Association between cardiac autonomic dysfunction and inflammation in type 1 diabetic patients: effect of beta-blockade

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Aims To assess the relationship between cardiac autonomic dysfunction and inflammation in patients with type 1 diabetes and whether beta-blocker therapy might improve both abnormalities in these patients.

Methods and results We studied 49 patients with type 1 diabetes (age 50.5 ± 11 years, 33 men). Serum levels of high-sensitivity C-reactive protein, as a marker of inflammation, and frequency-domain heart rate variability (HRV) on 24 h Holter monitoring, as a measure of cardiac autonomic function, were assessed in all patients. Twenty-one patients with depressed HRV were subsequently randomized to receive atenolol (50 mg daily) or no-beta-blockade. HRV and C-reactive protein were re-assessed after 3–4 weeks from randomization. An inverse correlation was found between C-reactive protein levels and HRV parameters, with the highest r coefficient shown with low-frequency (LF) power (r = −0.38; P = 0.007). Furthermore, C-reactive protein serum levels were significantly higher in patients with bottom quartile values of LF power compared with patients with values in the three top quartiles (4.64 ± 2.8 vs. 1.79 ± 1.6 mg/L, respectively; P = 0.003), also after adjustment for potential confounding variables (P = 0.013). HRV parameters improved significantly in patients treated with atenolol, but not in the no-atenolol group. Furthermore, C-reactive protein levels decreased in the beta-blockade group, but not in the no-beta-blockade group (P = 0.04 for changes between groups).

Conclusion In type 1 diabetic patients, serum C-reactive protein levels are significantly associated with depressed HRV; the favourable effects of beta-blockade on both HRV parameters and C-reactive protein serum levels suggest that autonomic nervous system may have significant modulator effects on inflammation.

KEYWORDS Type 1 diabetes; Heart rate variability; C-reactive protein

Introduction

Abnormal cardiac autonomic function1,2 and increased markers of inflammation3,4 are findings detectable in a sizeable proportion of patients affected by type 1 diabetes mellitus and are both associated with worse clinical conditions and outcome.5–8

Recent experimental data suggest that inflammatory reactions can be modulated by the activity of the autonomic nervous system (ANS). Specifically, vagal activity has been shown to have anti-inflammatory effects, which may mainly result from inhibition of macrophage activation through stimulation of macrophage nicotine receptors.9,10 On the other hand, possible effects of products and mediators of inflammation on the ANS activity have also been described.11,12

Accordingly, recent clinical studies have found a significant association between a reduced heart rate variability (HRV) and increased markers of inflammation in several populations, including apparently healthy subjects,13,14 and patients with heart disease.15–18 The exact relationship between cardiac autonomic function and inflammation, however, remains to be elucidated.

This study was aimed at assessing two questions: (i) whether a relationship between impaired cardiac autonomic function and inflammation also exists in patients with type 1 diabetes mellitus in the absence of any clinical evidence of heart disease; (ii) whether an intervention (i.e. beta-blocker therapy) known to have significant favourable effects on cardiac autonomic activity19–21 might also improve low-grade inflammation in these patients.

Methods

This study consisted of two phases. In the first phase, we assessed the relation between HRV parameters and C-reactive protein serum levels in a group of type 1 diabetic patients. In the second
phase, we randomized patients with reduced HRV to beta-blocker therapy or no-beta-blocker therapy. Specifically, we preventedly decided to enrol in the second phase of the study patients who showed values below the median of the HRV variable found to have the best correlation with C-reactive protein serum levels.

The study protocol complied with the Declaration of Helsinki and has been approved by institutional research committee. All participants gave written informed consent to both phases of the study.

**Study phase I**

**Patients**

About 400 patients with a diagnosis of type 1 diabetes mellitus according to the American Diabetes Association (ADA) guidelines are regularly followed at the Diabetes Care Unit of our Hospital. In this study, we enrolled a consecutive group of such patients who fulfilled all the following inclusion criteria: (i) no symptoms suspected for heart disease in clinical history; (ii) normal physical examination; (iii) normal 2D-echocardiographic colour-Doppler examination; (iv) normal symptom-limited exercise stress test (Bruce protocol); (v) no clinical evidence of any acute or chronic inflammatory disease. All patients were treated on a regular basis with three subcutaneous injections of insulin at meal times and an injection of neutral protamine or glargine insulin at bedtime. Of 51 patients screened for the study, two were excluded because of exercise-induced ST-segment depression. Thus, the study group included 49 diabetic type 1 patients, whose main clinical characteristics are summarized in Table 1.

