Heart Failure With Anemia

Novel Findings on the Roles of Renal Disease, Interleukins, and Specific Left Ventricular Remodeling Processes

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Background—Anemia is a highly prevalent and strong independent prognostic marker in heart failure (HF), yet this association is not completely understood. Whether anemia is simply a marker of disease severity and concomitant chronic kidney disease or represents the activation of other detrimental pathways remains uncertain. We sought to determine which pathophysiological pathways are exacerbated in patients with HF, reduced ejection fraction (HFrEF) and anemia in comparison with those without anemia.

Methods and Results—In a prospective study involving 151 patients, selected biomarkers were analyzed, each representing proposed contributive mechanisms in the pathophysiology of anemia in HF. We compared clinical, echocardiographic, and circulating biomarkers profiles among patients with HFrEF and anemia (group 1), HFrEF without anemia (group 2), and chronic kidney disease with preserved EF, without established HF (chronic kidney disease control group 3). We demonstrate here that many processes other than those related to chronic kidney disease are involved in the anemia–HF relationship. These are linked to the pathophysiological mechanisms pertaining to left ventricular systolic dysfunction and remodeling, systemic inflammation and volume overload. We found that levels of interleukin-6 and interleukin-10, specific markers of cardiac remodeling (procollagen type III N-terminal peptide, matrix metalloproteinase-2, tissue inhibitor of matrix metalloproteinase 1, left atrial volume), myocardial stretch (NT-proBNP [N-terminal probrain natriuretic peptide]), and myocyte death (troponin T) are related to anemia in HFrEF.

Conclusions—Anemia is strongly associated not only with markers of more advanced and active heart disease but also with the level of renal dysfunction in HFrEF. Increased myocardial remodeling, inflammation, and volume overload are the hallmarks of patients with anemia and HF.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00834691.

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Key Words: anemia ■ biomarkers ■ heart failure ■ inflammation ■ renal insufficiency, chronic ■ ventricular remodeling

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*A list of all ANCHOR study participants is given in the Appendix.

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diabetes mellitus, and elevated systolic blood pressure were independent predictors of lower hemoglobin.4

**Study Objectives**

By comparing the clinical, echocardiographic, and biomarker profiles of patients with HF with and without anemia with those of patients with CKD without systolic dysfunction, we aimed to improve our understanding of the mechanisms involved in the relationship between anemia and HF severity. More specifically, we wished to clarify the importance of CKD, left ventricular (LV) remodeling, and inflammation further in patients with anemia and HFrEF in comparison with those without anemia.

**Methods**

**Study Design**

We conducted the ANemia in CHronic heart failure: Etiology, comparisons with renal disease, and relationships with biomarkers and LV Remodeling (ANCHOR) study in 7 Canadian centers from 2008 to 2010; where the study had been approved by institutional review committees. The subjects gave informed consent. Measurements of stored biomarkers samples were subsequently completed, and statistical analyses were finalized in September 2013. As shown in Figure 1, using a cross-sectional study design, we compared 3 groups of patients. One had HFrEF and anemia, defined as hemoglobin <120g/L in women and <130g/L in men (World Health Organization 2011), a second had HFrEF without anemia, and the third had CKD (estimating glomerular filtration rate [eGFR], <60 mL/min per 1.73 m²) with preserved EF (≥50%) and were not identified based on HF symptoms.

**Patient Population: Inclusion and Exclusion Criteria**

Patients in groups 1 and 2 had an HF diagnosis ≥6 months before enrollment, were in New York Heart Association functional class II to IV, and received recommended HF therapy at stable doses for ≥1 month at the time of enrollment. Other inclusion criteria were age ≥18 years, LVEF ≥50% within 6 months.

In group 3, patients recruited from outpatient clinics or echocardiography laboratories had CKD with or without anemia, and a LVEF ≥50% documented within 6 months.

In all groups, patients were excluded if they had a recent acute renal failure episode (<1 month); red blood cells, other blood component transfusions, or erythropoietin therapy within 3 months; iron, B12, or folic acid supplements used to treat anemia (<3 months); inflammation syndrome or decompensated HF episode (<1 month); complex congenital heart disease; known malignant hematologic or other active neoplasia; immunosuppressive therapy, chemotherapy, or radiotherapy within 3 months; recent acute uncompensated failure of a chronic inflammatory disease within 3 months; recent viral or bacterial infection (<2 weeks); and active or recent viral hepatitis (<3 months).

Pregnant women and patients unable to provide informed consent were also excluded.

**Data Collection**

Two visits were performed within 2 weeks. During the first one, we collected demographics, medical history, current medications, New York Heart Association class, Canadian Cardiovascular Society angina class, cardiovascular physical examination, vital signs, weight, height, waist measurement, oxygen saturation, eGFR, electrolytes, urea, creatinine, complete blood count, and an ECG. During the second visit, the patient’s clinical stability was reviewed and repeat vital signs, weight, oxygen saturation, biomarkers for local and central laboratory measurements, and echocardiography were obtained. Figure 2 summarizes the rationale for the choice of selected biomarkers through the pathophysiology of anemia in HF. The samples were stored at the Montreal Heart Institute at −80°C until central laboratory analyses were performed.

**Renal Function**

Renal function was evaluated by measuring serum creatinine in our central laboratory and by eGFR, using the CKD-Epidemiology Collaboration Group equation.10 Cystatin C (Siemens Healthcare Diagnostics) and neutrophil gelatinase–associated lipocalin (R&D Systems) were measured as potential novel indicators of renal function.

