CHAPTER 6

Sympathetic Denervation is Associated with Microvascular Dysfunction in Non-infarcted Myocardium in Patients with Cardiomyopathy

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ABSTRACT

Aims: Sympathetic denervation typically occurs in the infarcted myocardium and is associated with sudden cardiac death. Impaired innervation was also demonstrated in non-infarcted myocardium in ischemic and dilated cardiomyopathy (ICMP and DCMP). Factors affecting sympathetic nerve integrity in remote myocardium are unknown. Perfusion abnormalities, even in the absence of epicardial coronary artery disease, may relate to sympathetic dysfunction. This study was aimed to assess the interrelations of myocardial blood flow (MBF), contractile function, and sympathetic innervation in non-infarcted remote myocardium.

Methods and results: Seventy patients with ICMP or DCMP and LVEF ≤35% were included. \[^{15}\text{O}]\text{H}_2\text{O}\text{- and }[^{11}\text{C}]\text{hydroxyephedrine (HED)} – PET was performed to quantify resting MBF, hyperemic MBF, and sympathetic innervation. CMR imaging was performed to assess left ventricular function, mass, wall-thickening, and scar size. Wall-thickening, \[^{11}\text{C}]\text{HED retention index (RI), and MBF were assessed in remote segments without scar, selected on CMR. }[^{11}\text{C}]\text{HED RI was correlated with resting MBF (r=0.41, p<0.001) and hyperemic MBF (r=0.55, p<0.001) in remote myocardium in both ICMP and DCMP. In addition, LV volumes (r=−0.40, p=0.001), LV mass (r=−0.31, p=0.008), and wall-thickening (r=0.45, p<0.001) correlated with remote[^{11}\text{C}]\text{HED RI. Multivariable analysis revealed that hyperemic MBF (B=0.79, p<0.001), wall-thickening (B=0.01, p=0.03), and LVEDV (B=−0.03, p=0.02) were independent predictors for remote[^{11}\text{C}]\text{HED RI.}}

Conclusion: Hyperemic MBF is independently associated with sympathetic innervation in non-infarcted remote myocardium in patients with ICMP and DCMP. This suggests that microvascular dysfunction might be an important factor related to sympathetic nerve integrity. Whether impaired hyperemic MBF is the primary cause of this relation remains unclear.
INTRODUCTION

Impaired cardiac sympathetic innervation in cardiomyopathy is associated with progression of heart failure and increased mortality.\(^1\) Furthermore, it was recently demonstrated that the total denervation size as assessed with \([^{11}\text{C}]\text{Hydroxyephedrine positron emission tomography (\([^{11}\text{C}]\text{HED PET})\text{)) predicts sudden cardiac death in patients with ischemic cardiomyopathy (ICMP).}^2\) Sympathetic denervation typically results from myocardial infarction and often exceeds infarct size as myocardial nerves are more susceptible to ischaemia than myocardium itself.\(^2\)\(^-\)\(^6\) Indeed, patients with chronic multivessel coronary artery disease (CAD) but without MI demonstrate ischemia-induced regional areas of denervation as well.\(^7\)\(^,\)\(^8\) As most studies have focused on denervation in infarcted or ischemic areas, sympathetic innervation of non-infarcted remote myocardium has not been studied extensively. Nonetheless, the integrity of the innervation system in remote myocardium may provide important prognostic information and is related to cardiac remodeling.\(^9\)\(^-\)\(^11\) In addition, these observations are not precluded to patients with ICMP but can similarly be observed in patients with dilated cardiomyopathy (DCMP).\(^12\)\(^,\)\(^13\) It has been suggested that heterogeneity of sympathetic innervation may serve as an arrhythmic substrate preceding sudden cardiac death.\(^14\)\(^,\)\(^15\) It is therefore of interest to gain more insight into factors influencing sympathetic nerve integrity in remote non-infarcted myocardium. Perfusion abnormalities, even in the absence of obstructive epicardial CAD, may play a role as a causal agent of sympathetic dysfunction. In fact, impaired perfusion has also been linked to an unfavorable prognosis in both ICMP and DCMP.\(^16\)\(^-\)\(^18\) The aim of the present study was therefore to explore the interrelations between myocardial perfusion, contractile function, and sympathetic innervation in non-infarcted remote myocardium in patients with ICMP and DCMP.

