic process and the CT phenotype of vulnerable plaques.

The objective of this study was to demonstrate the correlation between the severity of periodontal disease and the grade of vulnerability of coronary plaques, the calcium score and subclinical markers of atherosclerosis in patients with unstable angina and concomitant periodontal disease. 52 patients were enrolled, for which the total calcium score and the morphology and composition of the plaques were assessed, through Angio-CT. Following the complex dental examination, the periodontal index was established. According to the mean value of this index, established at 22, the study population was divided in two groups: group 1- 26 patients with low periodontal index and group 2- 26 patients with high periodontal index. Total periodontal index significantly presented a positive correlation with the total coronary calcium score ($r=0,45$, $p=0,0008$). Moreover, a higher calcium score was identified in patients with high periodontal index compared with those with low periodontal index ($505,29 \pm 478,64$ vs. $93,28 \pm 233$, $p=0,0001$). Also, in patients with high periodontal index the plaque volume ($p=0,019$), and the non-calcified volume ($p=0,002$) were more increased. Furthermore, in patients with high risk features in the culprit plaques (positive remodeling, punctiform calcifications, low density plaque and the napkin ring sign), the severity of the periodontal disease was more expressed compared with those with low periodontal index ($28,20 \pm 13,34$ vs $18,71 \pm 11,31$, $p=0,001$). From all periodontal indices, loss of attachments ($3,6 \pm 2,91$ vs. $1,66 \pm 1,8$, $p=0,009$) and the papillary bleeding index ($4,5 \pm 3,06$ vs. $2,04 \pm 1,96$, $p=0,002$) were significantly correlated with the plaque vulnerability. Thus, we can state that in patients with unstable angina, periodontal disease leads to the occurrence of a more vulnerable phenotype of the coronary plaques, placing the patient at risk of developing acute coronary events. Nevertheless, there were no significant differences between the subclinical markers of atherosclerosis and periodontal disease, between the two study groups, excepting the plaques identified at the level of the left carotid system ($p=0,03$).

Study no. 2: Correlation between the pathogenic germs and systemic inflammation in patients with acute coronary syndromes- study based on the new generation sequencing technique.

The aim of the second study was to demonstrate the correlation between the presence of specific pathogenic germs (determined by next generation sequencing technique) and systemic inflammation. As such, during the study, 17 patients were divided into two groups, taking in to account the identified germs

and the intensity of the presence of the germs from the red zone. Group 1 was formed of 8 patients with mild forms of PD, respectively group 2 was formed of 9 patients with advanced forms of PD. In patients with acute coronary syndromes are more expressed germs from the red complex (*P. gingivalis*, *T. Forsythia*, *T. denticola*) compared to those from the orange complex ($p=0.0008$). there was no significant correlation between red complex and those from red-orange complex group and inflammatory markers. Even though, P-selectin levels were significantly higher ($185,4 \pm 66,73$ vs $89,62 \pm 40,94$ ng/ml, $p=0.005$) in patients with germs included in the red complex, compared to those from red-orange complex, respectively the alkaline phosphatase levels at baseline ($47,83 \pm 12,53$ vs $75,72 \pm 24,42$ UI/l, $p=0.006$) and in day 7 ($52,83 \pm 6,122$ vs $70,95 \pm 9,71$ UI/l, $p=0.01$) were more expressed in patients with germs from both complexes (red and orange). Another important aspect observed was the association of the germs from the red complex with higher mortality rates in patients with myocardial infarction, expressed by the lymphocyte/monocyte ratio ($3,2 \pm 1,44$ vs $10,94 \pm 12,75$, $p=0.01$) in patients with mixed germs compared to those only from the red zone.

Study no.3: Correlation between periostin and inflammatory markers in patients with acute coronary syndrome and periodontal disease.

The aim of the third study was to demonstrate the correlation between PD and atherosclerosis by determining the inflammatory status in patients with acute coronary syndromes, centering the research on the relationship between periostin and inflammatory markers. According to median value of periostin, the study population was divided into two groups: group 1 comprising 46 patients with low values of the periostin and group 2, comprising 46 patients with high values of periostin (Pn). It was observed that patients with high periostin levels presented more frequent ST elevation myocardial infarction ($p=0.0004$), more frequently complicated with heart failure ($p=0.002$) or atrial fibrillation ($p=0.005$). Moreover, in these patients, the myocardial necrosis was more pronounced, being proven by the serum levels of total CK ($p=0.0004$) and CK-MB fraction ($p=0.0096$) and needed prolonged hospitalization ($p=0.008$) and prolongs monitoring in the advanced cardiac intensive care unit ($p=0.004$). Regarding the inflammatory process, it was observed that patients in group 2 presented significantly higher serum levels of MMP9 ($132,7 \pm 112,2$ pg/ml vs $201 \pm 69,37$ pg/ml, $p=0.003$), serum albumin (Ab) ($3,08 \pm 1,46$ mg/dl vs $4,08 \pm 0,34$ mg/dl, $p=0.02$), as well as higher alkaline phosphatase levels, both at baseline and in the 7th day after the acute event ($47,36 \pm 16,36$ UI/l vs $53,84 \pm 16,2$, $p=0.022$; $42,56 \pm 16,42$ UI/l vs $58,73 \pm 29,91$, $p=0.0001$). The lipidic profile, expressed through de LDL cholesterol fraction ($p=0.0008$) and triglycerides ($p=0.02$) is more expressed in patients with high Pn levels, as such, the cardiovascular risk is higher in these patients. However, the serum level of the C reactive protein both at baseline ($26,61 \pm 25,59$ mg/dl vs $9,2 \pm 12,87$ mg/dl, $p=0.0009$) and in the 7th day ($31,84 \pm 29,4$ mg/dl vs $19,25 \pm 26,77$ mg/dl, $p=0.043$) was significantly lower in group 2 compared to group 1, but with a significant increase in between the two moments ($p=0.007$). The level of periostin in patients with acute coronary syndromes and periodontal disease is correlated with more expressed inflammation and worse clinical outcomes, being especially associated with the serum level of MMP9.

CONCLUSIONS:

In conclusion, the present thesis demonstrated that the inflammatory status is more expressed in patients with PD and acute coronary syndrome, significantly contributing to the responsible mechanisms for the occurrence of acute coronary events, through various mechanisms. On one hand, patients with severe forms of periodontal disease present a more important atherosclerotic process, with a more vulnerable phenotype of the plaques, being an important cardiovascular risk factor. On the other hand, the predominance of the germs from the red complex, highly pathogenic germs, associated with severe forms of the disease, support the inflammatory process through repeated bacteriemias, in the same time producing endothelial dysfunction, being responsible for the occurrence of acute coronary events. Patients with ACS and concomitant PD are more vulnerable, at higher risk for clinical outcomes both on short and long term, with a high mortality rate, having inflammation as leading mechanism. At the same time, both PD and the increased levels of serum periostin can be considered cardiovascular risk factors, implied in the process of plaque vulnerability, but also in the long term evolution of patients with acute coronary syndromes.