Oral inflammatory load in patients with coronary artery disease

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Abstract: Periodontitis is an oral inflammatory disease that may have an association with coronary artery disease (CAD). Oral inflammatory load (OIL) can be quantified by assessment of oral polymorphonucleocytes (oPMN) in an oral rinse assay. The aim of the present study was to prospectively correlate OIL with CAD on angiography assessed in terms of SYNTAX score in patients presenting with stable angina or acute coronary syndrome (ACS). Consecutive eligible patients at a cardiac center were prospectively recruited. Two sets of oral rinse samples were collected before and after angiography, and the relationship between oPMN and SYNTAX score was assessed. Of the 137 patients recruited, 32.8% (n = 45) were female and 34.3% (n = 47) had diabetes mellitus. The overall mean oPMN count was low (mean 1.3 × 10⁵ cells/mL), and the mean SYNTAX score was 7.4 ± 8.5. Most of the patients presented with stable angina (89.8%, n = 123). Patients with oPMN ≥1.45 × 10⁵ (periodontitis threshold) were more likely to be elderly males presenting with stable angina. No significant correlation was established between oPMN and SYNTAX score. Although this prospective study did not demonstrate a correlation between OIL and the severity of CAD, most patients had low mean oPMN values. Larger studies are required before definite conclusions can be drawn.

Keywords: periodontitis; coronary artery disease; polymorphonucleocytes.

Introduction

Periodontitis is an inflammatory disease of gingival and bone tissues (1) that increases the oral inflammatory load (OIL). Left untreated, it can lead to significant morbidity (2,3). It has been suggested that periodontitis may be associated with various oral and non-oral (‘systemic’) conditions including coronary artery disease (CAD) by contributing to systemic inflammation. OIL can be quantified by measuring the levels of oral polymorphonucleocytes (oPMN) using a simple oral rinse assay for these cells. This quantitative assay precisely measures the degree of OIL.

The severity of CAD can be quantified on the basis of coronary angiography (CA) by use of the SYNTAX score—an online, validated tool (4). It is known that low-grade systemic inflammation is a risk factor for cardiovascular disease (5) and accordingly it could be hypothesized that the presence of inflammatory periodontal destruction (i.e. periodontitis) could lead to widespread dissemination of bacterial pathogens, endotoxins and inflammatory mediators into the systemic circulation, eventually increasing the risk of atherosclerosis and coronary plaque rupture (5).

In fact, the literature is replete with publications that have demonstrated a correlation between the presence
of periodontitis and increased risk for various forms of cardiovascular disease including CAD (4-7). As opposed to reliance on cross-sectional and retrospective analyses that have prevailed so far in the literature, the present study was designed to assess more directly the correlation between periodontitis (measured by OIL) and CAD on angiography (assessed by the SYNTAX score) in patients undergoing coronary angiography for stable angina or acute coronary syndrome (ACS).

**Materials and Methods**

Consecutive eligible patients with stable angina and ACS patients undergoing coronary angiography at University Hospital Network (Toronto General Hospital and Toronto Western Hospital) were prospectively included, as described previously (8). Inclusion and exclusion criteria were as follows:

**Inclusion criteria**
All patients with stable angina or ACS presenting for angiography and PCI at a cardiac center cardiac catheterization laboratory.

1) >18 years old
2) able to provide written informed consent

**Exclusion criteria**
1) <18 years of age
2) presence or history of other inflammatory diseases (rheumatoid arthritis, HIV, malignancy)
3) cardiogenic shock
4) known mechanical complications (e.g. ventricular septal defect, mitral regurgitation) of myocardial infarction (MI)
5) history of antibiotic use in the last three months

The study was ethically approved by the University Research and Education Board (REB CAPCR-ID: 14-8654) and adhered to all the principals for medical research outlined in the Declaration of Helsinki (9,10).

Two oral rinse samples were collected before angiography and two after angiography from each patient. Pre-procedure and post-procedure, patients gave two consecutive oral rinse samples (30 s each), 2 min apart using purified water (four samples per patient in total). Samples were maintained on ice and transferred within a period of 4 h to the laboratory for analysis in order to measure the levels of oPMN.

