Diabetes mellitus type 2 and angina pectoris: novel insights in diagnosis, prognosis and treatment

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Chapter 4

Diabetes mellitus type 2 is associated with higher levels of myeloperoxidase

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Abstract

Background
Diabetes mellitus type 2 is linked to augmented endothelial dysfunction and accelerated atherosclerosis. Myeloperoxidase (MPO) plays an important role in the initiation, progression and complications of atherosclerosis. We investigated whether MPO levels are increased in diabetic patients.

Methods
We compared baseline plasma MPO levels in diabetic and non-diabetic patients with mild, stable anginal complaints (Canadian Cardiovascular Society (CCS) I-II/IV) and performed multivariate linear regression analysis to adjust for possible confounding factors.

Results
A total of 440 patients were recruited from the outpatient clinic of Cardiology, 268 patients with and 172 without diabetes mellitus type 2. Levels of MPO were significantly higher in the diabetic patients (141 (115-171) pM (median, interquartile range) vs. 126 (105-167) pM, p=0.01). Diabetes mellitus type 2, age in years, current smoking, hypercholesterolemia, Calcium antagonists and ACE inhibitors were associated with logarithmically transformed MPO levels. Of these variables, diabetes mellitus type 2 (β 0.096, SE 0.038, p=0.01); age (β 0.01, SE 0.002, p<0.001) and current smoking (β 0.166, SE 0.05, p=0.001) remained independently associated with MPO-levels in multivariate analysis. The linear regression coefficient of diabetes mellitus type 2 in relation to MPO was 0.092 in univariate linear regression and 0.078 after adjustment for age, current smoking, the use of ACE inhibitors and calcium antagonists.

Conclusions
Diabetes mellitus type 2 is associated with mildly increased levels of MPO, independent of other clinical variables. This association may contribute to the accelerated progression of atherosclerosis in diabetics.
Introduction

Low grade chronic inflammation and endothelial dysfunction are contributing factors in the initiation and progression of cardiovascular disease. Recent evidence suggests that myeloperoxidase (MPO) contributes to the inflammatory process in atherosclerosis and its complications 1-3.

MPO is a pro-oxidant enzyme that is released from granules of activated leukocytes, monocytes and macrophages at inflammatory sites. When released as part of the innate host defence, it generates free radicals and reactive oxygen species (ROS) 2. However, the antimicrobial activity of MPO can also lead to oxidative damage of the endothelium and vessel wall. This is illustrated by the fact that both MPO and its oxidants are found in atheroma and atherosclerotic lesions 4.

Several mechanism via which MPO can promote atherosclerosis have been described. First, MPO activity leads to oxidation of low density lipoprotein (LDL)-cholesterol, which increases its atherogenicity 4, 5. Second, MPO-induced oxidation of high density lipoprotein (HDL)-cholesterol can reduce its capacity for reversed cholesterol transport 6. Finally, MPO activity leads to consumption of endothelial derived nitric oxide, which can lead to plaque formation and endothelial dysfunction 4, 5, 7, 8.

Literature regarding MPO activity in diabetes is scarce and conflicting, demonstrating both higher and lower levels of MPO in different tissues and clinical situations 9-11. However, it is known that endothelial dysfunction develops early in diabetes mellitus, preceding clinically detectable atherosclerosis and that diminished NO levels and enhanced oxidative stress are important factors in the pathogenesis of diabetic vascular complications 12-16. Furthermore, white blood cell count (WBC) is associated with micro- and macrovascular events in type 2 diabetics 17.

We therefore hypothesized that levels of MPO as a marker of endothelial dysfunction and generator of ROS are increased in diabetic patients. To test our hypothesis we compared baseline MPO levels in diabetic and non-diabetic patients with mild stable anginal complaints.

Methods

A total of 440 consecutive patients with stable anginal complaints (Canadian Cardiovascular Society (CCS) class I-II/IV) were recruited at the outpatient clinic of Cardiology, 268 patients with and 172 without type 2 diabetes mellitus. All patients...
underwent clinical examination and resting ECG. Baseline fasting blood samples were collected in EDTA tubes and stored within 1 hour at -80°C for further analysis. Patients were defined as having type 2 diabetes mellitus (DM2) when (i) two or more non-fasting glucose sample of ≥11.0 mmol/L or (ii) fasting samples of ≥7.0 mmol/L were measured on separate days or (iii) when patients were treated with anti-diabetic medication. Hypertension was defined as a systolic pressure ≥ 180mmHg or diastolic > 100mmHg after treatment. The absence or presence of hypercholesterolemia was based on a documented clinical diagnosis made by the treating physician for which patients required dietary and lipid lowering treatment. A history of coronary artery disease was present when a previous myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting was documented. Plasma MPO levels were measured in duplicate by high-sensitivity sandwich ELISA (PrognostiX, Inc, Cleveland, Ohio, USA). Each plate included a standard curve with isolated human MPO and controls were used on every plate to correct for interplate variability. Baseline characteristics are presented as mean (standard deviation), as median (interquartile range) or as number (percentage) and are compared by Student’s unpaired t test and χ²-test where appropriate. MPO-levels were not normally distributed and were compared using the Mann-Whitney test. For regression analysis, MPO levels were logarithmically transformed to a normal distribution. Multivariate linear regression analysis was used to adjust for all possible confounding factors, thereby demonstrating the independence of the relation between diabetes mellitus type 2 and MPO levels. Variables that were significantly related to MPO in univariate analysis were added as covariates into the model. Criterion for entry of variables into the linear regression analysis was set on p ≤0.2.