METHODS

Study population

Patients with ICMP (n=52) and DCMP (n=18) with left ventricular ejection fraction (LVEF) ≤35% were prospectively included. Patients with a cardiac rhythm other than sinus rhythm were excluded. None of the patients displayed ischemia or viability amendable for (further) revascularization at time of inclusion in the current study. All patients underwent \([^{15}\text{O}]\text{H}_2\text{O}\) and \([^{11}\text{C}]\text{HED PET imaging to assess resting MBF, hyperemic MBF, and cardiac sympathetic innervation. Furthermore, late gadolinium-enhanced cardiovascular magnetic resonance imaging (LGE-CMR) was performed. The study was approved by the Ethics Committee of the VU University Medical Center, in accordance with the Declaration of Helsinki and written informed consent was obtained from all patients. \)}}
CMR image acquisition

CMR studies were performed as described previously. In short, a clinical 1.5-T MRI scanner was used with a dedicated phased-array body coil. After survey scans, cine imaging was performed using a retrospectively ECG-gated, steady-state free precession sequence during breath holds in mild expiration. Standard four-, three- and two-chamber orientations were obtained and subsequently, a stack of 10-12 consecutive short-axis slices was acquired, fully covering the left ventricle. Approximately 10-15 min after administration of 0.2 mmol.kg\(^{-1}\) gadolinium, LGE images were acquired in similar orientations as the cine images, using a two-dimensional segmented inversion-recovery prepared gradient echo sequence. In case of difficulties with breath holding during LGE imaging, a single-shot sequence was used instead of a segmented sequence.

CMR image analysis

Images were analysed using dedicated software packages MASS (Mass v.5.1 2010-EXP beta, Medis, Leiden, the Netherlands) and QMass (QMass v.7.5, Medis). Volumes, mass, and function were calculated on the end-diastolic and end-systolic phases of the cine images. In addition, regional wall-thickening was calculated as the percentage increase in wall-thickness during the systole. Infarct size was calculated on LGE images by using the full-width-at-half-maximum method. All segmental analyses were quantified according to the 17-segment American Heart Association model, excluding the apex. Using segmental LGE results, myocardial remote segments without scar were selected. Regional LV function in non-infarcted myocardium was calculated as the mean LV wall-thickening in myocardial remote segments.

PET protocol and image acquisition

All PET studies were performed using a PET/CT device (Philips Gemini TF 64, Philips Healthcare, Best, The Netherlands). Patients were instructed to refrain from intake of products containing caffeine or xanthine 24 h prior to the scan. All patients received a radial artery catheter for arterial blood sampling during the \(^{11}C\)HED PET scan. The scanning protocol consisted of two dynamic \(^{15}O\)H\(_2\)O scans, followed by a \(^{11}C\)HED scan. A 5 mL (0.8 mL.s\(^{-1}\)) bolus injection of \(^{15}O\)H\(_2\)O (370MBq) followed by a 35 mL saline flush (2 mL.s\(^{-1}\)) was administered simultaneously with the start of a 6-min emission scan. To correct for attenuation, a respiration-averaged low-dose CT scan was performed immediately after the dynamic scan (55 mAs; rotation time, 1.5 s; pitch, 0.825; collimation, 64x0.625; acquiring 20 cm in 11 s) during normal breathing. After a 10-min interval to allow for decay of radioactivity, an identical PET sequence was performed under infusion of intravenous adenosine (140 µg.kg\(^{-1}\).min\(^{-1}\)). Adenosine infusion was started 2 minutes prior to the start of the dynamic stress scan and was terminated after the low-dose CT. Dynamic images were reconstructed into 22 frames (1x10, 8x5, 4x10, 2x15, 3x20,
2x30, and 2x60 s) using the 3-dimensional row-action maximum likelihood algorithm (3D RAMLA), applying all appropriate corrections. After an interval of ~15 minutes, a 5 mL (0.8 mL·s\(^{-1}\)) bolus \([^{11}\text{C}]\)HED (370 MBq) followed by a 35 mL saline flush (2 mL·s\(^{-1}\)) was injected simultaneously with the start of a 60-minutes emission scan. During this scan, 7 mL arterial samples were collected manually at 2.5, 5, 10, 20, 30, 40, and 60 min to determine plasma and whole blood activity concentrations, and radiolabelled plasma \([^{11}\text{C}]\)HED metabolites. The dynamic scan was immediately followed by a respiration-averaged low-dose CT scan. Images were reconstructed into 36 frames (1x10, 8x5, 4x10, 3x20, 5x30, 6x60, 4x150, 4x300, and 2x600 s) using the 3D RAMLA with application of all appropriate corrections.