**Clinical assessment**

For each patient, HbA1c mean values were obtained by high-performance liquid chromatography analysis performed on Diamat BioRad (BioRad, Milan, Italy). The Hba1c reference range for performance liquid chromatography analysis performed on Diamat Clinical assessment summarized in Table 1.

**Table 1 Main clinical data of patients with type 1 diabetes mellitus included in the study**

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.5 ± 11</td>
</tr>
<tr>
<td>Men, n(%)</td>
<td>33 (67)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>22.2 ± 14</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>7.9 ± 1.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9 ± 4</td>
</tr>
<tr>
<td>Insulin requirement (IU/kg)</td>
<td>0.60 ± 0.20</td>
</tr>
<tr>
<td>Microalbuminuria n(%)</td>
<td>16 (33)</td>
</tr>
<tr>
<td>Retinopathy, n(%)</td>
<td>26 (53)</td>
</tr>
<tr>
<td>CAD risk factors</td>
<td></td>
</tr>
<tr>
<td>Familiar history of CAD, n(%)</td>
<td>13 (26)</td>
</tr>
<tr>
<td>Systemic hypertension, n(%)</td>
<td>13 (26)</td>
</tr>
<tr>
<td>Active smoking, n(%)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Total cholesterol levels (mg/dL)</td>
<td>197 ± 33</td>
</tr>
<tr>
<td>Triglyceride levels (mg/dL)</td>
<td>91.6 ± 44</td>
</tr>
<tr>
<td>Drug therapy</td>
<td></td>
</tr>
<tr>
<td>Statins, n(%)</td>
<td>9 (18)</td>
</tr>
<tr>
<td>ACE-inhibitors, n(%)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>ARBs, n(%)</td>
<td>6 (12)</td>
</tr>
</tbody>
</table>

| CAD = coronary artery disease; ACE = angiotensin-converting enzyme; ARBs = angiotensin receptor blockers. |

**Study phase II**

**Heart rate variability**

A 24 h electrocardiographic Holter monitoring (HM) was performed in all patients, using 3-channel tape recorders (Oxford Medilog FDS) and monitoring the bipolar chest leads CM5, CM1 and modified aVF lead. All HM tapes were analysed by an expert cardiologist, using the Oxford Medilog Excel 3.0 device (Oxford Instruments, Abingdon, UK).

Cardiac autonomic function was assessed by frequency-domain HRV analysis on the entire 24 h in the frequency range of 0 to 0.5 Hz using a fast Fourier transform spectral analysis algorithm, with a spectral resolution of 0.0005 Hz. Data were analysed in 10 min epochs throughout the 24 h, and results from all epochs were averaged to form a composite spectrum. The power of the RR-interval variations in the whole frequency range of the spectrum (total power frequency 0–0.5 Hz) and in the range of very-low frequency (VLF, 0.0033–0.04 Hz), low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.40 Hz) were obtained. Furthermore, the LF/HF ratio was calculated, and the average RR interval in the 24 h was obtained.

**C-reactive protein measures**

A venous blood sample was collected in all patients before starting HM. Blood was centrifuged and serum and plasma samples were frozen at −80°C until assayed. C-reactive protein serum levels were measured, using a high-sensitivity immunonephelometric method (Behring Nephelometric 100 Analyzer, Scoppito, Italy), the lowest detection limit of which was 0.05 mg/L.

**Statistics**

Between-group comparisons of continuous variables were done by the Mann–Whitney U test, whereas proportions were compared by Fisher’s exact test. Correlation analyses were done by the Spearman test. Owing to skewed distribution, C-reactive protein and HRV variables were transformed into natural logarithmic values for parametric analyses.
To better define the relation between impaired HRV and subclinical inflammation, patients were divided into quartile groups according to LF power values, and C-reactive protein levels were compared among these groups by analysis of variance (ANOVA), with multiple comparisons done by the Bonferroni test. As ANOVA showed that C-reactive protein serum levels were significantly lower in the bottom quartile group, but did not differ among the three other quartile groups, the latter were grouped together and C-reactive protein levels were also compared between the bottom LF power quartile group and all other patients, both by unadjusted and adjusted ANOVA, with variables associated with C-reactive protein at univariate analysis (P < 0.1) included as covariates.

The response of HRV variables and C-reactive protein serum levels to atenolol, when compared with the randomized control group (atenolol–C-reactive protein interaction), was assessed by two-way ANOVA with a repeated measure design. Correction for possible intra-group correlation was done by the Greenhouse-Geisser method, and the Bonferroni correction was applied for multiple comparisons.

