 Effect of Atrial Fibrillation on Hematopoietic Progenitor Cells

A Novel Pathophysiological Role of the Atrial Natriuretic Peptide?

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Background—Injury to the heart causes hematopoietic progenitor cells (HPCs) to migrate to the site of damage and to undergo cell differentiation. Studies suggest that myocardial progenitor cells invade atrial tissue. So far it is unclear, however, whether an atrial disease per se affects circulating HPCs.

Methods and Results—Seventeen patients with persistent atrial fibrillation (persistAF), 12 with paroxysmal AF (paroxAF), and 17 matched patients with sinus rhythm (SR) were studied. HPCs (CD34+/CD117+) were quantified with the use of a fluorescence-activated cell sorter; stromal cell–derived factor-1α (SDF-1α), vascular endothelium growth factor (VEGF), and atrial natriuretic peptide (ANP) were determined by immunoassays. In patients with persistAF, blood samples were obtained before as well as 10 minutes, 24 hours, and 48 hours after electrical cardioversion. CD34+HPCs (AF, 7.0±2.3×10^3/mL versus SR, 5.0±1.6×10^3/mL; P<0.01) were increased during persistAF only. Highest SDF-1α levels were also observed during persistAF. Successful and unsuccessful cardioversion decreased CD34+HPCs temporarily (7.0±2.3×10^3/mL versus 24 hours: 5.0±1.5×10^3/mL; P<0.05). Forty-eight hours after successful cardioversion, SDF-1α and CD34+HPC levels started to decline, reaching control levels after 59±19 days. CD34+/CD117+ and VEGF levels, however, were increased by DC energy but not by AF. ANP levels correlated with CD34+HPC (r=0.76; P<0.01) and SDF-1α (r=0.56; P<0.01). HPCs from patients with AF had a greater tendency to differentiate into cells expressing (cardio)myocyte markers ANP and myocyte enhancer factor-2.

Conclusions—PersistAF appears to increase the potential of HPCs for (cardio)myogenesis. Restitution of CD34+HPC levels, mediated by SDF-1α and possibly ANP, occurs within several weeks after successful cardioversion.

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Key Words: arrhythmia ■ blood cells ■ cardioversion ■ fibrillation ■ natriuretic peptides

Effect of Atrial Fibrillation on Hematopoietic Progenitor Cells (HPCs) to migrate to the site of damage and to undergo progenitor cell differentiation.1,2 Recent studies suggest that multipotent cells can transdifferentiate into cardiomyocytes.1-3 Interestingly, Quaini et al2 have demonstrated a high proportion of progenitor cells, especially in atrial tissue. It is not known so far whether atrial fibrillation (AF), which is known to cause structural atrial changes, affects HPCs.

The purpose of the present study was to quantify CD34+ and CD34+/CD117+HPCs, stromal cell–derived factor-1α (SDF-1α), vascular endothelium growth factor (VEGF), and atrial natriuretic peptide (proANP 1-98) in patients with persistent (persistAF) and paroxysmal AF (paroxAF). In addition, the effect of electrical cardioversion on HPCs and HPC factors was determined.
## Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sinus Rhythm</th>
<th>Persistent AF</th>
<th>Paroxysmal AF</th>
</tr>
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<tbody>
<tr>
<td>Subjects, n</td>
<td>17</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±18</td>
<td>53±6</td>
<td>60±15</td>
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<tr>
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<td>12/5</td>
<td>5/7</td>
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<tr>
<td>History of AF, mo</td>
<td>...</td>
<td>11±6</td>
<td>22±23</td>
</tr>
<tr>
<td>CAD, % (n)</td>
<td>12 (2)</td>
<td>18 (3)</td>
<td>25 (3)</td>
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<tr>
<td>MI, % (n)</td>
<td>12 (2)</td>
<td>6 (1)</td>
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<tr>
<td>LVEF, %</td>
<td>56±6</td>
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<td>LAD, cm</td>
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<td>4.6±0.4*</td>
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<tr>
<td>Hypertension, % (n)</td>
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<td>88 (15)</td>
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<td>Diabetes, % (n)</td>
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<td>HLP, % (n)</td>
<td>71 (12)</td>
<td>53 (9)</td>
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<td>β-Blockers, % (n)</td>
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<td>65 (11)</td>
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<td>Calcium antagonists, % (n)</td>
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<tr>
<td>ACE inhibitors, % (n)</td>
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<td>59 (10)</td>
<td>42 (5)</td>
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</tbody>
</table>

CAD indicates coronary artery disease; MI, history of myocardial infarction; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; HLP, hyperlipidemia; and class I/II AA, class I/III antiarrhythmic drugs. Numbers in parentheses represent the absolute number.

