123I-mIBG assessed cardiac sympathetic activity: standardizing towards clinical implementation
Verschure, D.O.

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Myocardial $^{123}$I-mIBG scintigraphy in relation to markers of inflammation and long-term clinical outcome in patients with stable chronic heart failure

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R Lutter
BL van Eck-Smit
GA Somsen
HJ Verberne
ABSTRACT

Aim
Chronic heart failure (CHF) results in both increased cardiac sympathetic activity and myocardial inflammation. The aim of this study was to identify the relationship between severity of heart failure (i.e. NT-proBNP and LVEF), cardiac sympathetic activity (¹²³I-mIBG scintigraphy) and measures of inflammation in subjects with stable, optimally treated CHF. In addition, the predictive value for cardiac events (i.e. ventricular arrhythmia, progression of CHF and cardiac death) of ¹²³I-mIBG parameters and these inflammatory markers was evaluated.

Materials and methods
Fifty-five CHF patients (age 66.3±8.0 years, 78% male, LVEF 22.4±6.3) referred for cardiac ¹²³I-mIBG imaging were included. At 15 minutes (early) and 4 hours (late) after i.v. administration of ¹²³I-mIBG (185 MBq), planar images were acquired. Early heart-to-mediastinum (H/M) ratio, late H/M ratio and ¹²³I-mIBG washout (WO) were calculated. NT-proBNP and markers of inflammation (i.e. C-reactive protein (CRP), IL-1β, IL-6, IL-10, IL-12p40, tumor necrosis factor-α (TNF-α), soluble (s)E-selectin, myeloperoxidase (MPO), plasminogen activator inhibitor-1 (PAI-1), tPA, tumor necrosis factor receptor (TNFR) 1 and 2 and interferon (IFN) α and β) were measured in blood plasma samples, taken just before ¹²³I-mIBG administration.

Results
Mean early H/M ratio was 2.12 ± 0.39, late H/M ratio was 1.84 ± 0.40 and ¹²³I-mIBG WO was 13.0 ± 10.9. LVEF was the only independent predictor of late H/M ratio (adjusted R² = 0.100, p = 0.011). NT-proBNP was an independent predictor of ¹²³I-mIBG WO (adjusted R² = 0.090, p = 0.015). CRP, IL12p40, TNF-α, sE-selectin, MPO, PAI-1, tPA and TNFR2 were not related to late H/M ratio and ¹²³I-mIBG WO.

During a median follow-up of 34 months (2-58 months), 13 patients experienced a cardiac event (ventricular arrhythmia (4), progression of CHF (4) and cardiac death (5)). Univariate Cox regression analysis showed that the risk of a cardiac event was associated with CRP (HR 1.047 [1.013-1.081]), NT-proBNP (HR 1.141 [1.011-1.288]), MPO (HR 0.998 [0.996-1.000]) and late H/M ratio (HR 0.182 [0.035-0.946]). Multivariate Cox regression analysis showed that only CRP, NT-proBNP, MPO and IL-12p40 were predictors of a cardiac event.

Conclusion
Inflammation and cardiac sympathetic activity seem not to be related in stable CHF patients. This is corroborated by the finding that they both provide prognostic information in this specific CHF population. The current findings should be regarded as insightful but preliminary.
INTRODUCTION

Chronic heart failure (CHF) is a complex syndrome characterized by increased activity of the sympathetic nervous system, increased NT-proBNP levels and increased pro-inflammatory cytokines in plasma and myocardial tissue. Inflammation plays an important role in the pathogenesis and progression of many forms of heart failure (HF). With progression of CHF, the plasma levels of pro-inflammatory cytokines increase. In addition, cardiac sympathetic hyperactivity is associated with progression of CHF. However, there is only limited information on the relation between the sympathetic nervous activity and the inflammatory status in CHF patients.