Statistical analyses were done by the SPSS 12.01 statistical software. Data are reported as mean ± SD, unless differently indicated. All tests were two-sided, and a P < 0.05 was required for statistical significance.

**Results**

**Heart rate variability parameters and C-reactive protein levels**

An inverse statistically significant, or just above statistical significant, correlation was found between C-reactive protein levels and HRV variables (Table 2), the most significant association being found with LF power (r = −0.39, P = 0.005, Figure 1). Other variables associated with C-reactive protein serum levels included age, duration of diabetes, body mass index, triglyceride blood levels and microalbuminuria (inverse relation), with active smoking and hypertension being of borderline statistical significance (Table 3).

When assessed according to quartile values of LF power, C-reactive protein serum levels were significantly lower in patients in the bottom quartile (4.64 ± 2.8 mg/L), compared with those in the second (1.76 ± 1.6 mg/L, P < 0.001), third (2.42 ± 1.4 mg/L, P = 0.04), and top (1.20 ± 1.62 mg/L, P < 0.001) quartiles (Figure 2, P = 0.003 by ANOVA), whereas no statistically significant differences were found among the three upper quartile groups. The difference in C-reactive protein levels between the bottom quartile group and the three top quartile groups of LF power combined (1.79 ± 1.6 mg/L) was highly significant.

Table 2 Correlation data between C-reactive protein serum levels and heart rate variability parameters (Spearman rank test)

<table>
<thead>
<tr>
<th>r coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RR interval</td>
<td>0.06</td>
</tr>
<tr>
<td>Total power frequency</td>
<td>−0.28</td>
</tr>
<tr>
<td>Very-low frequency</td>
<td>−0.27</td>
</tr>
<tr>
<td>Low frequency</td>
<td>−0.39</td>
</tr>
<tr>
<td>High frequency</td>
<td>−0.31</td>
</tr>
<tr>
<td>Low-frequency/high-frequency ratio</td>
<td>−0.14</td>
</tr>
</tbody>
</table>

Continuous variables are related to C-reactive protein levels by the Spearman correlation analysis, whereas C-reactive protein levels in patients with or without individual nominal variables are compared by the Mann–Whitney U test.

Figure 1 Relationship between C-reactive protein serum levels and low-frequency power in 49 patients with type 1 diabetes mellitus.

![Figure 1](https://academic.oup.com/eurheartj/article-abstract/28/7/814/2887774/91282264/816754)
Cardiac autonomic dysfunction and inflammation in type 1 diabetic patients

(P = 0.001) and persisted after adjustment for potentially confounding variables (i.e. age, duration of diabetes, body mass index, triglyceride blood levels, microalbuminuria, active smoking, and hypertension) (P = 0.013).

Effect of beta-blockade

Of the 21 patients enrolled in the beta-blocker trial, 11 were randomized to receive atenolol and 10 to no-beta-blocker therapy. There were no differences in the main clinical and laboratory variables between the two groups of patients (Table 4). Both patients in the treatment group and those in the no-treatment group were re-studied at a median follow-up time of 1 month from the first study (interquartile time intervals 1–8 and 1–9, respectively; P = 1.0).

The results of HRV parameters and serum C-reactive protein levels at the end of the trial period in the two groups are shown in Table 5. There were no significant differences in HRV values at the basal evaluation. At follow-up, however, HRV variables were higher in the beta-blocker group when compared with no-beta-blocker patients, with the most significant difference being found for VLF power (P = 0.016).

Moreover, HRV variables did not show any significant changes at follow-up in the no-beta-blocker group, whereas they improved, almost all significantly, in patients treated with atenolol (Figure 3).

C-reactive protein serum levels at baseline were not significantly different between the two groups. At follow-up, on the other hand, there was a significant reduction of C-reactive protein levels in the atenolol group, but not in the no-beta-blocker group (P = 0.04 by two-way ANOVA) (Figure 3).