*P<0.05, persistent vs paroxysmal AF.

hours, and 48 hours after cardioversion. In 7 patients, blood samples were also drawn 59±19 weeks after cardioversion.

Analysis of HPCs was performed according to recent publications. In brief, each of the 50-μL EDTA blood samples was incubated with a nucleic acid dye, CD45-PerCP, and CD34-PE (ProCOUNT CD34 reagent, BD Immunocytometry Systems) in bead-containing TruCount tubes (BD). The control reagent (nucleic acid dye, yl-PE and CD45-PerCP) was used to assess the amount of nonspecific antibody binding. After red cell lysis with diluted FACS Lysing Solution (BD), the samples were measured with a FACS Calibur flow cytometer (BD). The absolute number of CD34<sup>+</sup> cells in the sample was determined by dividing the number of CD34<sup>+</sup> cellular events by the number of fluorescent bead events and then multiplying with the bead concentration. The ProCOUNT software system (BD) was used to acquire and analyze data.

For CD34<sup>+</sup>/CD117<sup>+</sup> quantification, 50-μL peripheral blood samples were labeled with FITC-CD34 and PE-CD117 (BD). After red cell lysis, flow cytometric analyses were performed on a FACS Calibur analytical flow cytometer (BD). List mode data were acquired by using the CellQuest software (BD).

### Immunoassays

Ice-cooled EDTA blood was used to determine human SDF-1α, VEGF, and proANP(1-98) plasma levels. Blood samples were centrifuged at 1000g within 30 minutes after collection. Human SDF-1α, VEGF, interleukin (IL)-6, and IL-10 (R&D Systems) as well as proANP(1-98) (Biomedica) were determined by commercially available immunoassays according to the manufacturer’s specifications.

### Cell Culture and Immunostaining

In 8 patients (4 persistAF, 4 SR) mononuclear cell fraction was separated with Ficoll-Paque Plus (Amersham Pharmacia) centrifugation and then cultured at a concentration of 1×10<sup>5</sup>/mL in Iscove’s modified Dulbecco’s medium with 2% fetal bovine serum (Stem Cell Technologies) and methylcellulose (Method HGF H4434). Methods for CFU-GM assays were previously described. After 4 days in culture, cells were transferred onto slides by cytopsin centrifugation, fixed in methanol-acetone-formalin-mixture, and stained for the myocyte enhancer factor (MEF-2) (Santa Cruz), ANP (Peninsula Laboratories), and CD31 (DAKO), by use of the APAAP method (Dako En Vision System).

### Electrical Cardioversion

Electrical cardioversion was performed under sedation with intravenous midazolam (2 mg) and etomidate (0.2 mg/kg). Three hundred sixty–Joule shocks (LIFEPAK 10, Physio-Control) were applied to the patients by self-adhesive skin electrodes (Physio-Control) in an anterior-posterior position.

### Statistical Analysis

All values are expressed as mean±SD. ANOVA analyses were used to assess differences between groups and within groups. A value of P<0.05 was considered statistically significant.

### Results

#### Effect of AF and Electrical Cardioversion

Baseline CD34<sup>+</sup> levels were significantly elevated in patients with persistAF (7.0±2.3×10<sup>3</sup>/mL versus SR, 5.0±1.6×10<sup>3</sup>/mL; P<0.01). In contrast, there was no difference between paroAF and SR (3.6±1.3×10<sup>3</sup>/mL versus SR, 5.0±1.6×10<sup>3</sup>/mL; P=NS) (Figure 1A). CD34<sup>+</sup>/CD117<sup>+</sup> levels were not different (persistAF, 0.19±0.12×10<sup>3</sup>/mL; paroAF, 0.14±0.11×10<sup>3</sup>/mL; SR, 0.26±0.21×10<sup>3</sup>/mL; P=NS). Heavy sedation alone did not change CD34<sup>+</sup> or CD34<sup>+</sup>/CD117<sup>+</sup> levels (data not shown).