**Echocardiographic Data**

Echocardiography was performed within 96 hours of blood sample collection, using a phased-array imaging system equipped with a transducer with second harmonics capability. Images were obtained in the parasternal long- and short-axis and apical views. All images were stored on CD-ROM and analyzed off-line (ProSolv). All participating sites received a detailed imaging manual and were certified by the core laboratory at the Montreal Heart Institute. Three sonographers blinded to study group (supervised by 2 cardiologists) performed the measurements according to the American Society of Echocardiography standards. Each parameter was analyzed on 5 consecutive cycles (5 if atrial fibrillation). Inter-reader and intrareader variability was excellent, as previously reported.11

**Serum Biomarkers**

The prognostic serum markers troponin T and NT-proBNP were measured by electrochemiluminescent immunoassays (Roche Diagnostics). Analyses of aldosterone, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-10, erythropoietin, transforming growth factor-β1, and hepcidin were performed using ELISA, according to manufacturer’s instructions.

The following circulating markers of ventricular remodeling were measured: the matrix metalloproteinases (MMP-2 and MMP-9), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1; R&D Systems), and procollagen type III N-terminal peptide (Orion Diagnostics). The oxidative stress marker F2-isoprostane was measured according to manufacturer’s instructions.12 For selected novel biomarkers, samples from 30 healthy volunteers (25–72 years old, no known disease and taking no medications) were analyzed to obtain local references for normal values. Standard biochemical and hematologic analyses were performed at participating centers.

**Sample Size Calculation**

Sample size was based on TNF-α, which was the most described inflammatory biomarker (in terms of statistics) in HF and in CKD at the time our study was designed. On the basis of biochemical methodology and references available,8,13,14 we had aimed at detecting a difference in TNF-α concentration of 10 pg/mL between any 2 groups and assumed a SD of 12 pg/mL. A sample size of 40 patients per group would then provide ≥90% power with a type I error rate of 0.0167 (to adjust for multiple comparisons). Considering a potential 20% rate of uncompleted tests, 50 patients per group were required for this study.
some variables were log-transformed before the analysis to respect the normality assumption better. These variables are presented as median (interquartile range) and the P values reported come from models based on log-transformed data. Statistical analysis was performed using SAS version 9.1, and the significance level was set to 0.05.

Results

Baseline Characteristics

The characteristics of the study population are presented in Table 1. When compared with patients in group 2 (n=54), patients with anemia and HFrEF (group 1; n=48) were older, had more documented coronary artery disease, diabetes mellitus, hypertension, and atrial fibrillation or flutter on ECG. They also had been more frequently hospitalized for HF and for other causes within the previous year and had more signs of congestion and lower arterial blood pressure. In terms of HF therapy, patients in group 1 more often received mineralocorticoid receptor antagonists, digitalis, long-acting nitrates, oral anticoagulants, and amiodarone compared with patients in group 2. There was no difference in cardiac resynchronization therapy: 16.7% in group 1 and 13.0% in group 2. Patients in group 3 (CKD-preserved EF; n=49), when compared with patients in group 2, had more documented coronary artery disease, diabetes mellitus, hypertension, and atrial fibrillation or flutter on ECG. They also had been more frequently hospitalized for HF and for other causes within the previous year and had more signs of congestion and lower arterial blood pressure. In terms of HF therapy, patients in group 1 more often received mineralocorticoid receptor antagonists, digitalis, long-acting nitrates, oral anticoagulants, and amiodarone compared with patients in group 2.

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Table 1. Baseline Characteristics of Patients

<table>
<thead>
<tr>
<th>Patients Characteristics (Mean±SD or % Patients)</th>
<th>Group 1 (n=48), HF/LVSD/Anemia</th>
<th>Group 2 (n=54), HF/LVSD/No Anemia</th>
<th>Group 3 (n=49), CKD/No LVSD</th>
<th>P Value (1, 2, and 3)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics and medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>71.6±8.4</td>
<td>64.7±10.6</td>
<td>74.9±7.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Men</td>
<td>87.5</td>
<td>88.9</td>
<td>61.2</td>
<td>0.0006</td>
</tr>
<tr>
<td>Ethnic origin/whites</td>
<td>91.7</td>
<td>94.4</td>
<td>93.9</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.9±5.6</td>
<td>29.3±4.8</td>
<td>30.3±7.4</td>
<td>0.15</td>
</tr>
<tr>
<td>HF of ischemic cause</td>
<td>72.9</td>
<td>61.1</td>
<td>N/A</td>
<td>0.15</td>
</tr>
<tr>
<td>Documented CAD</td>
<td>95.0</td>
<td>73.8</td>
<td>78.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>45.8</td>
<td>20.4</td>
<td>36.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypertension</td>
<td>79.2</td>
<td>44.4</td>
<td>83.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of cancer</td>
<td>22.9</td>
<td>11.1</td>
<td>14.3</td>
<td>0.25</td>
</tr>
<tr>
<td>Current smoking</td>
<td>6.2</td>
<td>13.0</td>
<td>8.2</td>
<td>0.48</td>
</tr>
<tr>
<td>Atrial fibrillation and flutter/ECG</td>
<td>25.0</td>
<td>5.6</td>
<td>29.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Hospitalization for HF within 1 y</td>
<td>27.1</td>
<td>5.6</td>
<td>10.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Any hospitalization within a year</td>
<td>56.3</td>
<td>35.2</td>
<td>34.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Current clinical status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA class III–IV, %</td>
<td>22.9</td>
<td>16.7</td>
<td>22.4</td>
<td>0.34</td>
</tr>
<tr>
<td>LVEF, %†</td>
<td>30.1±7.7</td>
<td>28.5±8.1</td>
<td>58.7±6.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68.3±10.4</td>
<td>66.5±10.0</td>
<td>65.0±8.9</td>
<td>0.27</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>109.9±17.6</td>
<td>113.8±14.7</td>
<td>129.4±24.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>61.5±8.8</td>
<td>67.1±9.5</td>
<td>68.3±11.7</td>
<td>0.002</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>147.4±40.0</td>
<td>133.4±38.0</td>
<td>112.0±34.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eGFR (CKD-EPI, mL/min per 1.73 m²)</td>
<td>47.1±17.6</td>
<td>70.1±23.1</td>
<td>40.9±12.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Anemia</td>
<td>100.0</td>
<td>0.0</td>
<td>55.1%</td>
<td>N/A</td>
</tr>
<tr>
<td>Signs of HF (present, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jugular vein distension</td>
<td>43.8</td>
<td>24.1</td>
<td>12.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Pulmonary rales/crackles</td>
<td>6.3</td>
<td>1.9</td>
<td>0.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>45.8</td>
<td>18.5</td>
<td>46.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Natriuretic peptides and troponins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP, ng/L</td>
<td>1904 (1189, 3577)</td>
<td>745 (309, 1609)</td>
<td>557 (232, 1402)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Troponin T ≥0.01, μg/L</td>
<td>62.5</td>
<td>20.4</td>
<td>34.7</td>
<td>0.0004</td>
</tr>
<tr>
<td>HF therapy, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blocker</td>
<td>97.9</td>
<td>100</td>
<td>81.6</td>
<td>N/A</td>
</tr>
<tr>
<td>ACEI</td>
<td>62.5</td>
<td>79.6</td>
<td>49.0</td>
<td></td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>89.6</td>
<td>98.2</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>100.0</td>
<td>81.5</td>
<td>59.2</td>
<td></td>
</tr>
<tr>
<td>Spironolactone</td>
<td>58.3</td>
<td>29.6</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Other diuretics</td>
<td>12.6</td>
<td>7.5</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>39.6</td>
<td>24.1</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Long-acting nitrates</td>
<td>60.4</td>
<td>37.0</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>Other specified medications, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>75.0</td>
<td>70.4</td>
<td>59.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>10.5</td>
<td>9.3</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>56.3</td>
<td>37.0</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>Lipid-lowering drug</td>
<td>85.4</td>
<td>81.5</td>
<td>83.7</td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>29.2</td>
<td>11.1</td>
<td>6.1</td>
<td></td>
</tr>
</tbody>
</table>