**Results**

The clinical characteristics of the 440 enrolled patients in relation to the presence of diabetes mellitus type 2 are depicted in table 1. Approximately half of all patients had a history of cardiovascular disease event. Patients with diabetes were more often male, had more other classical cardiac risk factors and were treated more aggressively for their anginal complaints and concomitant other risk factors. The median duration of diabetes was 6.6 years (interquartile range (IQR) 3.4-11 years), median HbA1c levels
Table 1. Clinical characteristics in patients with stable angina with and without type 2 diabetic patients

<table>
<thead>
<tr>
<th></th>
<th>no diabetes, n=172</th>
<th>diabetes, n=268</th>
<th>p(value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>99 (58)</td>
<td>183 (68)</td>
<td>0.02</td>
</tr>
<tr>
<td>Age in years</td>
<td>66 (11)</td>
<td>65 (9)</td>
<td></td>
</tr>
<tr>
<td>CCS II/IV</td>
<td>98 (57)</td>
<td>103 (38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>110 (64)</td>
<td>227 (85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statins</td>
<td>91 (53)</td>
<td>199 (74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>34 (20)</td>
<td>111 (41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>100 (58)</td>
<td>193 (72)</td>
<td>0.003</td>
</tr>
<tr>
<td>Long-acting nitrates</td>
<td>63 (37)</td>
<td>102 (38)</td>
<td></td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>51 (30)</td>
<td>116 (43)</td>
<td>0.004</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>74 (43)</td>
<td>144 (54)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>85 (49)</td>
<td>172 (64)</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking</td>
<td>27 (16)</td>
<td>46 (17)</td>
<td></td>
</tr>
<tr>
<td>Previous smoking</td>
<td>80 (55)</td>
<td>139 (62)</td>
<td></td>
</tr>
<tr>
<td>Family history CAD</td>
<td>85 (49)</td>
<td>96 (36)</td>
<td>0.005</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CAD events</td>
<td>81 (47)</td>
<td>133 (50)</td>
<td></td>
</tr>
<tr>
<td>Previous MI</td>
<td>60 (35)</td>
<td>83 (31)</td>
<td></td>
</tr>
<tr>
<td>Previous PCI</td>
<td>53 (31)</td>
<td>75 (28)</td>
<td></td>
</tr>
<tr>
<td>Previous CABG</td>
<td>23 (13)</td>
<td>50 (19)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Values are presented as number (percentage), except for age which is presented as mean (standard deviation). CAD= coronary artery disease; MI=myocardial infarction; PCI=percutaneous coronary intervention; CABG= coronary artery bypass grafting.

were 7.4 mmol/L (IQR 6.6-8.3), and 100 patients (39%) needed insulin therapy for adequate glycemic control.

Median levels of MPO were significantly higher in diabetic patients compared with non-diabetic patients (141 (115-171) pM vs. 126 (105-167) pM, p=0.01 (figure 1). Furthermore, a positive correlation was found with age (Spearman's correlation coefficient r=0.21, p<0.001). MPO levels did not significantly differ with gender, classical risk factors or with a previous history of CAD or CVD. Furthermore, in the diabetic patients, no correlation was found with duration of diabetes mellitus, treatment with insulin or with HbA1c levels.

Clinical variables associated with logarithmically transformed MPO levels are diabetes mellitus type 2 (unstandardized regression coefficient (β) 0.092; standard error (SE) 0.039, p<0.02); age in years (β 0.009, SE 0.002, p<0.001); current smoking (β 0.108, SE 0.052, p<0.04); hypercholesterolemia (β -.025, SE 0.04, p=0.05); Calcium antagonists (0.073, SE 0.04, p=0.1) and ACE inhibitors (0.068, SE 0.041, p<0.07). In multivariate
analysis, diabetes mellitus type 2 ($\beta$ 0.096, SE 0.038, $p=0.01$); age ($\beta$ 0.01, SE 0.002, $p<0.001$) and current smoking ($\beta$ 0.166, SE 0.05, $p=0.001$) remained independently associated with MPO-levels. Statins were not significantly related ($\beta$-0.001, SE 0.02, $p=0.97$).

The linear regression coefficient of diabetes mellitus type 2 remained unchanged after adjustment for variables related to MPO: 0.092 in univariate analysis and 0.078 after adjustment for age, smoking status, hypercholesterolemia, the use of calcium antagonists and ACE-inhibitors. This indicates that the relation of diabetes mellitus type 2 with MPO cannot be explained by unequal distributions of other variables.