Cardiac sympathetic activity can non-invasively be assessed with cardiac £I-meta-iodobenzyguanidine (£I-mIBG). mIBG is a norepinephrine (NE) analog that shares the same presynaptic uptake, storage and release mechanism as NE. Radiolabeling of mIBG with £I allows imaging with gamma cameras. The heart-to-mediastinum (H/M) ratio reflects presynaptic myocardial uptake of £I-mIBG. The early H/M ratio reflects predominantly the integrity of sympathetic nerve terminals (i.e. number of functioning nerve terminals and intact uptake-1 mechanism). The late H/M ratio offers predominantly information about neuronal function resulting from uptake, storage and release. The myocardial £I-mIBG washout (WO) reflects predominantly neuronal integrity of sympathetic tone/adrenergic drive. The late H/M ratio and £I-mIBG WO have been shown to be of clinical value, especially for the assessment of prognosis, in many cardiac diseases. Decreased late H/M ratio and increased £I-mIBG WO are associated with poor outcome in subjects with CHF.

The aim of this study was to identify the relationship between severity of HF (i.e. NT-proBNP and LVEF), cardiac sympathetic activity assessed with £I-mIBG scintigraphy and measures of inflammation in patients with stable CHF. In addition, we evaluated the prognostic value of these inflammatory markers and myocardial £I-mIBG-derived parameters (i.e. late H/M ratio and £I-mIBG WO).

MATERIAL AND METHODS

Subjects

Patients with stable CHF (New York Heart Association (NYHA) class II to III) who were referred for £I-mIBG scintigraphy in their work-up for primary ICD implantation between 2010 and 2015 to the department of Nuclear Medicine of the Academic Medical Center in Amsterdam were enrolled. The inclusion criteria were as follows: stable heart failure (i.e. no myocardial infarction, hospitalization or progression of heart failure) at least 3 months before inclusion and treatment with optimal medical therapy.
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according to the European HF guidelines including beta-blockers and angiotensin-converting-enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) and if necessary loop diuretics. Exclusion for participation was pregnancy or intolerance for iodine. All subjects provided written informed consent. The study was approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonization–Good Clinical Practice.

123I-mIBG scintigraphy acquisition and analysis
All patients continued their HF medication prior to 123I-mIBG scintigraphy. To block uptake of free 123I by the thyroid gland, subjects were pretreated with 250 mg oral potassium iodide 30 min before intravenous (IV) injection of 185 MBq 123I-mIBG (GE Healthcare, Eindhoven, the Netherlands). Fifteen minutes (early acquisition) and 4 hours (late acquisition) after administration of 123I-mIBG, 10-min planar images were acquired with the subjects in supine position using a gamma camera equipped with a medium energy (ME) collimator.

All planar 123I-mIBG images were analysed by one experienced observer (D.O.V.) blinded to patient data. H/M ratios were calculated from the 123I-mIBG images using a region-of-interest (ROI) over the heart and the upper part of the mediastinum. The H/M ratio was calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI. The 123I-mIBG WO was calculated as follows:

\[
WO = \left\{ \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right\} \times 100
\]

Markers of inflammation
Before administration of 185 MBq 123I-mIBG, 2 × 4.0 ml of venous blood was collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Immediately after collection of the required blood volume, one EDTA tube was analysed for CRP and NT-proBNP by standard procedures. The second EDTA tube was mixed with the sampled blood and immediately cooled on ice. Within 30 min of collection, the tubes were centrifuged at 1700 g for 10 min. The plasma was transferred to cryovials and stored in upright position at -70 °C till analysis. All samples were analysed at the end of the study for IL-1β, IL-6, IL-8, IL-10, IL-12p40, TNF-α, sE-selectin, MPO, PAI-1, tPA, TNFR1 and TNFR2 and INF α and β by luminex. Samples were measured with ProCarta reagents (eBioscience) following the supplier’s protocol and read on a Bioplex 200 (BioRad).