**PET image analysis**

PET image analysis was performed using in-house developed software. For both \([^{15}\text{O}]\)H\(_2\)O and \([^{11}\text{C}]\)HED, image-derived input functions were derived by placing 1 cm diameter regions of interest (ROIs) over the ascending aorta in at least 5 transaxial planes showing the first pass of the injected bolus. These ROIs were combined in one volumes of interest (VOI) for the ascending aorta. Furthermore, a right ventricular VOI was obtained by drawing a second set of ROIs in at least three transaxial planes over the right ventricle. Subsequently, both VOIs were transferred to the full dynamic images to obtain arterial whole blood and right ventricular time-activity curves (TACs). For \([^{11}\text{C}]\)HED image analysis, parent fractions and ratios of plasma/whole blood concentrations derived from the manual blood samples were fitted to a sigmoid function. Subsequently, the arterial whole blood TAC was multiplied by the fitted plasma/whole blood ratio and parent fraction curves. For MBF analysis, parametric images of MBF, anatomic tissue fraction, perfusable tissue fraction (PTF), and arterial and venous blood volume fractions were generated as previously described.\(^{22, 23}\) Subsequently, segmental VOIs were defined manually according to the 17-segment model of the American Heart Association\(^{21}\) on short-axis images. For MBF analysis, short-axis PTF images were used, whereas for \([^{11}\text{C}]\)HED analysis segmental VOIs were drawn on short-axis images in the final frame of the dynamic scan. Finally, for MBF analysis, segmental VOI templates were projected onto the entire dynamic emission scans to extract segmental TACs that were fitted to a single tissue compartment model.

MBF was expressed in mL·min\(^{-1}\)·g\(^{-1}\) of perfusable tissue. Coronary flow reserve (CFR) was defined as the ratio of hyperemic and resting MBF. Myocardial \([^{11}\text{C}]\)HED uptake was expressed using the retention index (RI) which was calculated as the uptake at the last frame (50-60 min) divided by the integral of the arterial plasma-corrected TAC. Mean resting MBF, hyperemic MBF, CFR, and \([^{11}\text{C}]\)HED RI were assessed in myocardial segments without scar, selected during LGE-CMR image analysis (excluding the apex). In case no enhancement was present in patients with DCMP, global myocardium was considered the remote area.
**Statistical analysis**

Continuous data are presented as means with standard deviations (SD), whereas categorical data are expressed as frequencies with percentages. Medians with interquartile range (IQR) were presented for non-parametric data. Histograms were used to evaluate whether continuous data were normally distributed. Patient groups were compared using unpaired Student’s t-tests for continuous data and χ² tests for categorical data. Levene’s test was used to verify whether the equal variances assumption of the unpaired Student’s t-test was appropriate. In addition, a non-parametric Mann-Whitney U test was used when appropriate. For comparisons of paired continuous data, paired samples t-test was applied. The association between two continuous variables was analyzed using the Pearson’s correlation coefficient. To assess the association of all demographic and imaging variables with [¹¹C]HED RI in remote myocardium, univariate linear regression analyses were performed. Furthermore, a multivariable linear regression analysis was performed using a backward selection procedure including all variables that were associated with [¹¹C]HED RI in remote myocardium in univariate analysis (with p-value <0.1). LVEDV and LVESV were analyzed in separate models due to the interdependency (Model 1 and Model 2, respectively). Furthermore, as preserved innervation may result in preserved regional wall-thickening, a third multivariable analysis was performed excluding remote wall-thickening and LVESV (Model 3). All tests were performed two-sided and were considered statistically significant if P-value <0.05. All statistical analyses were performed using SPSS software package (SPSS 20.0, IBM Corporation, Chicago, IL, USA).