N/A: considering the study design, comparisons not indicated for these variables. ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; eGFR, estimating glomerular filtration rate; EPI, Epidemiology Collaboration Group; HF, heart failure; LVEF, left ventricular ejection fraction; N/A, not applicable; NT-proBNP, N-terminal prohormone natriuretic peptide; and NYHA, New York Heart Association.

*Groups 1, 2, and 3 compared unless otherwise specified.
†LVEF reported in clinical setting within 6 mo of inclusion.
Commonly Used Circulating Biomarkers in HF

Patients in group 1 had higher NT-proBNP levels and more often elevated troponin T values when compared with patients in group 2 (Table 2). We assessed both New York Heart Association class and NT-proBNP in all patients, including group 3. Despite their renal dysfunction (which would be expected to raise NT-proBNP levels) and frequent anemia, only 18.4% of group 3 patients had both New York Heart Association ≥2 and elevated NT-proBNP; suggesting that HF with preserved EF was not a major contributor to our findings. A portrait of

Table 2. Comparison of Circulating Biomarkers Between Study Groups

<table>
<thead>
<tr>
<th>Characteristics of anemia</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value (Groups 1 vs 2)*</th>
<th>Group 3</th>
<th>P Value (Groups 1 vs 3)</th>
<th>P Value (Groups 2 vs 3)</th>
<th>P Value (Groups 1, 2, and 3)</th>
<th>Healthy Volunteers: Novel Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>117.1±8.5</td>
<td>142.3±9.0</td>
<td>&lt;0.0001</td>
<td>126.7±16.1</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Platelet count &lt;150×10⁹/L ( % patients)</td>
<td>25.0</td>
<td>13.0</td>
<td>0.12</td>
<td>8.2</td>
<td>0.03</td>
<td>0.43</td>
<td>0.06</td>
<td>...</td>
</tr>
<tr>
<td>Serum hepcidin, ng/mL</td>
<td>9.8 (5.7, 15.5)</td>
<td>7.6 (5.0, 12.9)</td>
<td>0.33</td>
<td>4.9 (3.3, 10.5)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.03</td>
<td>8.0 (2.1, 9.9)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate, mm/h</td>
<td>24.2±17.3</td>
<td>11.7±9.6</td>
<td>&lt;0.0001</td>
<td>23.8±17.3</td>
<td>1.0</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>EPO, pg/mL</td>
<td>20.4 (13.5, 31.2)</td>
<td>12.7 (8.5, 18.5)</td>
<td>0.003</td>
<td>13.0 (9.5, 16.6)</td>
<td>0.0005</td>
<td>0.82</td>
<td>0.0003</td>
<td>8.6 (6.6, 11.5)</td>
</tr>
<tr>
<td>TSH&gt;4.20, μU/L</td>
<td>19.1</td>
<td>9.4</td>
<td>0.16</td>
<td>12.2</td>
<td>0.35</td>
<td>0.85</td>
<td>0.35</td>
<td>...</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>47.1±17.6</td>
<td>70.5±23.1</td>
<td>&lt;0.0001</td>
<td>40.9±12.3</td>
<td>0.12</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>BUN, mmol/L</td>
<td>12.6±5.7</td>
<td>7.8±3.5</td>
<td>&lt;0.0001</td>
<td>11.3±4.2</td>
<td>0.42</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Creatinine (IDMS, μmol/L)</td>
<td>141.7±49.0</td>
<td>105.1±38.6</td>
<td>0.0003</td>
<td>142.5±50.3</td>
<td>1.0</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>BUN/IDMS creatinine ratio, mg/dL</td>
<td>22.2±7.0</td>
<td>18.4±3.7</td>
<td>0.0036</td>
<td>19.8±5.7</td>
<td>0.17</td>
<td>0.30</td>
<td>0.004</td>
<td>...</td>
</tr>
<tr>
<td>Cystatin C, mg/L</td>
<td>1.5 (1.2, 1.8)</td>
<td>1.0 (0.8, 1.2)</td>
<td>&lt;0.0001</td>
<td>1.5 (1.2, 1.7)</td>
<td>1.0</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>N/A</td>
</tr>
<tr>
<td>NGAL, ng/mL</td>
<td>146.9 (107.6, 185.4)</td>
<td>118.8 (82.7, 140.0)</td>
<td>0.003</td>
<td>136.2 (111.9, 161.2)</td>
<td>0.92</td>
<td>0.009</td>
<td>0.001</td>
<td>92.7 (78.0, 108.8)</td>