**Methodology for oral rinse analysis**
Patients were asked to rinse with 10 mL of USP water (water, USP/EP Purified, CAT#9190-5HP) supplemented with 0.075% sodium benzoate for 30 s and expectorate it into a 50-mL centrifuge tube (with screw caps, Cat NO:110708) with 0.5 mL of 18% saline.

The samples were kept on ice and processed for oPMN within 4 h of collection by using the ABTS (2,2’-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid or ABTS) assay (11). Briefly, ABTS is a substrate that reacts with hydrogen peroxide and enables assessment of peroxidase enzyme kinetics. The ABTS reaction was started by adding 1 mL of ABTS solution (pH 6.5) and 1 mL of hydrogen peroxide (pH 2.6) to each oral rinse sample. The samples were then incubated for 3 min at room temperature, and over time the solution (if oPMNs were present) developed an increasingly blue color reaction. One milliliter of 6% sodium dodecyl sulfate (SDS solution) was added to stop the reaction. To read the absorbance, 200 μL of each sample was placed into single wells in 96-well plates (Nunclon LOT#110984, NUNC A/S, Roskilde, Denmark). The absorption was read at 405 nm (FluoStar Optima, BMG Labtech, Ortenberg, Germany). For each sample, three readings were obtained. As shown previously (11), staining intensity correlates directly with oPMN number, and in order to assure proper quantification, standard curves were used by testing for known numbers (at various levels) of PMN cells so that staining intensity could be converted to specific numbers of oPMN and presented as a continuous variable (× 10^5 cells/mL).

**SYNTAX score**
The SYNTAX score is a quantitative index of the severity of coronary artery disease based on findings obtained during angiography. Scoring points are assigned based on the location of the lesion in the coronary tree and the angiographic severity of the lesion (4). The SYNTAX score is divided into tertiles based on the actual score. A score of 0-22 = low severity, 23-32 = intermediate severity and ≥33 = high severity (4).

**Subgroup analysis**
Subgroup analysis was performed in four patient subsets: Age ≥70 years, gender, DM, and oPMN ≥1.45 × 10^5 based on published (lower limit) levels of oPMN that correlate with periodontitis (12).

**Statistical analysis**

*Sample size calculation*
In the absence of published literature for oPMN in oral rinse samples, sample size calculation was based on previous studies that had quantified blood neutrophil levels in CAD patients.
Standard deviation of blood neutrophil count = 11.95 × 10^5. Using these data, the sample size was calculated as:

\[ n = \left( Z_{\frac{\alpha}{2}} \sigma \div E \right)^2 \]

Where:
- \( n \) = Sample size
- \( Z_{\frac{\alpha}{2}} = 1.96 \) (for 95\% confidence interval)
- \( \sigma \) = Standard deviation of oral neutrophil count
- \( E \) = Error

Average error value is 2 × 10^5

Therefore \( n = 137 \)

In order to ensure that the 95\% confidence interval estimate for oPMN in adults with CAD undergoing angiography and PCI was within 11.95 × 10^5 of the true mean oPMN value, a sample size of 137 patients was employed.

Analysis was performed with SPSS version 22. Categorical variables are presented as percentages and continuous variables as means (± SD). The correlation between oPMN and SYNTAX score was assessed by Pearson’s test. A receiver operating curve (ROC) was plotted to assess the correlation between the values of oPMN and the re-coded binary SYNTAX score (0 = low and 1 = intermediate or high score).

In addition, modeling was performed by multivariate backward-stepwise logistic regression analysis with the (binary) SYNTAX score as the dependent variable. The following independent variables were used: age, gender, hypertension, diabetes mellitus, previous myocardial infarction (MI), previous percutaneous coronary intervention (PCI), ethnicity, smoking habit, previous stroke, peripheral vascular disease (PVD) and mean oPMN. The goodness of fit of the model was ensured by the Nagelkerke R square and Hosmer and Lemeshow tests.