**RESULTS**

**Study population**

A total of 70 patients were included in this study. Clinical baseline characteristics are presented in table 1. Fifty-two (74%) patients suffered from ICMP whereas 18 (26%) patients were classified as DCMP. Patients with ICMP were more likely to be male (89 vs. 61%, p=0.03) and more frequently use statins (90 vs. 39%, P<0.001). Figure 1 shows examples of PET and CMR imaging in two ICMP patients.
<table>
<thead>
<tr>
<th>Characteristics (N (%), mean ± SD, or median (IQR))</th>
<th>Total (n=70)</th>
<th>Ischemic CMP (n=52)</th>
<th>Dilated CMP (n=18)</th>
<th>P-value (between CMP groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66 ± 9</td>
<td>66 ± 9</td>
<td>66 ± 11</td>
<td>0.91</td>
</tr>
<tr>
<td>Male gender</td>
<td>57 (81%)</td>
<td>46 (89%)</td>
<td>11 (61%)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Medication:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE/ARB</td>
<td>64 (91%)</td>
<td>46 (89%)</td>
<td>18 (100%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>62 (89%)</td>
<td>47 (90%)</td>
<td>15 (83%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Ca channel blocker</td>
<td>7 (10%)</td>
<td>4 (8%)</td>
<td>3 (17%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Diuretics</td>
<td>47 (67%)</td>
<td>32 (62%)</td>
<td>15 (83%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Statin</td>
<td>54 (77%)</td>
<td>47 (90%)</td>
<td>7 (39%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>3 (4%)</td>
<td>2 (4%)</td>
<td>1 (6%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12 (17%)</td>
<td>10 (19%)</td>
<td>2 (11%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25 (36%)</td>
<td>20 (39%)</td>
<td>5 (28%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>16 (23%)</td>
<td>13 (25%)</td>
<td>3 (17%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>51 (73%)</td>
<td>51 (98%)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PCI/CABG</td>
<td>47 (67%)</td>
<td>45 (87%)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Heart failure:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA I</td>
<td>20 (29%)</td>
<td>16 (31%)</td>
<td>4 (22%)</td>
<td>0.31</td>
</tr>
<tr>
<td>NYHA II</td>
<td>42 (60%)</td>
<td>32 (62%)</td>
<td>10 (56%)</td>
<td>0.31</td>
</tr>
<tr>
<td>NYHA III</td>
<td>8 (11%)</td>
<td>4 (8%)</td>
<td>4 (22%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>85 (75-104)</td>
<td>89 (76-111)</td>
<td>82 (74-86)</td>
<td>0.19*</td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme; ARB, angiotensin-II-receptor blockers; Ca, calcium; PCI, percutaneous coronary intervention; CABG, coronary arterial bypass graft surgery; CMP, cardiomyopathy; NYHA, New York Heart Association. *tested with Mann-Whitney U test.
Figure 1. Examples of PET and CMR imaging in two patients (A-D and E-H) with ischemic cardiomyopathy. Both patients demonstrated a large myocardial infarction at LGE-CMR (D and H) with corresponding PET perfusion and sympathetic innervation defects (A-C and E-G). Quantitative PET results of Patient 2 (E-H) revealed a preserved hyperemic MBF (E) and sympathetic innervation (F) in the non-infarcted remote myocardium as compared with hyperemic MBF and sympathetic innervation of Patient 1 (A and B, respectively). Note the difference in MBF and $[^{11}C]$HED RI scaling.
Table 2 summarizes hemodynamic parameters of all patients during PET at baseline and hyperemic conditions. Overall, during adenosine-induced hyperemia, heart rate and rate-pressure product increased significantly compared with baseline, whereas no significant change in both diastolic and systolic blood pressures was observed. These results did not differ significantly between patients with ICMP and DCMP, although a trend was observed towards a higher heart rate during hyperemia in DCMP patients (P=0.09).

Table 2. Hemodynamics at baseline and hyperemia during PET

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (n=70)</th>
<th>Ischemic CMP (n=52)</th>
<th>Dilated CMP (n=18)</th>
<th>P-value (between CMP groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>67 ± 12</td>
<td>66 ± 12</td>
<td>70 ± 12</td>
<td>0.22</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>80 ± 13</td>
<td>78 ± 12</td>
<td>84 ± 15</td>
<td>0.09</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>115 ± 15</td>
<td>115 ± 14</td>
<td>117 ± 18</td>
<td>0.66</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>115 ± 18</td>
<td>113 ± 16</td>
<td>118 ± 24</td>
<td>0.41</td>
</tr>
<tr>
<td>P-value</td>
<td>0.67</td>
<td>0.45</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>65 ± 8</td>
<td>65 ± 8</td>
<td>65 ± 7</td>
<td>0.90</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>66 ± 10</td>
<td>66 ± 10</td>
<td>65 ± 10</td>
<td>0.51</td>
</tr>
<tr>
<td>P-value</td>
<td>0.30</td>
<td>0.21</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>82 ± 9</td>
<td>82 ± 9</td>
<td>82 ± 9</td>
<td>0.86</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>82 ± 12</td>
<td>82 ± 11</td>
<td>82 ± 14</td>
<td>0.96</td>
</tr>
<tr>
<td>P-value</td>
<td>0.63</td>
<td>0.63</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Rate-pressure product (mmHg⋅min⁻¹):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7729 ± 1665</td>
<td>7547 ± 1487</td>
<td>8192 ± 2026</td>
<td>0.17</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>9149 ± 2221</td>
<td>8819 ± 1726</td>
<td>9991 ± 3058</td>
<td>0.14</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

CMP, cardiomyopathy
PET imaging results in remote myocardium are summarized in table 3. Mean resting MBF was $0.87 \pm 0.23 \text{ mL}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ and increased to $1.85 \pm 0.53 \text{ mL}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ during hyperemic conditions ($P<0.001$), resulting in a CFR of $2.21 \pm 0.64$. Patients with DCMP demonstrated a significantly higher hyperemic MBF compared with ICMP patients ($P=0.049$). However, resting MBF and CFR did not differ between both groups (figure 2). Mean $[^{11}\text{C}]$HED RI in remote myocardium was $3.53 \pm 0.91$ and did not differ between patients with ICMP and DCMP as demonstrated in table 3 and figure 3 ($P=0.83$).