</tr>
<tr>
<td>Infammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>7.8 (5.3, 12.5)</td>
<td>2.9 (1.3, 6.4)</td>
<td>0.0009</td>
<td>4.3 (3.1, 5.6)</td>
<td>0.07</td>
<td>0.03</td>
<td>0.001</td>
<td>0.5 (0.1, 1.2)</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>2.6 (1.1, 5.2)</td>
<td>1.6 (0.9, 3.7)</td>
<td>0.53</td>
<td>2.6 (1.1, 4.8)</td>
<td>0.97</td>
<td>0.69</td>
<td>0.53</td>
<td>...</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>1.5 (1.2, 2.4)</td>
<td>1.5 (0.9, 2.2)</td>
<td>0.82</td>
<td>2.2 (1.2, 3.6)</td>
<td>0.49</td>
<td>0.18</td>
<td>0.20</td>
<td>1.1 (0.6, 1.8)</td>
</tr>
<tr>
<td>TGF-β1, ng/mL</td>
<td>23.0±6.2</td>
<td>24.8±6.6</td>
<td>0.28</td>
<td>21.0±4.7</td>
<td>0.24</td>
<td>0.005</td>
<td>0.007</td>
<td>27.9±6.8</td>
</tr>
<tr>
<td>IL-1β (% with detectable levels)</td>
<td>31.3</td>
<td>20.4</td>
<td>0.21</td>
<td>10.2</td>
<td>0.01</td>
<td>0.16</td>
<td>0.04</td>
<td>13.3</td>
</tr>
<tr>
<td>IL-10, pg/mL</td>
<td>13.0 (9.3, 17.1)</td>
<td>11.4 (7.5, 14.9)</td>
<td>0.05</td>
<td>10.4 (8.4, 15.2)</td>
<td>0.99</td>
<td>0.04</td>
<td>0.02</td>
<td>10.3 (5.4, 14.9)</td>
</tr>
<tr>
<td>Left ventricular remodeling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIIINP, μg/L</td>
<td>6.0 (4.6, 8.5)</td>
<td>5.1 (3.9, 6.9)</td>
<td>0.02</td>
<td>5.8 (4.4, 7.0)</td>
<td>0.24</td>
<td>0.54</td>
<td>0.02</td>
<td>3.3 (2.8, 3.6)</td>
</tr>
<tr>
<td>MMP-2, μg/L</td>
<td>279.5 (224.6, 333.4)</td>
<td>226.4 (194.9, 279.4)</td>
<td>0.009</td>
<td>236.6 (217.6, 275.3)</td>
<td>0.05</td>
<td>0.82</td>
<td>0.009</td>
<td>N/A</td>
</tr>
<tr>
<td>MMP-9, μg/L</td>
<td>60.2 (46.2, 91.0)</td>
<td>56.8 (37.5, 137.0)</td>
<td>0.70</td>
<td>58.8 (38.0, 131.4)</td>
<td>0.92</td>
<td>0.93</td>
<td>0.71</td>
<td>N/A</td>
</tr>
<tr>
<td>TIMP-1, μg/L</td>
<td>223.3 (201.8, 255.0)</td>
<td>195.7 (167.2, 215.8)</td>
<td>0.0002</td>
<td>217.2 (203.3, 245.2)</td>
<td>0.83</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>184.7 (168.8, 201.7)</td>
</tr>
<tr>
<td>MMP-2/TIMP-1 ratio</td>
<td>1.2 (1.0, 1.4)</td>
<td>1.2 (1.0, 1.6)</td>
<td>0.97</td>
<td>1.1 (1.0, 1.2)</td>
<td>0.19</td>
<td>0.27</td>
<td>0.16</td>
<td>N/A</td>
</tr>
<tr>
<td>MMP-9/TIMP-1 ratio</td>
<td>0.3 (0.2, 0.4)</td>
<td>0.3 (0.2, 0.8)</td>
<td>0.12</td>
<td>0.2 (0.2, 0.5)</td>
<td>0.82</td>
<td>0.44</td>
<td>0.14</td>
<td>N/A</td>
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<tr>
<td>Natriuretic peptides and troponins</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP, ng/L</td>
<td>1904 (1189, 3577)</td>
<td>745 (309, 1609)</td>
<td>&lt;0.0001</td>
<td>557 (232, 1402)</td>
<td>&lt;0.0001</td>
<td>0.85</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Troponin T ≥0.01, μg/L</td>
<td>62.5</td>
<td>20.4</td>
<td>&lt;0.0001</td>
<td>34.7</td>
<td>0.006</td>
<td>0.10</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2-isoprostanes, ng/L</td>
<td>63.0 (47.0, 76.0)</td>
<td>57.5 (46.0, 75.0)</td>
<td>0.88</td>
<td>59.0 (46.0, 75.0)</td>
<td>0.69</td>
<td>0.94</td>
<td>0.72</td>
<td>39 (32, 45)</td>
</tr>
</tbody>
</table>

BUN indicates blood urea nitrogen; eGFR, estimating glomerular filtration rate; EPO, erythropoietin; hs-CRP, high-sensitivity-C-reactive protein; IL, interleukin; MMP, matrix metalloproteinase; NGAL, neutrophil gelatinase–associated lipocalin; NT-proBNP, N-terminal probrain natriuretic peptide; PIIINP, procollagen type III N-terminal peptide; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; TGF, transforming growth factor; TNF, tumor necrosis factor; and TSH, thyroid stimulating hormone.

