Chapter 5

Differential effects of statin therapy on CRP in patients with type 2 diabetes with and without the metabolic syndrome

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submitted
ABSTRACT

Objective
C-reactive protein (CRP) is a marker for the inflammatory process of atherosclerosis. We evaluated the effect of statin therapy on CRP in patients with type 2 diabetes mellitus (DM2) without manifest cardiovascular disease.

Research Design and Methods
A randomized, placebo-controlled double-blind trial was performed in 250 patients with DM2 without manifest cardiovascular disease. Patients were given 0.4 mg cerivastatin or placebo daily. The primary endpoint was the change in high sensitivity CRP after 2 years.

Results
CRP in the statin group was 1.58 mg/L at baseline and 1.69 mg/L at 2 years (p= 0.413), in the placebo group it increased from 2.03 mg/L at baseline to 2.54 mg/L at 2 years (p = 0.058) (p= 0.269 for comparison between the groups). In a high-risk subgroup with the metabolic syndrome and LDL levels > 2.6 mmol/L (40 % of the cohort) CRP levels increased significantly in the placebo group (from 2.97 mg/L at baseline to 3.99 mg/L at 2 years, p=0.036) in comparison to the statin group (from 2.13 mg/L at baseline to 2.10 mg/L at 2 years, p=0.885) (p=0.042 for comparison between the groups)

Conclusions
There was no effect of two year statin therapy on CRP in patients with DM2 without manifest cardiovascular disease, except in a subgroup with the metabolic syndrome and LDL > 2.6 mmol/L. Studies supporting risk stratified therapy in primary prevention in DM2 are needed.
INTRODUCTION

Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2). C-reactive protein (CRP) is a marker for the chronic inflammatory process in atherosclerotic plaques, and probably has pro-atherogenic properties of its own. When measured with high sensitivity assays, CRP levels are highly reproducible, unaffected by food intake and with no circadian variation. CRP is a strong predictor of future cardiovascular events, independent of traditional risk factors such as LDL cholesterol. CRP levels are associated with components of the metabolic syndrome (MS) such as triglycerides, obesity and insulin sensitivity. Finally, CRP might be predictive of incident DM2.

In DM2 without coronary artery disease, levels of CRP are higher than in non-diabetic controls. CRP levels independently predict future cardiovascular events in DM2 in some studies. Importantly, in the Hoorn study, the association of CRP with future CVD events in DM2 was not independent of classical risk factors.

A meta-analysis of intervention studies with statins in the setting of secondary prevention after a cardiovascular event has shown a correlation between reduced cardiovascular events and reduction in CRP, independent of LDL cholesterol lowering. Results from intervention studies on the effects of statin therapy on CRP in DM2 have shown contradictory results. The present study is an analysis of CRP within a randomized, placebo-controlled trial that has evaluated the effect of 2 years’ statin therapy on CRP as a pre-specified secondary endpoint in patients with DM2 without CVD.

RESEARCH DESIGN AND METHODS

Subjects and design

The study design and baseline characteristics of the original patient population have been described elsewhere. Briefly, 250 patients with DM2 for at least one year, aged 30-80 years, without CVD were included between August 1999 and February 2001 in this randomized, double-blind, clinical trial. Patients were given 0.4 mg cerivastatin (Bayer B.V., Mijdrecht, The Netherlands) or placebo daily for 2 years. After the withdrawal of cerivastatin from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin (Merck Sharp & Dome, Haarlem, the Netherlands), without unblinding the study. At that moment, all the patients had been randomized with a mean follow-up of 15 months (range 6-23 months).

Eligible patients gave their written informed consent. The study was performed at the HAGA Hospital, The Hague. The study was approved by the hospital’s Medical Ethics Committee.
**Study Objectives**

The primary endpoint of this sub-study was the change in CRP between 24 months and baseline. The relationship between CRP and MS score was a secondary endpoint.

**Follow-up**

Patients returned to the study site after 12 hours fast at 3, 6, 12, 18 and 24 months when blinded lipid and safety measurements (creatinnin kinase, ALT) were performed. CRP was measured at baseline and at 24 months.

**Laboratory investigations**

Lipid and safety measurements were performed at the Department of Clinical Chemistry and Hematology of the HAGA Hospital, according to ISO 15189 standard procedures. Blood samples were collected from the subjects after a 12 hour fast. EDTA tubes were used for the determination of HbA1c. Liver enzymes and lipids were measured in serum. A urine sample was collected for the determination of the albumin-to-creatinin ratio.