![Figure 2](image1.png)

**Figure 2.** Scatter plot comparison demonstrating resting MBF, hyperemic MBF, and CFR in remote myocardium of patients with ischemic- and dilated cardiomyopathy.

![Figure 3](image2.png)

**Figure 3.** Scatter plot comparison demonstrating $[^{11}\text{C}]$HED retention index in remote myocardium of patients with ischemic- and dilated cardiomyopathy.
### Table 3. PET imaging in remote myocardium

<table>
<thead>
<tr>
<th>Characteristics (mean ± SD)</th>
<th>Total (n=70)</th>
<th>Ischemic CMP (n=52)</th>
<th>Dilated CMP (n=18)</th>
<th>P-value (between CMP groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting MBF (mL·min⁻¹·g⁻¹)</td>
<td>0.87 ± 0.23</td>
<td>0.87 ± 0.24</td>
<td>0.89 ± 0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>Hyperemic MBF (mL·min⁻¹·g⁻¹)</td>
<td>1.85 ± 0.53</td>
<td>1.78 ± 0.51</td>
<td>2.06 ± 0.53</td>
<td>0.049</td>
</tr>
<tr>
<td>CFR</td>
<td>2.21 ± 0.64</td>
<td>2.14 ± 0.65</td>
<td>2.38 ± 0.61</td>
<td>0.17</td>
</tr>
<tr>
<td>[¹¹C]HED RI</td>
<td>3.53 ± 0.91</td>
<td>3.52 ± 0.87</td>
<td>3.57 ± 1.04</td>
<td>0.83</td>
</tr>
</tbody>
</table>

MBF, myocardial blood flow; CFR, coronary flow reserve; CMP, cardiomyopathy; HED, hydroxyephedrine; IQR, interquartile range; PET, positron emission tomography; RI, retention index; SD, standard deviation.

### Table 4. CMR imaging variables

<table>
<thead>
<tr>
<th>Characteristics (mean ± SD or median (IQR))</th>
<th>Total (n=70)</th>
<th>Ischemic CMP (n=52)</th>
<th>Dilated CMP (n=18)</th>
<th>P-value (between CMP groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV (mL)</td>
<td>288.0 ± 73.0</td>
<td>278.7 ± 60.6</td>
<td>314.9 ± 97.9</td>
<td>0.07</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>209.7 ± 68.1</td>
<td>199.8 ± 53.4</td>
<td>238.2 ± 95.4</td>
<td>0.12</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>28.1 ± 6.9</td>
<td>28.6 ± 6.3</td>
<td>26.1 ± 8.5</td>
<td>0.15</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>140.4 ± 40.0</td>
<td>138.8 ± 38.9</td>
<td>145.0 ± 43.8</td>
<td>0.57</td>
</tr>
<tr>
<td>Scar size (g)</td>
<td>14.6 (5.3-22.7)</td>
<td>16.8 (12.0-25.4)</td>
<td>0.0 (0.0-5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Scar size (%)</td>
<td>12.1 (3.1-17.7)</td>
<td>14.7 (7.9-19.6)</td>
<td>0.0 (0.0-3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remote segments (n)</td>
<td>5.5 (4.0-9.3)</td>
<td>5.0 (4.0-7.0)</td>
<td>17.0 (5.8-17.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remote LVED wall-thickness (mm)</td>
<td>7.1 ± 1.2</td>
<td>7.3 ± 1.2</td>
<td>6.5 ± 1.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Remote LVES wall-thickness (mm)</td>
<td>9.6 ± 1.9</td>
<td>10.1 ± 1.7</td>
<td>8.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remote LV wall-thickening (%)</td>
<td>36.2 ± 17.5</td>
<td>40.0 ± 17.8</td>
<td>25.0 ± 10.8</td>
<td>&lt;0.001</td>
</tr>
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</table>

CMP, cardiomyopathy; CMR, cardiovascular magnetic resonance; IQR, interquartile range; LV, left ventricle; LVED, left ventricular end-diastolic; LVES, left ventricular end-systolic; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; SD, standard deviation.
CMR

Table 4 presents LGE-CMR imaging results. Regional LV wall-thickening in non-infarcted area was higher in ICMP patients compared with DCMP patients (P<0.001), although LVEF did not differ. In addition, patients with DCMP showed lower wall-thickness of remote myocardium in both end-diastolic and end-systolic phase compared with patients with ICMP (P=0.02 and P<0.001, respectively). All patients with ICMP displayed enhancement at LGE-CMR with a median scar size of 14.7% (IQR 7.9-19.6%), whereas 8 of 18 (44%) patients with DCMP showed enhancement (median 0%, IQR 0-3.6%) (P<0.001). Consequently, the number of non-infarcted remote segments was lower in patients with ICMP as compared with DCMP (5.0, IQR 4.0–7.0 vs. 17.0, IQR 5.8–17.0, respectively, P<0.001).