Clinical follow-up and event adjudication.
Follow-up was based on telephone interviews (D.O.V.) and medical records. All subjects received standard clinical care and were followed up until: 1) subject death was confirmed; 2) the trial was terminated. The Clinical Adjudication Committee
reviewed data from case report forms and source documents to confirm the occurrence of CEs, specifically: 1) HF progression: increase in symptomatic status from NYHA functional class II to III or IV, or increase from NYHA class III to class I or 2) potentially life-threatening arrhythmic event, including documented episode of spontaneous sustained (30 s) ventricular tachyarrhythmia, resuscitated cardiac arrest, or appropriate ICD therapy (antitachycardia pacing or defibrillation); or 3) cardiac death (further classified as due to HF progression, sudden cardiac death [SCD]).

Statistical Analysis
All continuous variables are expressed as mean ± standard deviation. After demonstrating a normal distribution of variables, between-group comparisons were performed using independent-sample t-tests. Non-detectable levels of biomarkers were treated as zeroes for the analysis. Continuous data were compared using analysis of variance (ANOVA). The association between $^{123}$I-mIBG outcomes and biomarkers was assessed using Pearson's correlation coefficient (2-tailed). Multivariate linear regression analysis was performed to determine independent predictors of $^{123}$I-mIBG parameters. The overall goodness-of-fit for each model was expressed as the adjusted $R^2$. The F-test was used to assess whether a model explained a significant proportion of the variability. A p-value < 0.05 was considered to indicate a statistical significance. Univariate and multivariate Cox proportional hazards regression analysis was used to assess independent predictors of cardiac events. ROC analysis was used to determine the optimal cut-off value (i.e. highest product of sensitivity and specificity) for predictors of CEs. Statistical analyses were performed with SPSS, release 22.0 for Windows (SPSS Inc., Chicago, IL, USA 2003).
<table>
<thead>
<tr>
<th></th>
<th>All  ((n = 55))</th>
<th>Cardiac event ((n = 13))</th>
<th>No cardiac event ((n = 42))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>66.3 ± 8.0</td>
<td>66.4 ± 7.0</td>
<td>66.2 ± 8.4</td>
<td>0.118</td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>43 (78)</td>
<td>10 (77)</td>
<td>33 (79)</td>
<td>0.809</td>
</tr>
<tr>
<td><strong>Etiology heart failure</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.790</td>
</tr>
<tr>
<td>Ischaemic (%)</td>
<td>33 (60)</td>
<td>8 (62)</td>
<td>25 (60)</td>
<td></td>
</tr>
<tr>
<td>Non-ischaemic (%)</td>
<td>22 (40)</td>
<td>5 (38)</td>
<td>17 (40)</td>
<td></td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>125 ± 17</td>
<td>126 ± 14</td>
<td>125 ± 19</td>
<td>0.223</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>76 ± 10</td>
<td>77 ± 8</td>
<td>75 ± 11</td>
<td>0.148</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>22.4 ± 6.3</td>
<td>23.9 ± 6.6</td>
<td>22.1 ± 6.2</td>
<td>0.491</td>
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<tr>
<td><strong>NYHA</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.051</td>
</tr>
<tr>
<td>Class II (%)</td>
<td>41 (75)</td>
<td>8 (62)</td>
<td>33 (79)</td>
<td></td>
</tr>
<tr>
<td>Class III (%)</td>
<td>14 (25)</td>
<td>5 (38)</td>
<td>9 (21)</td>
<td></td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betablockers (%)</td>
<td>43 (78)</td>
<td>9 (69)</td>
<td>34 (81)</td>
<td>0.118</td>
</tr>
<tr>
<td>ACE-I/ARBs (%)</td>
<td>50 (91)</td>
<td>13 (100)</td>
<td>37 (88)</td>
<td>0.004</td>
</tr>
<tr>
<td>MRAs (%)</td>
<td>18 (33)</td>
<td>5 (38)</td>
<td>13 (31)</td>
<td>0.560</td>
</tr>
<tr>
<td>Loop diuretics (%)</td>
<td>38 (69)</td>
<td>10 (77)</td>
<td>28 (67)</td>
<td>0.122</td>
</tr>
<tr>
<td>Statines (%)</td>
<td>38 (69)</td>
<td>9 (69)</td>
<td>29 (69)</td>
<td>0.980</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>32 (58)</td>
<td>6 (46)</td>
<td>26 (62)</td>
<td>0.448</td>
</tr>
<tr>
<td><strong>¹²³I-mIBG parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Early H/M ratio</td>
<td>2.12 ± 0.39</td>
<td>2.00 ± 0.28</td>
<td>2.16 ± 0.42</td>
<td>0.140</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.84 ± 0.40</td>
<td>1.66 ± 0.28</td>
<td>1.90 ± 0.42</td>
<td>0.137</td>
</tr>
<tr>
<td><strong>¹²³I-mIBG WO</strong></td>
<td>13.0 ± 10.9</td>
<td>19.8 ± 10.6</td>
<td>11.8 ± 10.8</td>
<td>0.897</td>
</tr>
</tbody>
</table>
Cardiac sympathetic activity and inflammation in CHF