The high sensitive CRP assay was performed in the Leiden University Medical Center with the Tina Quant C-reactive protein (latex) high sensitive assay from Roche using particle enhanced immunoturbidimetry on a Roche Module P (Basel, Switzerland). The lower detection limit (analytical sensitivity) is 0.03 mg/L and the functional sensitivity 0.11 mg/L. The intra-assay CV is 1.34% at 0.55 mg/L and the inter-assay CV is 5.70% at 0.52 mg/L. All CRP assays were performed after completion of the study.

**Statistical analysis**

The primary treatment comparison was between placebo and statin therapy in patients completing the study, as on-treatment analysis. CRP values more than 15 mg/L were excluded. As CRP values were not normally distributed, logarithmic transformations were used. Changes within each treatment group were analyzed using Student’s paired t-test. Comparisons of the effects between the treatment groups were performed using Student’s independent samples t-test. Analysis of the baseline data was performed in all randomized patients. Stepwise regression techniques were used to investigate the effect of baseline characteristics on baseline CRP and on changes in CRP. ANOVA was used to investigate the relation between the MS score (1 point for every criterion (waist, triglycerides, HDL cholesterol and blood pressure) according to the NCEP/ATPIII criteria) and baseline CRP. In addition, the effect of statin treatment on CRP was analyzed in a high-risk patient group with 3 or 4 additional MS criteria on top of their diabetes and LDL cholesterol levels > 2.6 mmol/L. To test the equivalence of cerivastatin 0.4 mg and simvastatin 20 mg, LDL levels before and after the switch to simvastatin were compared using Student’s paired t-test. Correlation between changes in CRP and changes in other parameters were evaluated with Pearson’s correlation coefficients.
Analyses were performed using SPSS 11.0 for Windows software. All analyses were 2-sided, with a level of significance of $\alpha = 0.05$.

**RESULTS**

The characteristics of the study population are given in Table 1. No statistical differences between the groups were observed. 68 patients did not complete the study. This relatively high drop-out rate was mainly caused by the withdrawal of cerivastatin from the market\(^2\). There were no significant differences in demographic or lipid parameters between the full cohort (n=250) and the patients completing the study (n=182), except for race as more Caucasians than non-Caucasians completed the study (data not shown).

**Table 1** Baseline Characteristics of 250 Randomized Patients

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=125)</th>
<th>Statin (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>57 (46)</td>
<td>61 (49)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.2 ± 11.4</td>
<td>58.8 ±11.3</td>
</tr>
<tr>
<td>Ethnicity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>86 (69)</td>
<td>83 (66)</td>
</tr>
<tr>
<td>Asian-Indians</td>
<td>20 (16)</td>
<td>28 (22)</td>
</tr>
<tr>
<td>other</td>
<td>19 (15)</td>
<td>14 (11)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>33 (26)</td>
<td>28 (22)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>66 (53)</td>
<td>60 (48)</td>
</tr>
<tr>
<td>Diabetes duration (years)*</td>
<td>7 ± 8</td>
<td>6 ± 7</td>
</tr>
<tr>
<td>Insulin use</td>
<td>69 (55)</td>
<td>62 (50)</td>
</tr>
</tbody>
</table>

Data are means ± SD or numbers of patients (%). *Median ± SD.

**CRP measurements (Table 2)**

Baseline CRP was not significantly different between the groups. Baseline CRP in the dropouts did not differ from values in patients completing the study. A total of 149 patients had analyzable CRP data (i.e. < 15 mg/L) at baseline and 24 months.

There was no significant difference between the change in CRP in 2 years in the statin group and the placebo group (mean difference 0.53 mg/L [95% CI −0.42 to 1.48 mg/L] \(p=0.269\)). CRP in the placebo group increased from 2.03 mg/L at baseline to 2.54 mg/L at 2 years \(p = 0.058\), in the statin group it was 1.58 mg/L at baseline and 1.69 mg/L at 2 years \(p = 0.413\).

