Transcoronary Transplantation of Functionally Competent BMCs Is Associated With a Decrease in Natriuretic Peptide Serum Levels and Improved Survival of Patients With Chronic Postinfarction Heart Failure

Results of the TOPCARE-CHD Registry

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Abstract—Although intracoronary administration of bone marrow–derived mononuclear progenitor cells (BMCs) may be associated with improved cardiac function in patients with chronic postinfarction heart failure, the impact on prognosis and clinical outcome of these patients is unknown. To identify potential predictors for a favorable clinical outcome, we assessed natriuretic peptide serum levels as objective markers of heart failure and the occurrence of cardiac death in relation to functional capacity of the infused cells in a consecutive series of 121 patients with chronic ischemic heart disease treated with intracoronary infusion of BMCs. Our analyses show that both N-terminal pro–brain natriuretic peptide (NT-proBNP) and N-terminal pro–atrial natriuretic peptide (NT-proANP) serum levels were significantly reduced in patients with established postinfarction heart failure 3 months after transcoronary progenitor cell administration. NT-proBNP serum levels greater than or equal to median (735 pg/mL) at baseline and a high number of infused progenitor cells with colony-forming capacity were the only independent predictors of a favorable response 3 months after intracoronary administration of BMCs. During extended clinical follow-up (577±442 days), a total of 14 deaths occurred in the overall patient population. Kaplan–Meier curves for both all cause and cardiac mortality showed that patients receiving a higher number of colony-forming cells were significantly less likely to die than those patients receiving low numbers of colony-forming cells (P=0.01). Most importantly, infusion of a high number of cells with colony-forming capacity was associated with a complete abrogation of increased mortality in patients with elevated NT-proBNP serum levels (≥735 pg/mL; median) at baseline (P<0.001). Taken together, our results show that patients with objective evidence of postinfarction heart failure demonstrate a significant reduction of both NT-proBNP and NT-proANP serum levels within 3 months following intracoronary infusion of BMCs. Importantly, infusion of progenitor cells with a high functional capacity is associated with a significantly lower mortality during further follow-up. (Circ Res. 2007;100:1234-1241.)

Key Words: progenitors cells • ischemic cardiomyopathy • NT-proBNP

Chronic postinfarction heart failure remains a major challenge.1 Therapeutic strategies in patients with chronic postinfarction heart failure aim at restricting fluid retention, inhibition of activated neurohumoral systems, and resynchronization of cardiac contraction.2 However, despite the use of full conventional treatment, including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-blockers, aldosterone inhibitors, and diuretics, morbidity and mortality of patients with postinfarction heart failure remain high.2-4

Previous experimental studies suggested that the administration of bone marrow–derived progenitor cells (BMCs) may contribute to functional regeneration of infarcted myocardium and, hence, may beneficially modulate postinfarction remodeling processes.5 Moreover, a recent placebo-controlled clinical multicenter trial suggested that the enhanced contractile recovery following intracoronary administration of BMCs may be associated with reduced cardiovascular events in patients with acute myocardial infarction (MI).6,7 However, it is unknown whether such a treatment strategy may also be associated with improvements in cardiac function in patients with chronic heart failure resulting from healed MI with established scar formation.
Although a randomized controlled clinical pilot trial suggested a moderate short-term improvement in left ventricular (LV) ejection fraction, no data are available on objective markers of heart failure in patients with persistent LV dysfunction ≥3 months after acute MI treated with infusion of BMCs into the infarct-related artery. Most importantly, no study so far has addressed a potential interaction between functionality of the transplanted cells and long-term clinical outcome.

Therefore, it was the aim of the present study to investigate whether transcoronary transplantation of BMCs beneficially modulates serum levels of natriuretic peptides, which are well established independent indicators of LV remodeling and survival in patients with chronic postinfarction heart failure. Moreover, the present study was designed to determine a potential interaction between the functionality of the infused BMCs and clinical outcome.