**Results**

Of the 137 patients recruited, 32.8\% (\( n = 45 \)) were female and 34.3\% (\( n = 47 \)) were diabetic (Table 1). The mean oPMN level was 1.3 × 10^5 (± 1.25) and the mean SYNTAX score was 7.4 ± 8.5. Most patients had a SYNTAX score in the low tertile 0-22 (91.2\%; \( n = 125/137 \)).

The levels of oPMN before and after angiography were not significantly different (Fig. 1, 1.21 vs. 1.38, \( P = ns \)). Pearson’s test revealed no correlation between oPMN and overall SYNTAX score for the patients overall (\( P = ns \), table 2). The ROC between the binary SYNTAX score and oPMN values demonstrated that the area under the curve (AUC) was 0.51 (\( P = ns \), negative) (Fig. 2). Multivariate backward-stepwise logistic regression analysis showed that OIL (oPMN count) was not a predictor of SYNTAX score.

**Subgroup analysis**

Patients >70 years of age (\( n = 42 \), male = 26, female = 16) had significantly higher oPMN levels (1.74 ± 1.4 vs. 1.1 ± 1.3, \( P = 0.012 \)) than younger patients. Male patients in the entire cohort demonstrated significantly higher mean oPMN values than female patients (1.45 ± 1.36 vs 0.99 ± 0.90, \( P = 0.02 \)). There was no difference in the oPMN counts between patients with and without DM (1.38 ± 1.4 vs 1.26 ± 1.17, \( P = ns \)).

Patients with oPMN ≥1.45 (× 10^5 cells/mL) accounted for 35\% (\( n = 48 \), male = 34, female = 14) of the cohort and were significantly older (68.17 ± 10.8 years vs. 62.58 ± 10.4 years, \( P = 0.004 \)) and more likely to present with stable angina (97.9\% vs. 86.5\%, \( P = 0.03 \)) than those with oPMN levels of <1.45 (× 10^5 cells/mL).

The correlation between oPMN and CAD severity in terms of SYNTAX score was negative in all of the 4 subgroups.

**Discussion**

This prospective study, which included both patients with stable angina and ACS, did not demonstrate a correlation between the oPMN count in oral rinse samples and the severity of CAD in terms of the SYNTAX score on coronary angiography.

This study was an expansion of previous analysis (8) and included subgroup analysis. Most of the study cohort had overall low mean oPMN levels of <1.45 × 10^5 cells/mL (65\%, \( n = 89 \)), indicating that the overall burden of oral disease (periodontitis) was low in this population.
A majority of the patients were in the low SYNTAX tertile 0-22 (91.2%) and presented with stable angina (89.8%, \(n = 123\)). Patients with oPMN \(\geq 1.45 \times 10^5\) cells/mL were older males, and again presented more often with stable angina.

A systematic review has shown that patients with periodontitis had a 19% increase in the risk for cardiovascular events, the risk being greatest (44%) in patients younger than 65 years (6). Patients with stable angina in the present cohort were older and had a greater number of oPMN than those presenting with ACS. These results are similar to those of the Swedish National Study on aging and care, which demonstrated that periodontitis increased with advancing age, males being primarily affected (12).

Previous studies have examined the relationships between periodontal disease and myocardial infarction (MI) as well as atherosclerosis (i.e. not only CAD) (13-15). Poor periodontal health can be correlated with an increased risk for MI and CAD, independent of other traditional risk factors for CAD. Marfil-Alvarez et al.

Table 2 Correlation between SYNTAX and oPMN

| SYNTAX score Pearson Correlation | SYNTAX score | Average Pre and post oPMN count |
|----------------------------------|--------------|---------------------------------|
| SYNTAX score Pearson Correlation| 0.088         | 0.088                           |
| Sig.* (2-tailed)                 | 0.310         | 0.310                           |
| N\(^\d\)                         | 135          | 135                             |
| Pre and post oPMN count Pearson Correlation | 0.310    | 137                             |
| N\(^\d\)                         | 135          | 137                             |

Sig*: significance; N\(^\d\): number of patients.
(14) have demonstrated that the extent and severity of periodontal disease and inflammation can be correlated positively with the size of acute MI (AMI). It has been speculated that elevated leukocyte and neutrophil counts may account for the observed relationships between chronic periodontitis and serum cardiac marker levels (troponin I and myoglobin) (4), although in reality the inflammatory mechanisms that might explain these relationships are still unclear. Additionally, proteases released into the bloodstream in patients with periodontitis have the capacity to degrade fetuin, a protein that protects against vascular calcification and possibly atherogenesis (7).