Determinants for baseline CRP in univariate analysis were waist, Body Mass Index (BMI), HbA1c, age, gender (higher in women), ethnicity (higher in Caucasians and Asian-Indians), smoking, triglycerides, apoB/LDL cholesterol, diabetes medication and MS score. When included into a regression model, age (beta = -0.005, \(p= 0.031\)), BMI (beta = 0.019, \(p<0.001\)), HbA1c (beta = 0.049, \(p=0.016\)), gender( beta = 0.171, \(p=0.003\)), ethnicity ( beta = -0.196,
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Table 2 CRP and metabolic changes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>24 months</th>
<th>p 0-24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.03</td>
<td>2.54</td>
<td>0.058</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>33</td>
<td>35</td>
<td>0.783</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.62</td>
<td>7.57</td>
<td>0.764</td>
</tr>
<tr>
<td>BP syst (mmHg)</td>
<td>137</td>
<td>134</td>
<td>0.071</td>
</tr>
<tr>
<td>BP diast (mmHg)</td>
<td>77</td>
<td>74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2</td>
<td>30.3</td>
<td>0.696</td>
</tr>
<tr>
<td>Waist (m)</td>
<td>1.03</td>
<td>1.02</td>
<td>0.176</td>
</tr>
<tr>
<td>MS score</td>
<td>2.48</td>
<td>2.22</td>
<td>0.031</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>3.13</td>
<td>9.74</td>
<td>0.019</td>
</tr>
<tr>
<td>(g/mol creat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Statin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.58</td>
<td>1.69</td>
<td>0.413</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>25</td>
<td>26</td>
<td>0.410</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.49</td>
<td>7.63</td>
<td>0.235</td>
</tr>
<tr>
<td>BP syst (mmHg)</td>
<td>137</td>
<td>132</td>
<td>0.007</td>
</tr>
<tr>
<td>BP diast (mmHg)</td>
<td>77</td>
<td>74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.1</td>
<td>30.4</td>
<td>0.145</td>
</tr>
<tr>
<td>Waist (m)</td>
<td>1.02</td>
<td>1.01</td>
<td>0.369</td>
</tr>
<tr>
<td>MS score</td>
<td>2.18</td>
<td>2.04</td>
<td>0.141</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>2.48</td>
<td>5.81</td>
<td>0.053</td>
</tr>
<tr>
<td>(g/mol creat)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are geometric means for CRP and means for other parameters.

CRP (beta = 0.071, p=0.010) and MS score (beta = 0.011) and MS score (beta = 0.071, p=0.010) remained significant determinants and together explained 29% of the variance in baseline CRP. The relation between MS score and CRP was linear at baseline (ANOVA p for linearity <0.001) (Figure 1). After 2 years, this relation remained statistically significant only in the placebo group. Further analysis on this issue revealed that in a high-risk subgroup with 3 or more additional MS criteria (on top of their diabetes) and LDL levels > 2.6 mmol/L, which comprised 40% of the cohort (29 patients in the placebo group and 30 patients in the statin group), two years’ CRP levels increased significantly in the placebo group (from 2.97 at baseline to 3.99 at 2 years, p=0.036) in comparison to the statin group (from 2.13 mg/L at baseline to 2.10 mg/L at 2 years, p=0.885) (Figure 2, p=0.042 for comparison between placebo and statin).

Changes in CRP were not related to baseline characteristics, changes in lipid levels, body weight or Hba1c. The effect of the two statins used was analyzed by correcting the change in CRP for duration of simvastatin treatment (range 1 to 18 months). This did not change the results.

**Lipids**

LDL cholesterol was 3.44 ± 0.71 mmol/L at baseline and 2.58 ± 0.95 mmol/L at 2 years (-25 %, p < 0.001) in the statin group and 3.55 ± 0.71 mmol/L at baseline and 3.78 ± 0.81 mmol/L at 2 years (+8 %, p=0.003) in the placebo group (p < 0.001). HDL cholesterol was 1.23 ± 0.39
Effect of statins on CRP in DM2

Figure 1. Relation between MS score and CRP at baseline

mmol/L at baseline and 1.20 ± 0.36 mmol/L at 2 years in the statin group and 1.21 ± 0.37 mmol/L at baseline and 1.22 ± 0.38 mmol/L at 2 years in the placebo group. Triglycerides were 1.82 ± 0.97 mmol/L at baseline and 1.60 ± 1.38 mmol/L at 2 years in the statin group and 1.88 ± 0.79 mmol/L at baseline and 1.72 ± 1.22 mmol/L at 2 years in the placebo group. Changes in HDL cholesterol and triglycerides were not significantly different compared to baseline or compared to the placebo group, except for the reduction in triglycerides in the statin group after 2 years (p=0.043). Average LDL cholesterol levels were higher after the switch to simvastatin (2.34 before versus 2.56 mmol/L after the switch, p < 0.001).