*For the continuous variables, Tukey adjustment was used for pairwise comparisons (see Table II in the Data Supplement for adjusted comparisons between groups 1 and 2 for selected biomarkers).
the cardiac structure and function of patients in group 3 can be found in Table I in the Data Supplement.

Characteristics of Anemia

Despite having at least moderate CKD, only 55% of patients in group 3 had anemia, and hemoglobin was lower in patients in group 1 than in those in group 3. Erythropoietin values were significantly higher in group 1 (20.4 [13.5, 31.2] pg/mL) when compared with those in group 2 (12.7 [8.5, 18.5] pg/mL) and 3 (13.0 [9.5, 16.6] pg/mL). Vitamin B12 and folic acid were normal in the vast majority of patients and did not differ between groups (data not shown), as expected based on exclusion criteria. On the basis of the STIR/log (ferritin) ratio and ferritin levels,15 14.9% of patients in group 1 were found to have iron deficiency anemia when compared with only 3.7% of patients in group 3 (data not shown). Thyroid stimulating hormone levels above the central laboratory reference range of patients in group 3 (data not shown). Thyroid stimulating hormone levels above the central laboratory reference range of patients in group 3 (data not shown). Thyroid stimulating hormone levels above the central laboratory reference range of patients in group 3 (data not shown).

Renal Function

Patients in group 1 had more advanced CKD than patients in group 2 (mean eGFR, 47.1±17.6 versus 70.1±23.1 mL/min per 1.73 m²; P<0.0001), but their eGFR was not statistically different than that of patients in the CKD group 3 (40.9±12.3 mL/min per 1.73 m²; P=0.12 between groups 1 and 3). Both cystatin C and neutrophil gelatinase–associated lipocalin were significantly higher in group 1 than in group 2 (Table 2); however, these markers of renal dysfunction, as eGFR, did not differ between groups 1 and 3 (P=1.0 and 0.92, respectively). Correlation coefficients between hemoglobin and cystatin C were −0.56 (P<0.0001) in groups 1 and 2 combined and −0.33 (P=0.02) in group 3. All other markers of renal function (eGFR, blood urea nitrogen/creatinine ratio, and neutrophil gelatinase–associated lipocalin) had weaker correlations with hemoglobin, with eGFR (correlation coefficient, 0.49; P<0.0001) performing best after cystatin C (data not shown).

Inflammation

The IL-6 (P=0.0009) and IL-10 levels (P=0.05) were higher in group 1 when compared with those in group 2. IL-1β was the only inflammatory marker that differed significantly between groups 1 and 3 (higher in group 1). An inflammatory pattern was seen as frequently in group 1 as in group 3 on protein electrophoresis. A small proportion of patients was newly found to have monoclonal gammopathy in all 3 groups (3%–6%, data not shown).

LV Remodeling by Echocardiography and Serum Biomarkers

Patients in group 1 had greater LV remodeling when compared with patients in the other 2 groups. They had a higher LV mass index, more mitral regurgitation, and a larger left atrial end-systolic volume index (Table I in the Data Supplement). Also, right ventricular systolic pressure was highest in group 1 and lowest in group 3. LVEF (Table 1) was similar in groups 1 and 2 (30.1±7.7% and 28.5±8.1%, respectively; P=0.54) and normal in group 3 (58.9±6.7%).

Serum biomarkers related to collagen turnover and LV remodeling (Table 2) did not significantly differ between groups 1 and 3. However, procollagen type III N-terminal peptide, MMP-2, and TIMP-1 were all significantly higher in group 1 when compared with those in group 2.

Biomarkers Independently Related to Anemia in HF

When groups 1 and 2 were compared after adjustment for several cofactors or comorbid conditions (Table II in the Data Supplement), the markers of renal function (except neutrophil gelatinase–associated lipocalin), IL-10, and the MMP-9/TIMP-1 ratio remained statistically different between patients with anemia and nonanemia. We also examined the predictors of hemoglobin as a continuous variable in patients with HFrEF, including the same variables as in Table II in the Data Supplement in this analysis but with eGFR representing renal function. We also added IL-10 and the MMP-9/TIMP-1 ratio to this analysis, as per the above results. For groups 1 and 2 combined (HFrEF), lower eGFR (P<0.0001), female sex (P=0.007), and higher IL-10 levels (P=0.04) were independent predictors of lower hemoglobin.

Conducting the same multivariable analysis as above in group 3, we found that only female sex (P=0.01) and lower MMP-9/TIMP-1 ratio (P=0.001) were independent predictors of lower hemoglobin.

Discussion

The main objective of this study was to improve our understanding of how patients with HFrEF and anemia differ from subjects with the same condition but without anemia, and from subjects with CKD and preserved EF. Our specific aims were to clarify the role of CKD in anemia associated with HF further, and to understand how markers of LV remodeling and inflammation differ between patients with anemia and nonanemia with HFrEF. We think that these comparisons can help shed light on how anemia confers a worse prognosis in HF. In agreement with previous studies, we found that patients with anemia and HFrEF had more symptoms and higher levels of markers of advanced HF, but we also demonstrated that they had more adverse LV remodeling on echocardiography, despite having similar LVEF (groups 1 and 2). Patients with anemia also had higher erythropoietin values, worse renal function, and exhibited more inflammation and activation of biomarkers associated with LV remodeling. High erythropoietin levels have previously been demonstrated and may suggest an adequate response to anemia in patients with HFrEF; where erythropoietin resistance is most likely involved.16 Although the use of oral anticoagulants was higher in patients with anemia and HFrEF, this does not necessarily imply a causal relationship, particularly as iron deficiency anemia was infrequent in our cohort of patients.