**Discussion and conclusion**

In this study, we have shown that plasma MPO levels were mildly elevated in patients with diabetes mellitus type 2 with stable anginal complaints (CCS I-II/IV) compared to non-diabetics with similar complaints and that this positive relation was not the result of an unequal distribution of other variables associated with diabetes mellitus or MPO. Besides diabetes mellitus, age and current smoking were independently correlated with the levels of MPO.

**MPO and diabetes mellitus**

To our knowledge, only a limited number of studies specifically focused on the relation between MPO levels and the presence of diabetes. In total, 3 clinical studies found significantly higher MPO levels (twice measured in serum, once in plasma samples)
in diabetics, whereas 4 clinical studies found no correlation \cite{8, 11, 18-22}. The observed discrepancies with the negative studies may be explained, at least in part, by differences in populations, methods, and examined tissues, thereby hampering the interpretation of these findings.

Furthermore, some studies strived to identify the mechanisms of this possible relation between MPO and diabetes mellitus. One study demonstrated that adhered neutrophils, evaluated by MPO activity \textit{in vitro} are enhanced by insulin treatment and the authors therefore speculated that high MPO activity in diabetes might be related to hyperinsulinemia \cite{23}. Another study group stated that vascular-bound MPO could use high glucose–stimulated hydrogen peroxide to amplify high glucose–induced injury to the vascular wall \cite{24}. The findings in our study are in accordance with these reports demonstrating higher levels of MPO in a group of diabetic patients with a moderate glycemic control and treatment with insulin in 40% of these patients.

Although the difference in MPO level between diabetic and non-diabetic patients in our study was significant, levels were within the normal range of MPO (<539pM (95% upper percentile of middle-aged healthy population, \(n = 300\), Prognostix)). However, several recent studies have described similar low levels of MPO in healthy patients and in patients with cardiac risk factors but without overt cardiac disease. Moreover, even in several studies in patients with chest pain or acute coronary syndromes levels below approximately 350 pM are described \cite{18, 19, 25-27}. Nonetheless, these low levels do suggest that, in the individual type 2 diabetic patient with only stable anginal complaints, levels of MPO are of little value in the detection of instable plaques or coronary artery disease. However, it still may be an indication that MPO contributes to the accelerated progression of atherosclerosis in diabetics.

**MPO and inflammation**

The question whether MPO is a marker of an increased inflammatory state and leukocyte count in diabetics or that MPO plays an independent role in diabetic atherosclerosis can not be answered in the present study because levels of other inflammation markers such as leucocyte count, C-reactive protein or interleukin-6 were not available in this study.

However, based on the experimental study of Zhang et al. we might expect an increased effect or activity of MPO in diabetic subjects \cite{24}. Furthermore, hyperglycemia and diabetes mellitus have been shown to be associated with activation of leukocyte counts \cite{17, 28}. The present study clearly supports this concept showing that circulating MPO
levels, derived predominantly from leukocytes, are higher in diabetics compared to controls.

**Clinical variables and the level of MPO**

Similar to our findings, both age and smoking, have previously been linked to elevated MPO levels and increased inflammatory activity \(^{29,30}\). However, conflicting results on the unadjusted effect of these variables on MPO were presented, only 2 studies found an independent relation with age and one also reported a positive correlation with smoking \(^{8,18,19}\).

MPO has also been related to a history of CAD or CVD, but we could not establish this relation in our population with very stable patients with and without coronary artery disease \(^{18,19,22}\). This might be explained by the fact that the blood samples of the previous studies were obtained in patients presenting in an acute coronary situation which may have affected the MPO levels. Secondly, no relation could be found between the severity of the anginal complaints and the levels of MPO, although we only distinguished between patients with CCS I and II. Moreover, the diabetics in our study population, which had significant higher levels of MPO, more often presented with less overt anginal complaints compared with their non-diabetic counterparts.

The lack of relation between severity of anginal complaints and the level of MPO in these diabetic patients might be explained by the fact that diabetics more often present with atypical complaints and approximately 25% of the diabetics already have clinically relevant ischemia without any anginal complaints \(^{31}\). Unfortunately, no information on the severity of myocardial ischemia in relation to the anginal complaints was available in this study.

Finally, no relation was found between the use of statins and the level of MPO as has been described in a number of studies \(^{25,27,32,33}\). These studies suggested that a decrease in MPO level was a possible pleiotropic effect of statin use, however these possible pleiotropic effects of statins on MPO were not replicated in a recent study on statin therapy in hypercholesterolemic patients \(^{25}\). Whether or not the presented difference in MPO between diabetics and non-diabetics in our study was underrated due to statin therapy therefore remains unclear.

**Conclusion**

Diabetes mellitus type 2 is associated with mildly increased levels of MPO, independent of other clinical variables. This association may contribute to the accelerated progression of atherosclerosis in diabetics.
Reference List