CONCLUSIONS

Patients with DM2 have a high-risk of cardiovascular events. Many studies have been performed to evaluate new non-traditional risk factors for CVD. The number of studies in DM2
however is sparse. This is the first randomized controlled trial on the effect of long-term statin therapy on CRP in DM2. We did not find an effect of 2 years’ intermediate-dose statin therapy on CRP. Consistent with our results, Koh et al did not find an effect of 2 months of simvastatin 20 mg on CRP in a randomized, placebo-controlled crossover trial in DM2\textsuperscript{22}. In patients with DM2 and low HDL levels, both 40 and 80 mg simvastatin significantly reduced CRP levels\textsuperscript{19}. Other randomized placebo-controlled studies in DM2 show a dose dependent effect of atorvastatin on CRP\textsuperscript{18,20}. Similarly, pravastatin 40 mg decreased CRP levels in an open, randomized, crossover study in DM2\textsuperscript{21}. Balletshofer et al\textsuperscript{23} found no effect of 12 weeks cerivastatin 0.2 mg and 0.8 mg on CRP in DM2; this study however included only 20 patients per group because the study was terminated after the withdrawal of cerivastatin.

Possible explanations for the inconsistent findings on the effect of statin therapy on CRP in DM2 are differences in patient inclusion criteria, differential effects of statins, dose-depending effects and duration of statin treatment.

In line with another study\textsuperscript{9}, CRP in our asymptomatic DM2 group was related to the MS and to other aspects of glucose metabolism. Interestingly, BMI and MS score were both independently associated with CRP. This is in concordance with the findings of Putz\textsuperscript{27} and Mc Laughlin\textsuperscript{28}, implicating that the relationship between insulin resistance and CRP is only partly explained by obesity.

Data from NHANES III show that among people with DM2 and MS the prevalence of CVD was higher than among people with DM2 without MS\textsuperscript{29}. We were able to identify a high-risk phenotype, present in 40% of our cohort, with 3 or 4 additional MS criteria on top of their

\begin{figure}[h]
\centering
\includegraphics[width=0.6\textwidth]{figure2.png}
\caption{Effect of statin therapy on CRP, stratified by risk group}
\end{figure}

Changes in CRP (+ SE) after two years, stratified by high-risk (HR) (MS score 3 or 4 and LDL> 2.6 mmol/L) and standard risk (SR) (rest group).

p=0.042 for the difference in changes between placebo and statin in the high-risk group.
diabetes and LDL levels > 2.6 mmol/L. These patients showed a significant effect of statin therapy on CRP in comparison to placebo. Intriguingly, this effect occurred only when the combination of MS and higher LDL levels was present. This important observation suggests that statins are most effective at reducing low grade inflammation in high-risk groups and supports risk stratification in the prescription of statin therapy in primary prevention in DM2. As advocated by the NCEP\textsuperscript{26}, statin therapy should be prescribed to DM2 individuals without CVD with LDL levels > 2.6 mmol/L and at least one additional cardiovascular risk factor.

We explored the effect of diabetes related factors as a possible cause of the (non-significant) rise in CRP in the placebo group. We found no changes in HbA1c after 24 months; blood pressure was significantly lower after 24 months although there was progression of microalbuminuria; One has to realize however, that in our regression model only 29% of the variance in CRP could be explained and that the diabetic state itself and genetic factors\textsuperscript{30} might be major determinants of CRP.

Our study has limitations: the switch from cerivastatin to simvastatin was unplanned and we did not have the possibility to collect blood samples at the time of the switch. All our 24 month samples were collected after the switch, after 1-18 months of simvastatin treatment. In addition, we did not find an influence of the duration of simvastatin treatment on the 24 months results in regression analysis. The statin dose used was relatively low, but common in primary prevention during the time this study was started.

In conclusion, the present study showed no effect of two year statin therapy on CRP in patients with DM2 without manifest CVD. The beneficial effects of statin therapy on CRP in a high-risk subgroup with MS and LDL > 2.6 mmol/L supports the use of risk stratification in DM2. Prospective studies are needed to further substantiate these findings.
REFERENCES