The low overall oPMN count and the increased cohort of stable CAD patients would have obscured the relationship between OIL and CAD. Moreover, there was also a limited proportion of patients with ACS (10.2%), and consequently the overall SYNTAX scores were also low. The absence of active and more ‘severe’ disease (oral or cardiovascular) in this population would have definitely impeded any attempt to establish a correlation between OIL and CAD.

In a small cohort of patients with stable angina, Wlosowicz et al. (16) demonstrated higher values of the approximal plaque index (API), clinical attachment level (CAL), pocket depth (PD) and bleeding index (BI) on periodontal examination. They also demonstrated an increased prevalence of multi-vessel versus single-vessel disease on coronary angiography. In patients with stable CAD, periodontal disease is known to be associated with increased systemic inflammation, as demonstrated by increased blood interleukin levels and TNFα (17).

Other reports (e.g. the joint EFP/AAP workshop) have concluded that periodontitis has a strong and consistent association with, and poses an increased risk of future cardiovascular diseases (14). oPMN counts in patients with periodontitis range from $1.45 \times 10^5$-5.4 × 10$^5$ (11). In the present cohort, patients with oPMN ≥$1.45 \times 10^5$ accounted for only a third (35.03%, $n = 48/137$).

In addition, most of the patients presented with stable angina (89.8%), and the majority were also in the low SYNTAX tertile (81.8%, 112/137). To date, interventional trials designed to investigate whether periodontal treatment results in overall improvement of cardiovascular parameters have yet to demonstrate any causal relationships between these two disease groups, and thus additional more precisely designed studies are needed (14). Increased (blood) levels of matrix metalloproteinases (MMP) are associated with an increase in the incidence of MI and may be a predictive marker for future CAD (18). Eventually it might be possible to use OIL in a predictive way to help identify individuals at risk for CAD and the subsequent development of other cardiovascular disease conditions. It is possible that while oPMNs may not have a strong correlation with the severity of coronary disease, other markers including blood PMN counts may yield a stronger correlation.

Although this was a prospective study, there were some limitations. First, most patients were in the low SYNTAX tertile. While this bias could not be overcome because patients were recruited without prior knowledge of coronary anatomy, future studies could include patients with high SYNTAX scores or an equal proportion of patients with stable angina and ACS. Secondly, only the correlation with salivary oPMN count was examined. Assessment of blood oPMN in addition to salivary oPMN may have altered the outcome.

Larger studies including patients with higher SYNTAX scores may yield a different outcome or correlate with the blood concentrations of PMN (in addition to oPMN) with CAD on angiography. If a positive correlation is demonstrated, future studies could explore the feasibility of a simple semi-quantitative Near Patient Test (NPT) at the dental clinic to identify coronary disease. More importantly, subsequent investigations must be designed with larger sample sizes or more stratified samples so that patients who actually have disease, or at least a greater probability of either disease, can be studied, possibly allowing further subdivision into groups with varying levels of disease severity. In the present study, most patients were actually “healthy”, with low levels of periodontitis and limited coronary artery disease. However, the present findings show that it would be possible to measure CAD and OIL in appropriate patient populations in the future. Also, if a more targeted approach were to be adopted, it might be possible to demonstrate definitive correlations that could clarify some aspects of the pathophysiological mechanisms underlying the apparent correlation between cardiovascular and periodontal diseases.

This appears to be the first prospective study to have attempted to demonstrate a correlation between OIL and CAD.

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**Conflict of interest**

None.
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