Interrelations of PET and CMR variables

Scatter plots demonstrating the association between MBF and \(^{11}\)C-HED RI in remote myocardium are presented in figure 4. A correlation was observed between resting MBF and \(^{11}\)C-HED RI in non-infarcted myocardium (r=0.41, P<0.001). This correlation was primarily driven by the relation in DCMP patients specifically (r=0.68, P=0.002), whereas in ICMP patients this correlation was poor (r=0.30, p<0.001). Correcting resting MBF for rate-pressure product resulted in comparable correlations with innervation in remote myocardium (r=0.44, P<0.001). Furthermore, hyperemic MBF was significantly correlated with \(^{11}\)C-HED RI in both ICMP and DCMP patients (r=0.47, P<0.001 and r=0.77, P<0.001, respectively) resulting in an overall correlation coefficient of r=0.55 (P<0.001). CFR, however, did not correlate with \(^{11}\)C-HED RI in patients with ICMP or DCMP. Comparable correlation coefficients were obtained when correcting the CFR for rate-pressure product. Figure 5 demonstrates scatter plots of the correlation of remote \(^{11}\)C-HED RI with global left ventricular volumes, function, and mass. Both LVEDV and LVESV were negatively correlated with \(^{11}\)C-HED RI (both r=-0.40, P=0.001). Comparable significant correlations were obtained in ICMP and DCMP patients. In addition, a poor correlation was observed between LV mass and \(^{11}\)C-HED RI in patients with ICMP but not in patients with DCMP. With regard to LVEF, however, no correlation was found in ICMP and DCMP patients specifically although for the total study population a positive correlation was observed (r=0.27, P=0.02). Conversely, regional LV function in remote myocardium as assessed with wall-thickening was significantly related to remote innervation in patients with ICMP (r=0.54, P<0.001), but no significance was reached in DCMP patients (r=0.40, P=0.10) (figure 6).
Figure 4. Scatter plots demonstrating the association of remote $[^{11}C]$HED retention index with resting MBF (A), hyperemic MBF (B), CFR (C) assessed in remote myocardium in patients with ischemic- and dilated cardiomyopathy.
CHAPTER 6 Denervation is associated with microvascular dysfunction

Figure 5. Scatter plots demonstrating the association of remote $[^{11}\text{C}]$HED retention index with LVEDV (A), LVESV (B), LVEF (C), and LV mass (D) in patients with ischemic- and dilated cardiomyopathy.

Figure 6. Scatter plot demonstrating the association of remote $[^{11}\text{C}]$HED retention index with regional LV wall-thickening in non-infarcted myocardium in patients with ischemic- and dilated cardiomyopathy.
Table 5. Uni- and multivariable analysis for predicting remote sympathetic innervation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>B (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>-0.68 (-1.22 - -0.15)</td>
<td>0.01</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>0.58 (-0.09 - 1.25)</td>
<td>0.09</td>
</tr>
<tr>
<td>Diuretics</td>
<td>-0.63 (-1.07 - -0.20)</td>
<td>0.01</td>
</tr>
<tr>
<td>LVEDV (per 10 mL)</td>
<td>-0.05 (-0.08 - -0.02)</td>
<td>0.001</td>
</tr>
<tr>
<td>LVESV (per 10 mL)</td>
<td>-0.05 (-0.08 - -0.02)</td>
<td>0.001</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.04 (0.01 - 0.07)</td>
<td>0.02</td>
</tr>
<tr>
<td>LV mass (per 10 g)</td>
<td>-0.07 (-0.12 - -0.02)</td>
<td>0.01</td>
</tr>
<tr>
<td>Remote LV wall-thickening (%)</td>
<td>0.02 (0.01 - 0.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remote resting MBF (mL·min⁻¹·g⁻¹)</td>
<td>1.59 (0.72 - 2.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remote hyperemic MBF (mL·min⁻¹·g⁻¹)</td>
<td>0.94 (0.59 - 1.29)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Predictors for sympathetic innervation