Fourteen of 17 patients (82%) with persistAF were successfully converted to SR [CV(+); cardioversion was unsuccessful [CV(−)] in the remaining 3 of 17 patients (18%). None of the 14 patients with CV(+) had a recurrence of AF during follow-up. Ten minutes after application of 1 DC shock (mean, 1; range, 1 to 3; total mean energy, 487±253 J), the amount of CD34<sup>+</sup>HPCs decreased in all patients (Figure 1A). Interestingly, the decrease occurred regardless of the outcome of cardioversion [CV(+), 5.5±1.7×10<sup>3</sup>/mL versus CV(−), 5.0±0.9×10<sup>3</sup>/mL; P=NS]. The decrease in CD34<sup>+</sup>HPCs was greater in 4 patients who received more than 1 shock (1 shock, 1 DC, −17±14% versus ≥2 DC, −45±24%; P<0.05). After the transient drop, CD34<sup>+</sup> levels increased to baseline within 48 hours (24 hours, 5.0±1.5×10<sup>3</sup>/mL versus 48 hours, 7.4±2.7×10<sup>3</sup>/mL; P<0.05) in all patients. After CV(+), CD34<sup>+</sup> levels decreased during follow-up (−32±23%; P<0.05), reaching levels similar to SR [CV(+), 5.6±2.2×10<sup>3</sup>/mL versus SR, 5.0±1.6×10<sup>3</sup>/mL; P=NS]. In contrast, CD34<sup>+</sup> levels remained elevated after CV(−) (n=3) (Figure 1A). Baseline HPC levels did not predict the success of cardioversion.

CD34<sup>+</sup>/CD117<sup>+</sup>HPCs increased transiently 48 hours after cardioversion (0.19±0.12×10<sup>3</sup>/mL versus 48 hours, 0.59±0.39×10<sup>3</sup>/mL; P<0.01) and declined to 0.16±0.04×10<sup>3</sup>/cells/mL during follow-up. CD34<sup>+</sup>/CD117<sup>+</sup>HPCs were not affected by the success of cardioversion.

Numbers of HPCs did not correlate with clinical variables such as age, AF duration, atrial size, left ventricular ejection fraction, or medical therapy (data not shown).

### SDF-1α, VEGF, and ANP Levels/Culture Cells

SDF-1α levels were highest during persistAF (2057.4±514.9 versus SR, 1439.5±267.4 pg/mL; P<0.01). Patients with paroAF had intermediate levels (1891.1±341.8 versus SR, 2447.2±434.2 pg/mL; P>0.05). ANP levels were highest in persistAF (150.0±22.0 pg/mL versus SR, 80.8±17.1 pg/mL; P<0.05).
1439.5±267.4 pg/mL; *P < 0.05). SDF-1α levels started to decline after CV(+) (baseline, 2057.4±514.9 versus 48 hours, 1963.7±172.3 pg/mL; *P < 0.05) but remained elevated after CV(−) (Figure 1B). VEGF levels were not significantly elevated in patients with persistAF (27.9±30.0 versus SR, 15.0±5 pg/mL; *P < 0.05). Similar to the rise in CD34+/CD117+ HPCs, there was a trend toward increased VEGF levels 48 hours after cardioversion (baseline, 27.9±30.0 versus 48 hours, 61.8±52.0 pg/mL; *P = 0.07). VEGF levels returned to baseline (27.9±30.0 versus 15.2±1.2 pg/mL; *P = NS) during follow-up. The outcome of cardioversion did not affect VEGF levels significantly.