The association between anemia and adverse LV remodeling is particularly interesting, especially in light of our findings on inflammatory markers, as we will detail below.
Using multivariable analyses, we found that female sex, eGFR, and higher IL-10 levels were the strongest predictors of lower hemoglobin in patients with HFrEF. Patients with CKD and preserved EF differed in many ways from patients with anemia and HFrEF. The 10% rate of hospitalization for HF within 1 year in patients with CKD may reflect the presence of HF with preserved EF in this group. There is a recognized association between those 2 conditions, related to the reduced ability of patients with CKD to control (and adapt to) volume overload.

Renal dysfunction is a major contributor to the pathophysiology of anemia in HF, and it was also the strongest predictor of lower hemoglobin in our study. Cystatin C showed a stronger correlation with hemoglobin than did eGFR. Despite very similar levels of renal dysfunction, patients in groups 1 and 3 largely differed in their biomarkers profiles, demonstrating the many processes other than those related to CKD are involved in the development of anemia in the presence of LV systolic dysfunction. These mechanisms, which are directly involved in the pathophysiology of systolic HF, seem much more active in patients with anemia and HFrEF than in those without anemia. We indeed observed that levels of markers of inflammation, LV remodeling, myocardial stretch (NT-proBNP), and myocyte death (troponin T) are higher in patients with HFrEF and anemia than in those without anemia.

About inflammatory pathways, IL-6 was significantly higher in patients with HFrEF and anemia than in the other 2 groups, intermediate in patients with CKD, and the lowest in patients with HFrEF without anemia. The IL-6 levels reported here were lower than those obtained in the Coordinating Study Evaluating Outcomes of Advising and Counseling in HF (median, 12.0 [7.1–25.5] pg/mL), which included patients hospitalized for HF. This difference can be explained by the fact that patients in our study had chronic stable HF rather than acute HF. In Coordinating Study Evaluating Outcomes of Advising and Counseling in HF, high-sensitivity-C-reactive protein also differed between patients with anemia and without anemia with a median of 3.4 (1.3–6.6) mg/L in patients with acute HF and anemia. We observed lower levels of high-sensitivity-C-reactive protein in patients with chronic HFrEF and anemia (median value of 2.6 [1.1–5.2] mg/L), which did not differ from patients in either group 2 or 3. The IL-6 and high-sensitivity-C-reactive protein levels we observed were similar to those recently reported in stable chronic HF.

In patients with HFrEF, levels of TNF-α, transforming growth factor, and the proportion of patients with detectable levels of IL-1β did not significantly differ, regardless of the presence of anemia. However, variable differences in these biomarkers existed between patients with HFrEF and those of group 3. IL-6, involved in a major apoptosis signaling pathway that is upregulated in chronic HF, differed across groups: with group 1 having the highest values and group 2 having intermediate values. Levels of IL-10, which suppresses the production of TNF-α and other proinflammatory cytokines and counteracts many of the adverse effects of TNF-α on cardiac function, were higher in group 1 when compared with those in group 2 but were comparable between groups 1 and 3. Once known independent predictors of worse prognosis in HF were accounted for in patients with HFrEF, IL-10 was the only inflammatory marker that remained different between patients with anemia and non-anemia (adjusted \( P=0.035 \)). Similar results were observed in patients with chronic obstructive pulmonary disease, where higher levels were documented in anemic patients with chronic obstructive pulmonary disease (25.6 [1.9–95.2] pg/mL) than those observed here in patients with HFrEF and anemia. Interestingly, IL-10 was recently proposed as a potential therapeutic approach to limit the progression of pressure overload–induced adverse cardiac remodeling. However, volume overload seems to be a concern in patients with HF and anemia.

Our results indicate a active LV remodeling process in patients with anemia and HFrEF, as shown by increased procollagen type III N-terminal peptide, MMP-2, and TIMP-1 levels in group 1 when compared with those in group 2. The fact that patients in group 1 more frequently received mineralocorticoid receptor antagonists could have favorably modified the levels of remodeling biomarkers but the latter remained higher in this anemic group. The MMP-9/TIMP-1 ratio was the only marker of LV remodeling that remained significantly different between patients with and without anemia with HFrEF, after adjustment for well-established markers of prognosis, suggesting that this relationship is complex (Table II in the Data Supplement). The MMP-9/TIMP-1 ratio was lower in group 1, reflecting higher levels of inhibition of the degradation process (higher TIMP-1 levels; Table 2) in patients with anemia. This ratio has also been correlated to severity of disease and mortality in sepsis. In their study, Lorente et al. demonstrated that nonsurviving sepsis patients had a lower MMP-9/TIMP-1 ratio \( (P=0.003) \), and higher levels of IL-10 \( (P<0.001) \), than did survivors. The latter biomarker profiles of nonsurviving sepsis patients are thus similar to those of anemic HFrEF patients. In terms of interactions between pathophysiological pathways, the anti-inflammatory cytokine IL-10 has been shown to induce TIMP-1 and reduce MMP-9 expression in endothelium/macrophage cocultures and is proposed as an important modulator of monocyte–endothelial cell interactions. Cytokine-induced monocyte–endothelial interaction and vascular inflammation play a critical role in atherogenesis, another potential link between prognosis and anemia in HF.