Table 2. Biomarkers of CHF patients.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>All (n = 55)</th>
<th>Cardiac event (n = 13)</th>
<th>No cardiac event (n = 42)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>5.44 ± 11.07</td>
<td>9.4 ± 20.3</td>
<td>4.2 ± 5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>6.00 ± 2.41</td>
<td>5.56 ± 1.41</td>
<td>6.12 ± 2.65</td>
<td>0.483</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>26.33 ± 10.67</td>
<td>28.91 ± 10.83</td>
<td>25.54 ± 10.62</td>
<td>0.800</td>
</tr>
<tr>
<td>IL-12p40 (pg/ml)</td>
<td>7.51 ± 5.58</td>
<td>9.68 ± 6.81</td>
<td>6.83 ± 5.04</td>
<td>0.154</td>
</tr>
<tr>
<td>MPO (ng/ml)</td>
<td>72.60 ± 56.44</td>
<td>52.05 ± 32.31</td>
<td>78.96 ± 60.95</td>
<td>0.018</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>8.50 ± 1.69</td>
<td>8.50 ± 2.04</td>
<td>8.51 ± 1.60</td>
<td>0.355</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>1.56 ± 0.68</td>
<td>1.73 ± 0.83</td>
<td>1.51 ± 0.64</td>
<td>0.294</td>
</tr>
<tr>
<td>TNFR2 (pg/ml)</td>
<td>174 ± 57</td>
<td>191 ± 50</td>
<td>168 ± 58</td>
<td>0.602</td>
</tr>
<tr>
<td>NT-proBNP (ng/L)</td>
<td>1974 ± 3026</td>
<td>3280 ± 5402</td>
<td>1570 ± 1675</td>
<td>0.000</td>
</tr>
</tbody>
</table>

RESULTS

In total 252 patients with CHF were screened for enrollment. However the majority of the subjects did not meet the inclusion criteria of at least 3 months stable heart failure, already participate in other studies or refused to give informed consent. Baseline patient characteristics including $^{123}$I-mIBG-derived parameters are presented in Table 1. The study population comprised 55 stable CHF patients (43 male and 12 female) with a mean age of 66.3 ± 8.0 years. Forty-one (75%) patients were in NYHA class II and 14 (25%) we in NYHA class III. The mean LVEF was 22.4 ± 6.3% and 60% of the patients had ischaemic heart disease. The mean early H/M ratio was 2.12 ± 0.39, the late H/M ratio was 1.84 ± 0.40 and the mean $^{123}$I-mIBG WO was 13.0 ± 10.9. Most of the plasma levels of IL-1β, IL-6, IL-8, IL-10, TNFR1, IFN-α and IFN-β were below the detection limit. Table 2 shows only biomarkers with levels above lower limit of detection in all patients.