Discussion

In this study, we found a significant association between impaired cardiac autonomic function, as assessed by the HRV analysis, and low-grade inflammation, as assessed by C-reactive protein serum levels, in patients with type 1 diabetes mellitus. Furthermore, patients with low values of

### Table 4 Main clinical data of patients randomized in the beta-blocker trial

<table>
<thead>
<tr>
<th></th>
<th>Atenolol (n = 11)</th>
<th>No atenolol (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.2 ± 8</td>
<td>54.4 ± 10</td>
<td>0.96</td>
</tr>
<tr>
<td>Men/women</td>
<td>6/5</td>
<td>5/5</td>
<td>1.0</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>28.0 ± 15</td>
<td>29.1 ± 13</td>
<td>0.86</td>
</tr>
<tr>
<td>Glycosylated Hb (%)</td>
<td>7.5 ± 1.0</td>
<td>8.2 ± 1.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.5 ± 5</td>
<td>25.7 ± 3</td>
<td>0.34</td>
</tr>
<tr>
<td>Insulin requirement (IU/kg)</td>
<td>0.61 ± 0.2</td>
<td>0.69 ± 0.3</td>
<td>0.45</td>
</tr>
<tr>
<td>Microalbuminuria, n(%)</td>
<td>6 (54)</td>
<td>5 (50)</td>
<td>1.0</td>
</tr>
<tr>
<td>Retinopathy, n(%)</td>
<td>7 (64)</td>
<td>7 (70)</td>
<td>1.0</td>
</tr>
<tr>
<td>CAD risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>4 (36)</td>
<td>3 (30)</td>
<td>1.0</td>
</tr>
<tr>
<td>Systemic hypertension</td>
<td>5 (45)</td>
<td>4 (40)</td>
<td>1.0</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>191 ± 38</td>
<td>207 ± 25</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>91 ± 21</td>
<td>113 ± 74</td>
<td>0.34</td>
</tr>
<tr>
<td>Active smoking</td>
<td>3 (27)</td>
<td>3 (30)</td>
<td>1.0</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>3 (27)</td>
<td>2 (20)</td>
<td>1.0</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>3 (27)</td>
<td>1 (10)</td>
<td>0.59</td>
</tr>
<tr>
<td>ARBs</td>
<td>1 (9)</td>
<td>3 (30)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*P-values indicate basal comparisons between groups.

**P-values referring to beta-blocker x variable interaction according to two-way ANOVA on logarithmic transformed data.
LF power showed higher C-reactive protein serum levels also after adjustment for possible confounding variables.

Most important, we found that the significant improvement of HRV in the group of patients with a clear impairment of cardiac autonomic function treated with atenolol was associated with a parallel reduction of C-reactive protein serum levels. In contrast, no significant changes in both HRV variables and C-reactive protein serum levels were detected in patients not treated with atenolol.

Taken together, our data give further support to the evidence that an imbalance of the ANS activity, characterized by a predominance of sympathetic activity, may favor inflammatory reactions and suggest that intervention able to modify the sympatho-vagal balance towards a reduction of adrenergic activity and a relative increase of vagal tone may also modulate the inflammatory state.

Finding interpretation

The frequent impairment of autonomic nervous function in diabetic patients is well known and it is also associated with worse clinical conditions and outcome. More recent studies, on the other hand, have shown that inflammation is also increased in diabetic patients and might also contribute to a bad clinical outcome. In this study, for the first time, we show a significant association in type 1 diabetic patients between inflammation, as assessed by serum C-reactive protein levels, and impaired cardiac autonomic function, as assessed by frequency-domain HRV parameters.

In a very recent study, diabetic neuropathy, in type 1 diabetic patients, has been shown to be associated with tissue necrosis factor-alpha system, whereas no significant relation with C-reactive protein levels was found. However, in this study, only a minority of patients showed abnormalities in cardiac autonomic function, which was also assessed with methods (deep breathing, Valsalva manoeuvre) less sensitive than HRV in detecting early cardiac autonomic impairment. Our results, on the other hand, confirm some previous findings obtained in other populations, including healthy subjects, patients with stable or unstable coronary artery disease, and patients with heart failure. In particular, by showing significantly increased C-reactive protein serum levels specifically in patients with LF power values in the bottom quartile, the results of this study confirm our previous observation that the relation between cardiac autonomic function and inflammation may become particularly evident when clearly abnormal findings are detectable, whereas it may be less evident when subjects with HRV values and C-reactive protein levels still in the normal or near-normal range are considered.

The exact relationship between cardiac autonomic dysfunction and inflammation in the clinical setting remains to be elucidated. Indeed, whether impaired autonomic function may be a major causal factor for inflammation or vice versa or even whether both are caused by an independent third factor remains to be established.

Experimental findings suggest that the nervous autonomic system can significantly modulate inflammatory reactions. Autonomic fibres innervate the lymphoreticular system (e.g. spleen, liver, gut, bone marrow) and may influence tissue inflammatory cells in these organs as well as in inflamed tissues. Vagal stimulation, in particular, may reduce inflammatory reactions by inhibiting tissue macrophage activation through stimulation by acetylcholine and of macrophage nicotine receptors. High sympathetic activity, on the other hand, may favour, and sympathectomy thwart, inflammatory reactions.