The echocardiographic markers of remodeling, LV mass, and left atrial volume indices were increased in patients with anemia when compared with those with nonanemia and HFrEF (Table I in the Data Supplement). These parameters have consistently been associated with cardiovascular outcomes in HF and were markedly increased in our patients with anemia and HFrEF. In patients with chronic anemia but without HF, increased cardiac output, as a result of anemia, may lead to progressive cardiac enlargement, LV hypertrophy, and left atrial enlargement, as was found in our study. The higher values of NT-proBNP (reflecting myocardial stretch) in group 1 also favor the above remodeling hypotheses.

Troponin T was elevated in patients with HFrEF, suggesting an ongoing adverse cellular necrotic process. However, the proportion of patients with elevated troponin T levels did not...
remain statistically different between patients with and without anemia once other markers of prognosis were considered, suggesting that this elevation was multifactorial in nature.

In multivariable analysis, a recent hospitalization was identified as an independent predictor of lower hemoglobin, which, in addition to reflecting the severity and activity of the disease, may also indirectly point to congestion as a major contributor to anemia. In a study involving 196 patients with advanced HF, Androne et al directed assessed hemodilution, which was present in 46% of the subset of 37 patients with ambulatory anemia. In our study, 43.8% of patients with anemia and HFrEF had jugular vein distension, and 45.8% had peripheral edema. The prevalence of these markers of congestion was much lower in patients with HFrEF without anemia (24.1% and 18.5%). More signs of congestion observed in patients with anemia (increased jugular venous pressure, peripheral edema, higher bilirubin levels, and lower platelet counts) suggest that hemodilution and right HF were significant contributors to anemia. To compensate for hemodilution, jugular vein distension was included in our adjusted analysis of other potential drivers of anemia (Table II in the Data Supplement). These findings and those of previous mechanistic studies on hemodilution should prompt control of volume overload before considering other treatments of anemia in HF.

**Limitations**

We have used a cross-sectional study design, with a relatively small number of patients per group. Nevertheless, our sample size is similar to that of other studies on mechanisms of anemia in HF and was sufficient to observe significant, clinically meaningful differences in multiple biomarkers between groups. Sample size determination was based on TNF-α levels using older enzymatic assays in previous cohorts, leading to lower observed TNF-α concentrations. However, the observed levels of key inflammatory markers compared well with those reported in current literature. The results of our multivariable analysis need to be interpreted keeping the sample size in mind and will need to be replicated in future studies.

**Conclusions**

Anemia is strongly associated with the level of renal dysfunction in HFrEF, but more importantly, it is indicative of a complex interplay between pathophysiological mechanisms, portraying a more advanced and active heart disease. These mechanisms include increased myocardial remodeling, inflammation, and volume overload, hallmarkers of patients with anemia and HF.

**Appendix**

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Dr O’Meara holds a Junior Researcher scholarship (level 2) from FRQS and has received an unrestricted grant from Johnson & Johnson to perform this research. Dr Rouleau has received research grants or been a coinvestigator on grants supported by Novartis. Dr White holds the Carolyn and Richard Renaud Research Chair in Heart Failure of the Montreal Heart Institute. Dr Tardif holds the Canada Research Chair in translational and personalized medicine and the Université de Montréal endowed research chair in atherosclerosis. Dr de Denus has received research grants or been a coinvestigator on grants supported by AstraZeneca, Novartis, Roche, Pfizer. The other authors report no conflicts.

**References**


**CLINICAL PERSPECTIVE**

During the past decade, anaemia emerged as a highly prevalent and strong independent prognostic marker in heart failure (HF), yet this association is not completely understood and therapeutic options are still being investigated. By comparing the clinical, echocardiographic, and circulating biomarkers profiles of patients with HF with and without anaemia with those of patients with chronic kidney disease without systolic dysfunction, we aimed to improve our understanding of the mechanisms involved in the relationship between anaemia and HF. Our results demonstrate that anaemia is strongly associated with the level of renal dysfunction in HF with reduced ejection fraction, but more importantly, it is indicative of a complex interplay between pathophysiological mechanisms, portraying a more advanced and active heart disease. These mechanisms include increased myocardial remodeling, inflammation, and volume overload. Targeting these mechanisms should be considered in the treatment of anemic patients with HF, first through the application of evidence-based therapy, including management of volume overload. Theoretically, novel agents reducing inflammation could also have an effect on anaemia in HF, but whether these would lead to improved clinical outcomes remains to be demonstrated.
Heart Failure With Anemia: Novel Findings on the Roles of Renal Disease, Interleukins, and Specific Left Ventricular Remodeling Processes

Eileen O'Meara, Jean L. Rouleau, Michel White, Karine Roy, Lucie Blondeau, Anique Liszkowski, François Madore, Jean-Claude Tardif and Simon de Denus for the ANCHOR Investigators

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“Supplemental Material”
Table S1: Echocardiographic parameters*