Association between $^{123}$I-mIBG and markers of inflammation

The LVEF, NYHA functional class, NT-proBNP, CRP, TNFα, TNFR2, sE-selectin, IL12p40, MPO, PAI-1 and tPA were used as explanatory variables of late H/M ratio and $^{123}$I-mIBG WO. Late H/M ratio was significantly associated with LVEF (R = 0.342, p = 0.011) and NT-proBNP (R = -0.272, p = 0.045). $^{123}$I-mIBG WO was also significantly associated with LVEF (R = -0.286, p = 0.034), NYHA (R = 0.281, p = 0.038) and NT-proBNP (R = 0.325, p = 0.015). Multivariate regression analysis using both biomarkers and clinical parameters (i.e. LVEF, NYHA functional class) showed LVEF as the only independent predictor of late H/M ratio (adjusted R² = 0.100, p = 0.011). NT-proBNP was the only independent parameter associated with $^{123}$I-mIBG WO (adjusted R² = 0.090, p = 0.015) (Table 3).
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Table 3. Multivariate regression analysis to determine independent predictors for late H/M ratio (upper panel) and $^{123}$I-mIBG WO (lower panel).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient $b$</th>
<th>Standard error $b$</th>
<th>$p$-value</th>
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<tbody>
<tr>
<td>Constant</td>
<td>1.351</td>
<td>0.193</td>
<td></td>
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<tr>
<td>LVEF</td>
<td>0.022</td>
<td>0.008</td>
<td>0.011</td>
</tr>
<tr>
<td>Goodness-to-fit of the model</td>
<td>Adjusted $R^2$</td>
<td>$p$-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td></td>
<td>0.011</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient $b$</th>
<th>Standard error $b$</th>
<th>$p$-value</th>
</tr>
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<tbody>
<tr>
<td>Constant</td>
<td>10.667</td>
<td>1.678</td>
<td></td>
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<tr>
<td>NT-proBNP</td>
<td>0.001</td>
<td>0.000</td>
<td>0.015</td>
</tr>
<tr>
<td>Goodness-to-fit of the model</td>
<td>Adjusted $R^2$</td>
<td>$p$-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.090</td>
<td></td>
<td>0.015</td>
</tr>
</tbody>
</table>

Predictors of cardiac events

None of the patients were lost during a median follow-up of 34 months (2 - 58 months). Thirteen patients (24%) experienced a first CE: progression of HF ($n = 4$), arrhythmic event with appropriate ICD therapy ($n = 4$) and cardiac death ($n = 5$; four subjects due to sudden cardiac death (SCD) and one due to progression of HF). In addition, one patient had a non-cardiac death. There was a significant difference in plasma levels of NT-proBNP, CRP and MPO between patients with and without CEs (Table 2). However, there was no significant difference in late H/M ratio and $^{123}$I-mIBG WO between both groups. Univariate Cox regression analysis showed that the risk of a CE was associated with CRP, NT-proBNP, MPO and late H/M ratio (Table 4). Multivariate Cox regression analysis showed that CRP, MPO, IL-12p40 and NT-proBNP were independent predictors of a CE. CRP, MPO, IL12p40 and NT-proBNP did not show any mutual relationship with each other (data not shown). Figure 1 shows cumulative event curves for the late H/M ratio and CRP. ROC analysis showed that the optimal cut-off values for the late H/M ratio and CRP were 1.68 and 1.85 mg/l, respectively. These cut-off values resulted in a moderate discriminative power: AUC for late H/M ratio 0.69 and for CRP 0.64, respectively).
Figure 1. Examples of cumulative event curves for different parameters. A. Comparing CHF patients with late H/M ratio < 1.68 versus > 1.68 (p = 0.019). B. Comparing CHF patients with CRP < 1.85 mg/L versus CRP > 1.85 mg/L (p = 0.032).
DISCUSSION

The findings of this study show that in this specific, optimally treated and stable, CHF population general markers of inflammation were not associated with increased cardiac sympathetic activity assessed with $^{123}$I-mIBG. In addition, the cardiac sympathetic activity was associated with severity of heart failure (i.e. NT-proBNP and LVEF). In line with the lack of association between myocardial $^{123}$I-mIBG parameters and markers of inflammation, both were related to the prognosis of this specific CHF population.