Products of inflammation, however, have also the potential to influence nervous autonomic activity. Thus, IL-6 and TNF may reach the hypothalamus and the limbic system and stimulate autonomic-related centers. Furthermore, inflammatory mediators may stimulate peripheral afferent endings evoking autonomic responses finalized to modulate the inflammatory reaction (inflammatory reflex).

In this study, for the first time, we give some evidence in man that a causal link may exist between modulation of ANS activity and inflammation. Indeed, the beta-blocker atenolol, as expected, improved HRV (i.e. sympatho-vagal balance) in our patients, and this was associated with a parallel decrease in C-reactive protein in the absence of any change of the clinical status of patients.

Although we cannot exclude an effect of atenolol on inflammation, we suggest that the C-reactive protein reduction observed in our patients was actually mediated by the improvement of autonomic nervous function. Indeed, the direct effects of beta-blockers on cardiac autonomic activity are well known, and several studies have repeatedly showed its ability to improve HRV in several groups of patients, including type 1 diabetic patients.

Thus, although several other factors may variably contribute to inflammation and although we cannot exclude that inflammation may influence ANS activity, our data strongly support the hypothesis that short-term variations in ANS activity may significantly influence inflammation in diabetic type 1 patients and that this is likely to occur also in other clinical conditions.

It should be acknowledged that we cannot also exclude the possibility that the reduction in C-reactive protein by atenolol might be related to a local antagonist drug effect on a potential adrenergically stimulated C-reactive protein
production by liver cells. To our knowledge, however, there are no clinical or experimental findings supporting a direct effect of adrenergic activity on C-reactive protein release by the liver.

Clinical implications
The possibility to improve simultaneously autonomic nervous function and inflammation may suggest that beta-blockers may be helpful in diabetic patients with cardiac autonomic dysfunction, although our data deserve confirmation in larger studies, and only appropriately designed clinical trials may establish whether beta-blockers may improve clinical outcomes in these patients.

Recent data suggested that inflammation may be predictive of sudden death in some groups of patients with severe forms of heart disease. The association between HRV and C-reactive protein, however, may suggest that this relation may be mediated by the impaired autonomic function, frequently present and predictive of sudden death in these groups of patients.

Limitations of the study
It should be acknowledged that the beta-blocker study included a small group of patients and was not blinded or placebo-controlled. However, HRV variables and C-reactive protein serum levels were assessed independently and in a placebo-controlled. However, HRV variables and C-reactive protein measurements, whereas confirmation of the effect was not blinded or placebo-controlled.

Inflammation was assessed only by serum C-reactive protein measurements, whereas confirmation of the effect of atenolol on inflammatory state by other markers of inflammation might have given further strength to our study. However, C-reactive protein is the most largely used inflammatory biomarker in research studies and in clinical practice, and the large experience accumulated on C-reactive protein suggests that it can be used reliably to assess the inflammatory state.

Conflict of interest: none declared.

References
Clinical vignette

Kommerell’s aneurysm

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A 54-year-old man had sudden onset chest pain with radiation to back 4 years ago. Right-sided aortic arch, aberrant left subclavian artery, and type B dissection were noted. The symptoms resolved after conservative treatment. One month prior to this admission, he developed progressive dysphasia and exertional dyspnoea. Plain chest film showed widening mediastinum and marked anterior displacement of trachea. Computed tomography showed a huge Kommerell’s aneurysm with dissection flap and aberrant left subclavian artery. The trachea and oesophagus were significantly compressed. Thoracic aortic replacement from mid-arch to low thoracic aorta and reconstruction of both subclavian arteries were performed via right thoracotomy under partial cardiopulmonary bypass. The patient had an uneventful recovery.

Panel A. Chest X-ray shows an enlarged mediastinal silhouette.

Panel B. Lateral plain film shows anterior displacement of trachea (arrow).

Panel C. Chest CT-scan shows retrotracheal aneurysm with dissection flap.

Panel D. Reconstructed chest CT-scan shows aberrant left subclavian artery originated from the dissecting aneurysm.

Panel E. Post-operative MRI shows bypass graft (G) of thoracic aorta and four individual arch vessels (arrow).

Panel F. Post-operative reconstructed CT-scan shows patent left subclavian artery graft in the residual aneurysm cavity (arrow).