Table 5 summarizes the univariate- and multivariable linear regression analysis of all clinical and imaging variables associated with $[^{11}]$C]HED RI in remote myocardium. Univariate analysis demonstrated that male gender, use of diuretics, LVEDV, LVESV, LVEF, and LV mass were significant predictors for $[^{11}]$C]HED RI. Resting MBF, hyperemic MBF, and LV wall thickening assessed in remote myocardium, however, yielded the strongest predictive value (B=1.59, P<0.001, B=0.94, P<0.001, and B=0.02, P<0.001 respectively). Multivariable analysis revealed that LVEDV (B=-0.03, P=0.02), remote LV wall-thickening (B=0.01, P=0.03) and hyperemic MBF (B=0.79, P<0.001) were the only independent predictors for $[^{11}]$C]HED RI (Model 1, R²=0.45). In addition, multivariable analysis Model 2 (R²=0.46) demonstrated that remote LV wall-thickening (B=0.01, P=0.04), and hyperemic MBF (B=0.76, P<0.001) were independently associated with $[^{11}]$C]HED RI in remote myocardium. When excluding remote wall-thickening from the multivariable analysis (Model 3), hyperemic MBF and LVEDV remained independently associated with remote $[^{11}]$C]HED RI (B=0.87, P<0.001 and B=-0.04, P=0.001, respectively).
DISCUSSION

The current study was conducted to investigate the interrelations between myocardial perfusion, sympathetic innervation, and contractile function in non-infarcted myocardium in patients with ICMP and DCMP. Sympathetic myocardial function was found to be strongly related to hyperemic perfusion and contractile function. This relation was independent of CMP etiology, suggesting a common pathogenic pathway.

Sympathetic innervation

The observed $[^{11}\text{C}]$HED retention in remote non-infarcted myocardium in this study was comparable with previous studies using the RI in patients with ICMP and DCMP.\textsuperscript{12, 13, 24} Although the present study did not include healthy controls, the $[^{11}\text{C}]$HED retention was markedly reduced when compared with previous published data on $[^{11}\text{C}]$HED retention in a control group.\textsuperscript{13} In addition, remote $[^{11}\text{C}]$HED RI was equally impaired in both ICMP and DCMP groups. Interestingly, Vesalainen et al.\textsuperscript{25} found that patients with ICMP
displayed significantly lower \[^{11}\text{C}]\text{HED} \text{ retention compared with DCMP patients, even}

in the non-infarcted area specifically. However, non-infarcted remote myocardium was

selected based on the absence of wall motion abnormalities using echocardiography

whereas the current study used LGE-CMR allowing a more accurate definition of non-

infarcted myocardium. Although all patients suffered from CMP, a considerable variation

was observed in \[^{11}\text{C}]\text{HED} \text{ retention in non-infarcted areas. It is unclear, however, what}

factors may affect sympathetic nerve integrity in remote myocardium. Accordingly, the

current study evaluated the interrelations with myocardial perfusion and function in

remote myocardium in ICMP and DCMP.

**Myocardial perfusion and innervation**

The current study population demonstrated an impaired hyperemic perfusion and

CFR in remote myocardium when compared with previously published \[^{15}\text{O}]\text{H}_2\text{O-PET}

perfusion data in patients with preserved LVEF and no obstructive CAD. Patients with

ICMP showed a lower hyperemic MBF compared with DCMP patients, possibly reflect-

ing residual diffuse CAD not amenable to revascularization. Second, advanced stages

of atherosclerosis may result in more severe microvascular dysfunction. A significant

correlation of hyperemic MBF and \[^{11}\text{C}]\text{HED} \text{ retention was observed in non-infarcted}

myocardium. Fricke et al. found comparable correlations between impaired stress

perfusion and \[^{11}\text{C}]\text{HED} \text{ retention in non-infarcted segments of patients with advanced}

CAD. In the current study, none of the patients were considered candidates for (further)

revascularization. Epicardial CAD leading to ischemic damage of sympathetic nerves,

therefore, is less likely in remote non-infarcted myocardium. Furthermore, the relation

between hyperemic MBF and \[^{11}\text{C}]\text{HED} \text{ retention seemed more present in patients}

with DCMP. These results suggest that microvascular dysfunction might play a role in

the sympathetic nerve integrity. The observed discrepancy in strength of correlation

between hyperemic MBF and \[^{11}\text{C}]\text{HED} \text{ RI in ICMP versus DCMP may be the result}

from a more heterogeneous pattern of innervation in remote myocardium at the infarct

borderzone which might contain small areas of denervation in ICMP patients. However, as

segments were carefully selected that did not show any scar during LGE-CMR, the influ-

ence of heterogeneity at the infarct border on the total remote innervation is limited.