Irrespective of the etiology of CHF, pro-inflammatory cytokines are implicated in the progression of CHF.\textsuperscript{4,15,16} In CHF patients increased levels of TNF-α are associated with impaired LV function and consequently increased mortality.\textsuperscript{17,18} Although the exact mechanism of TNF-α in relation to CHF remains to be elucidated, it has been reported that TNF-α induces β-adrenergic receptor (βAR) desensitization.\textsuperscript{19} This phenomenon identifies a cross-talk between TNF-α and βAR function that at least in part contributes to a potential further reduction in cardiac contractility in CHF. In addition, increased levels of IL-6 have been reported in CHF patients and are correlated to plasma levels of NE ($R = 0.839, p < 0.0001$).\textsuperscript{20} In our study plasma levels of IL-1β, IL-6, IL-10,
Cardiac sympathetic activity and inflammation in CHF

IFN-α, IFN-β and TNFR1 were below the detection limit. We consider it unlikely that our procedure failed to detect these cytokines as we took great care to process the blood samples quickly and limit activation. In addition, the earlier spike experiments for these cytokines yielded good recoveries and the internal standards were correct. A possible explanation for these undetectable levels could be the treatment with statins (hydroxymethylglutaryl-CoA reductase inhibitors), aspirin, ACE-I, ARBs, mineralocorticoid receptor antagonists (MRAs) and beta-blockers. Statins have pleiotropic benefits independent of cholesterol levels including anti-inflammatory effects and it has been suggested that statins might reduce the production of TNF-α, IL-1, and IL-6.19,21-23 In addition aspirin, ACE-I/ARBs, MRAs and beta-blockers have been shown to decrease plasma levels of cytokines.24 Consequently, the use of these drugs could have influenced the plasma levels of cytokines in our population. In addition, these findings may suggest that our stable CHF patients were optimally treated.

In line with others, we showed increased cardiac sympathetic activity (i.e. decreased late H/M ratio and increased 123I-mIBG WO) in a stable CHF population.11,25,26 However, in contrast to previous studies with IDCM27,28, our study did not show a significant correlation between the most important markers of inflammation (i.e. TNF-α, IL-1β and IL-6) and cardiac sympathetic activity. IL-1β and IL-6 levels were below the lower limit of quantification, whereas TNF-α was detectable, but did not show a correlation. In conclusion, in this population of stable, optimally treated CHF markers of inflammation were subordinate to the more frequently used markers of prognosis in CHF (i.e. NT-proBNP, LVEF, NYHA class) in relation to sympathetic activity.

LVEF and NT-proBNP were moderately, but significantly related to late H/M ratio. In addition, LVEF, NT-proBNP and NYHA class were moderately related to 123I-mIBG WO. Recently, it has been shown that BNP modulates autonomic nervous function by inhibiting cardiac sympathetic activity in CHF.29 As in CHF prolonged increased cardiac sympathetic activity has a detrimental effect on the contractility of the myocardium, this influences the LVEF. This is in line with the found negative association between LVEF and 123I-mIBG WO.