ANP levels were higher during persistAF compared with paroxAF (2385.1±1497 versus 1200.8±450.9 fmol/mL; *P < 0.05). ParoxAF was not different compared with SR (1200.8±450.9 versus SR, 883.6±134.6 fmol/L; *P = NS). ANP levels declined after CV(+) (2385.1±1497 versus 48 hours, 1468.2±508.2 fmol/mL; *P < 0.05) (Figure 1C). Especially in paroxAF, ANP levels correlated with SDF-1α (r = 0.56; *P < 0.01) and CD34+ (r = 0.76; *P < 0.01).

Amounts of IL-6 and IL-10 were not elevated during persistAF (IL-6 10 pg/mL; IL-10 <5 pg/mL; n=5). Cardioversion did not alter IL-6 and IL-10 levels. Cell cultures from patients with AF showed more MEF-2–positive cells (85%; absolute 1.3%) and ANP-positive cells (53%; absolute 2.3%) compared with SR (0.7% and 1.5%) (Figure 2). There was no difference in the number of CD31 (platelet endothelial cell–adhesion molecule-1 [PECAM1])–positive cells (AF: absolute 44% versus 42%; n=8). PECAM-1 serves as a marker for endothelial differentiation.
To the best of our knowledge, this is the first study demonstrating increased CD34⁺ HPCs in the blood of patients with persistAF. Interestingly, application of DC shocks temporarily reduces the amount of CD34⁺ HPCs and increases CD34⁺/CD117⁺ HPCs regardless of the underlying cardiac rhythm. In contrast, CD34⁺ levels were comparable in patients with paroxAF and SR.

Recent reports suggest that blood levels of CD34⁺ HPCs and progenitor cells increase in response to cardiac injury. 1,2 Chemokines such as SDF-1α and VEGF modulate the homing of HPCs in the bone marrow, and overexpression of these factors results in mobilization of hematopoietic cells in vivo, whereas no such relation was found for IL-3 and IL-6. 1,8

AF triggers the release of ANP into the systemic circulation and causes degenerative atrial changes. 4 In the present study, successful restoration of SR caused SDF-1α and ANP levels to return to normal, which was followed by a decrease in HPCs. ANP levels were related to SDF-1α and CD34⁺ levels. Interestingly, after 4 days of culture, more HPCs from patients with AF expressed ANP (Δ53%) and MEF-2 (Δ85%). Pathophysiologically, these findings imply that AF affects transdifferentiation of HPCs into cardiomyocytes, and thereby it may contribute to myocardial (atrial) tissue repair. Importantly, large amounts of ANP stored as amyloid are found in fibrillating atria. 4 It is tempting to speculate that ANP contributes to increased amounts of MEF-2–positive cells in atrial tissue. This is supported by a recent study, which suggests a role of the oxytocin-ANP system in cardiomyogenesis. 9 Further studies are needed, however, to prove ANP to be a homing factor for HPCs. Besides potential beneficial effects of HPC differentiation, recent findings suggest that progenitor cell–derived cardiomyocytes may have proarrhythmic effects. Thus, CD34⁺ HPCs or specific subsets thereof may also contribute to AF or focal arrhythmias. 3

In contrast to myocardial ischemia, CD34⁺/CD117⁺ cells are not increased during AF, and absolute cell counts are low, implying that they are of minor importance in this setting. 1

In the present study, application of DC shocks caused a transient decrease of CD34⁺ HPCs. It is tempting to speculate that described vascular effects of DC energy increase the adhesion/invasion of circulating HPCs to vascular endothelium, which can also explain the energy-dependent, rapid decline of circulating HPCs after cardioversion. In addition to these rapid alterations, endothelial damage is known to induce a slower humoral response resulting in increased VEGF levels. 10 One previous study has reported increased VEGF levels in some patients with AF, suggesting that AF per se may affect VEGF. 11 In our study, increased VEGF levels were related to the amount of CD34⁺/CD117⁺ HPCs, explaining their increase 48 hours after cardioversion. However, endothelial differentiation of HPCs was not induced by AF.

In conclusion, AF and application of DC shocks have significant effects on blood HPCs. Further important studies are warranted to investigate the regulation of chemokine receptors such as CXCR4 during AF.

Acknowledgments
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References
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