No correlation of CFR and \[^{11}\text{C}]\text{HED} \text{ retention was demonstrated which is most likely the}

result of the poor but significant correlation between resting MBF and \[^{11}\text{C}]\text{HED} \text{ retention}

in remote myocardium. This might be explained by severe microvascular dysfunction

which may even be apparent in the assessment of resting MBF. In addition, the influ-

ence of flow-dependent tracer delivery of \[^{11}\text{C}]\text{HED could be involved in this relation.}

**LV function and innervation**

The population in the present study was characterized by an impaired LVEF in both ICMP

and DCMP groups. Regional LV function in remote myocardium was more preserved
in ICMP patients, reflecting the more globally impaired contractility in DCMP whereas
the function impairment in ICMP is predominantly present in the infarct area. \([^{11}C]\)HED retention in remote myocardium was correlated with global LV volumes, consistent with
the findings from Aoki et al.\(^{27}\). In addition, a poor correlation between global LVEF and
\([^{11}C]\)HED retention was found in the total study population as consistently demonstrated
in prior studies.\(^{12, 13, 27, 28}\) However, most studies did not focus on sympathetic innervation
in remote myocardium specifically. Regional wall-thickening in non-infarcted myocardium
was found to correlate with \([^{11}C]\)HED retention predominantly in ICMP patients, whereas
no significance was reached in DCMP patients. As previously mentioned, the contractility
of remote myocardium in ICMP is relatively preserved compared with DCMP which might
explain the observed discrepancy. Consistently, Bengel et al.\(^{12}\) found only a poor correla-
tion between regional LV function and \([^{11}C]\)HED RI in patients with DCMP. In patients
with ICMP, however, Aoki et al.\(^{27}\) demonstrated a stronger relation between regional LV
function and \([^{11}C]\)HED RI non-infarcted myocardium. Furthermore, sympathetic inner-
vation is importantly related with LV remodeling after myocardial infarction.\(^{9, 10}\)

**Predictors of sympathetic nerve integrity**

The present study showed relations of sympathetic innervation in remote myocardium
with LV volumes, wall-thickening, resting perfusion, and hyperemic perfusion. However,
hyperemic perfusion, regional wall-thickening, and LVEDV were the only independent
predictors for \([^{11}C]\)HED retention in remote myocardium. In particular, hyperemic MBF
demonstrated the strongest relation with \([^{11}C]\)HED retention, suggesting that micro-
vascular dysfunction plays an important role in sympathetic innervation in remote
myocardium. Previous studies have demonstrated that impaired hyperemic perfusion
independently predict mortality in patients DCMP and ICMP.\(^{16-18}\) Furthermore, a relation
was found between ventricular arrhythmia inducibility and impaired hyperemic MBF,
even when assessing in remote non-infarcted areas.\(^{19}\) Impaired sympathetic innervation
related with hyperemic MBF might contribute to the relation with electrical instability
resulting in a higher risk of (sudden) cardiac death.

**Limitations**

There are several limitations in this study. First, no cause-effect relation between
hyperemic MBF and innervation was established in this study. Consequently, whether
impaired hyperemic MBF is the primary cause of impaired sympathetic innervation
in remote myocardium remains unclear. Left ventricular remodeling may affect both
hyperemic MBF and innervation. However, after correction for markers of LV remodeling
in a multivariable analysis, hyperemic MBF remained strongly associated with remote
innervation. Secondly, comparisons of smaller regions of remote myocardium in each
patient might provide additional important correlative information. However, as all
imaging data were not exactly aligned, the current study explored the relation of mean
innervation and perfusion in the total non-infarcted tissue to minimize the influence of misalignment between CMR, $[^{11}C]$HED PET, and $[^{15}O]H_2O$ PET. Thirdly, Harms et al.\textsuperscript{29} demonstrated that calculation of the volume of distribution of $[^{11}C]$HED using a single tissue compartment model allows absolute quantification of $[^{11}C]$HED activity which may be a more sophisticated way to describe the measured tracer activity compared with the semi-quantitative RI from a technical point of view. However, as this model has not been clinically validated yet, it remains unclear whether the single tissue compartment model leads to a better assessment of actual presynaptic sympathetic nerve activity. The clinical importance of this model, therefore, remains unclear as most clinical studies have used the RI to assess sympathetic innervation.\textsuperscript{2-4, 12, 13, 25, 27, 28, 30} In addition, due to the lack of a control group, normal versus abnormal remote innervation could not be distinguished. Nonetheless, usage of the RI allowed comparisons with previous published data that used the RI in CMP patients as well as in control groups. Finally, the partial volume effect is a limitation of PET imaging, especially for $[^{11}C]$HED quantification and may be involved in the current study as patients have enlarged ventricles which are thin walled. However, the extent of the partial volume effects on the assessed uptake of $[^{11}C]$HED in remote myocardium is unclear.

**Conclusion**

Hyperemic MBF is independently associated with cardiac sympathetic innervation in non-infarcted remote myocardium in patients with both ICMP and DCMP. This suggests that microvascular dysfunction might be an important factor related to sympathetic nerve integrity in non-infarcted myocardium. Whether impaired hyperemic MBF is the primary cause of this relation remains unclear.
REFERENCES