Predictor of cardiac events

Increased cardiac sympathetic activity occurs early in the CHF disease process. Initially, βAR stimulation by the increased NE levels helps to compensate for impaired myocardial function, but long-term NE excess has detrimental effects on myocardial structure and gives rise to a down regulation of post-synaptic βARs.(30) This down regulation leads to left ventricle remodeling and poor prognosis. In our study decreased late H/M ratio was associated with CEs (Figure 1A). This is line with two large meta-analyses and a large prospective multicentre trial.11,25,26 However, in our study not late H/M ratio, but CRP, MPO, IL12p40 and NT-proBNP were the only independent predictors of CEs.
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CRP is regarded a non-specific marker for acute inflammation. Several studies have examined CRP levels in coronary heart disease and have demonstrated its prognostic value.\textsuperscript{31,32} Interestingly, in our study, CRP levels were significantly higher in patients with an event compared to those without CEs (Figure 1B). Similar results were reported in IDCM patients.\textsuperscript{33} The past decade high-sensitive (hs)CRP has been introduced and is commonly used for cardiovascular risk stratification. Increased hsCRP is associated with increased risk for cardiovascular disease and mortality.\textsuperscript{34}

MPO, an enzyme derived from neutrophilic granulocytes reflects inflammation and plaque destabilization. In addition to a possible candidate biomarker to predict future adverse events in patients with acute coronary syndromes (ASC) and myocardial infarction,\textsuperscript{35,36} MPO has been associated with the severity of CHF.\textsuperscript{4} In our study the levels of MPO were elevated. Interestingly, activation of neutrophils leads to the release of preformed IL-8 and MPO. As we found no IL-8 in conjunction with MPO, we consider it unlikely that MPO was generated during processing of blood samples.\textsuperscript{37} In contrast to previous studies,\textsuperscript{35,36} we found a negative association between these elevated levels of MPO and CEs, suggesting a protective mechanism of MPO. However, due to the relatively limited sample size, this extraordinary finding should be interpreted with great care.

IL-12p40 is elevated in an early stage of atherosclerosis both at the mRNA and protein level.\textsuperscript{38} Elevated levels of IL-12p40 have also been reported in patients with coronary artery disease (CAD).\textsuperscript{39,40} Interestingly in a murine atherosclerosis study, aspirin reduced the levels of IL-12p40, suggesting the involvement of IL-12p40 on vascular inflammation.\textsuperscript{41} Our study showed a prognostic value of IL-12p40 in a stable CHF population. This association is most likely explained by the high percentage (i.e. 60\%) of CAD in our CHF population.

NT-proBNP is a widely used powerful predictor of clinical outcome and a better marker for efficacy of drug treatment in patients with HF than other biomarkers or clinical parameters. Its clinical use has been endorsed in clinical practice guideline.\textsuperscript{13} NT-proBNP was an independent predictor of CEs, in line with previous results showing almost exponentially raising risk with increasing levels of NT-proBNP.\textsuperscript{42}

Our study has several limitations. The major limitation of the study is the relative small number of patients included. This may have resulted in a limited statistical power. Therefore, the results of the study should be regarded as insightful but preliminary and larger studies are necessary to confirm our findings. Second, the study population was heterogeneous including ischaemic and non-ischaemic heart disease. Although markers of inflammation were elevated in both groups, it is conceivable that elevation of these markers is more pronounced in the presence of atherosclerosis (i.e. ischaemia). Third, the event rate was relatively low in this stable heart failure population, resulting in non-significant result for the individual endpoints. Therefore, a combination of the individual endpoints into a single endpoint was chosen. Finally treatment with statins, aspirin, ACE-I or ARBs, MRAs and beta-blockers may have influenced cytokine
production. However, treatment of CHF with these drugs is followed according to the international HF guidelines. Therefore, our results are an accurate reflection of this specific stable CHF population.

In conclusion, this study demonstrated that some markers of inflammation were undetectable most likely reflecting adequate medical treatment in clinically stable and optimally treated CHF patients. However, there is no association between detectable general markers of inflammation and cardiac sympathetic activity in this stable CHF population. This was corroborated by the fact that both markers of inflammation and cardiac sympathetic activity were prognostic indicators of CEs.

**New knowledge gained**
The results of this study support the notion that inflammation and cardiac sympathetic activity are both important markers reflecting the multifactorial aspects of heart failure progression.
REFERENCES


Cardiac sympathetic activity and inflammation in CHF


Chapter 9