Measured echocardiographic parameters were compared between the 3 groups.
*A majority of patients had a complete central laboratory analysis of their echocardiogram. However, the number of available patients per group varies for each parameter, according to the quality of each recording (see methods section). § Since only groups 1 and 2 had left ventricular systolic dysfunction (LVSD), selected parameters were compared between these two groups when judged more appropriate. Tukey adjustment was used for the comparisons.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>30.1 ± 7.7</td>
<td>28.5 ± 8.1</td>
<td>58.9 ± 6.7</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Cardiac index (L/min/m²)</td>
<td>2.0 ± 0.5</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 0.5</td>
<td>0.0006</td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume index (ml/m²)</td>
<td>95.1 ± 33.1</td>
<td>83.8 ± 23.5</td>
<td>43.8 ± 8.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Left ventricular end-systolic volume index (mL/m²)</td>
<td>63.5 ± 26.7</td>
<td>54.7 ± 20.9</td>
<td>20.1 ± 5.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Left ventricular mass index (g/m²)</td>
<td>153.2 ± 35.8</td>
<td>134.0 ± 38.4</td>
<td>102.8 ± 33.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Left atrial end-systolic volume index (ml/m²)</td>
<td>39.0 ± 15.7</td>
<td>27.2 ± 10.3</td>
<td>28.6 ± 15.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mitral regurgitation grade ≥ 3/4</td>
<td>10.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Right ventricular myocardial performance index (Tei index)</td>
<td>0.34 ± 0.17</td>
<td>0.33 ± 0.15</td>
<td>0.25 ± 0.11</td>
<td>0.01</td>
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<tr>
<td>Right ventricular estimated systolic pressure (mmHg)</td>
<td>45.8 ± 14.9</td>
<td>41.7 ± 13.2</td>
<td>36.7 ± 8.6</td>
<td>0.01</td>
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<tr>
<td>Tricuspid regurgitation grade ≥ 2/4</td>
<td>12.5</td>
<td>9.3</td>
<td>14.3</td>
<td>0.73</td>
</tr>
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</table>
Table S2: Adjusted comparisons of selected† biomarkers in patients with HF with reduced EF (groups 1 and 2)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n=48)</th>
<th>Group 2 (n=54)</th>
<th>P-value Unadjusted**</th>
<th>P-value Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>117.1 ± 8.5</td>
<td>142.3 ± 9.0</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15.5 ± 1.9</td>
<td>13.8 ± 1.4</td>
<td>&lt; 0.0001</td>
<td>0.2040</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>12.2 ± 3.7</td>
<td>14.9 ± 5.2</td>
<td>0.0039</td>
<td>0.2965</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>20.7 ± 7.6</td>
<td>26.0 ± 9.7</td>
<td>0.0043</td>
<td>0.2645</td>
</tr>
<tr>
<td>EPO (pg/mL)</td>
<td>20.4 (13.5,31.2)</td>
<td>12.7 (8.5,18.5)</td>
<td>0.0030</td>
<td>0.2392</td>
</tr>
<tr>
<td>Sedimentation rate (mm/h)</td>
<td>24.2 ± 17.3</td>
<td>11.7 ± 9.6</td>
<td>&lt; 0.0001</td>
<td>0.0199</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‡ Blood urea nitrogen (mmol/L)</td>
<td>12.6 ± 5.7</td>
<td>7.8 ± 3.5</td>
<td>&lt; 0.0001</td>
<td>0.0102</td>
</tr>
<tr>
<td>‡ IDMS creatinine (µmol/L)</td>
<td>141.7 ± 49.0</td>
<td>105.1 ± 38.6</td>
<td>&lt; 0.0001</td>
<td>0.0156</td>
</tr>
<tr>
<td>‡ BUN/IDMS creatinine ratio (mg/dL)</td>
<td>22.2 ± 7.0</td>
<td>18.4 ± 3.7</td>
<td>0.0014</td>
<td>0.0348</td>
</tr>
<tr>
<td>‡ Cystatin C (mg/L)</td>
<td>1.5 (1.2,1.8)</td>
<td>1.0 (0.8,1.2)</td>
<td>&lt; 0.0001</td>
<td>0.0029</td>
</tr>
<tr>
<td>NGAL (ng/mL)</td>
<td>146.9 (107.6,185.4)</td>
<td>118.8 (82.7,140.0)</td>
<td>0.0018</td>
<td>0.1841</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>7.8 (5.3, 12.5)</td>
<td>2.9 (1.3,6.4)</td>
<td>0.0003</td>
<td>0.1175</td>
</tr>
<tr>
<td>Interleukin-10 (pg/mL)</td>
<td>13.0 (9.3,17.1)</td>
<td>11.4 (7.5,14.9)</td>
<td>0.0217</td>
<td>0.0350</td>
</tr>
<tr>
<td>LV remodeling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procollagen type III amino-terminal propeptide (PIIINP; µg/L)</td>
<td>6.0 (4.6,8.5)</td>
<td>5.1 (3.9,6.9)</td>
<td>0.0092</td>
<td>0.8740</td>
</tr>
<tr>
<td>Matrix metalloproteinase 2 (MMP-2; µg/L)</td>
<td>280 ( 225,333)</td>
<td>226 (195,279)</td>
<td>0.0070</td>
<td>0.6126</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>223.3 (201.8,255.0)</td>
<td>195.7 (167.2,215.8)</td>
<td>&lt; 0.0001</td>
<td>0.4733</td>
</tr>
<tr>
<td>MMP-9/TIMP-1 ratio</td>
<td>0.26 (0.20,0.44)</td>
<td>0.31 (0.21,0.75)</td>
<td>0.0475</td>
<td>0.0484</td>
</tr>
<tr>
<td>Clinically established markers of prognosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-terminal pro brain natriuretic peptide (NT-proBNP; ng/L)</td>
<td>1904 (1189,3577)</td>
<td>745 (309,1609)</td>
<td>&lt; 0.0001</td>
<td>0.2596</td>
</tr>
<tr>
<td>Troponin T ≥ 0.01 µg/L (%)</td>
<td>62.5</td>
<td>20.4</td>
<td>&lt; 0.0001</td>
<td>0.1936¶</td>
</tr>
</tbody>
</table>

Comparisons of biomarkers with significantly different levels between groups 1 and 2, representing different pathophysiological mechanisms in systolic heart failure.

*P-values adjusted for age, sex, CAD, Diabetes, Hypertension, AF or flutter on ECG, eGFR, hospitalization for HF within previous year, jugular vein distension.

**Unadjusted p-values may differ from those in table II since the Tukey adjustment was not necessary for the 2 group comparison here presented.

‡ For the analysis of these markers of renal function, eGFR was not included in the model.

¶Sex was not included as a covariable for the analysis of troponin T since not enough women had troponin T levels ≥ 0.01.