**Patients and Methods**

**Patients**

Between January 2002 and April 2006, a total of 121 consecutive patients who experienced MI ≥3 months previously were treated with intracoronary infusion of BMCs at a single center. In the current analysis, patients were pooled from the TOPCARE-CHD pilot and crossover trial and from an ongoing registry having the same inclusion criteria as the randomized trial. Briefly, patients between 18 and 85 years of age with ischemic heart disease were eligible for inclusion in the study, if they had had a documented MI at least 3 months before inclusion and a residual well-demarcated region of LV systolic dysfunction. There were no requirements for specific signs or symptoms of heart failure at inclusion, because we purposely aimed to include a broad range of LV systolic dysfunction to identify potential predictors of a beneficial effect of intracoronary BMC infusion on cardiac function. Patients had to be on stable (fixed) postinfarction pharmacological therapy for at least 3 months before inclusion in the study. Exclusion criteria were the presence of acutely decompensated heart failure with New York Heart Association status IV, and a history of hematological or malignant diseases.

The ethics review board of the Johann Wolfgang Goethe University of Frankfurt, Germany, approved the protocols. The randomized TOPCARE-CHD crossover trial was registered according to the University of Frankfurt, Germany, approved the protocols. The randomized TOPCARE-CHD crossover trial was registered according to the

**Study Design**

To evaluate potential beneficial effects of intracoronary infusion of BMCs on cardiac function in patients with healed MI, after completion of the randomized trial, a prospective registry database was initiated. There was no randomized control group, given that no power calculations could be performed to predict a positive outcome because of the lack of data for assessing the effect of BMC administration in patients with healed MI.

The primary end point assessed within the registry was the change in serum natriuretic peptide levels as an objective marker of cardiac functional capacity, measured at baseline and at follow-up 3 months after intracoronary cell infusion. In addition, potential predictors of a beneficial effect of intracoronary BMC administration on N-terminal pro–brain natriuretic peptide (NT-proBNP) serum levels were assessed by post hoc analyses.

**Preparation of Progenitor Cells and Cell Infusion Procedure**

Bone marrow aspirate (50 mL) was obtained under local anesthesia in the morning of the cell transplantation day. BMCs were isolated by Ficoll density gradient centrifugation as previously reported. A mean of 214±98×10⁶ BMCs were infused.

The functional capacity of the infused BMCs was determined by measuring their colony-forming unit (CFU) capacity, as described previously. The number of infused colony-forming cells was calculated by multiplying the CFU capacity with the number of infused cells (mean 0.66±0.46×10⁶, median 0.54×10⁶ colony-forming cells).

For cell administration, after puncture of the femoral artery, 58% of the patients received 7500 to 10 000 U of heparin and a bolus of abciximab (0.25 mg/kg). Using a standard over-the-wire balloon catheter advanced into the infarct-related coronary artery via a guiding catheter, cells were infused into the vessel supplying the most dyskinetic LV area using the stop-flow technique as described.

**Clinical Follow-Up and Definition of Events**

Clinical data, medication, and safety laboratory data were prospectively collected by study nurses. Twenty-four hours after progenitor cell infusion, troponin T and creatine kinase serum levels were measured to assess potential myocardial injury associated with the progenitor cell infusion catheterization procedure. Potential bleeding complications at the bone marrow puncture site were documented.

Follow-up visits were scheduled after 3 months and were performed by physicians. Further follow-up was performed every 12 months up to 5 years after intracoronary cell therapy. In case of unwillingness to undergo a follow-up visit in our outpatient clinic, patients and their primary physicians were contacted via telephone, E-mail, or fax. The primary clinical end point was death. Death was categorized as cardiac (attributable to MI, heart failure, sudden death, documented arrhythmia, or other cardiac-related problems eg, pericardial rupture, tamponade) and noncardiac.

**Measurement of Natriuretic Peptides**

Blood for serum analysis was collected from every patient before bone marrow aspiration at the day of cell therapy as well as at 3 months follow-up. As the active natriuretic peptides are rapidly cleaved with a half-life of 3 to 4 minutes, we determined the serum levels of N-terminal pro–atrial natriuretic peptide (NT-proANP[1–98]) and NT-proBNP using a 1-step enzyme-immunoassay based on electrochemiluminescence technology (Elecys 2010, Roche Diagnostics). Reproducibility and precision of this assay is well below 5%, even at high concentrations of NT-proBNP.

**LV Angiography**

LV angiograms were obtained at the baseline procedure and at 3 months follow-up. Quantitative analysis of paired LV angiograms (LV ejection fraction and calculation of stroke volumes) was performed as described previously.

**Statistical Analysis**

Continuous variables are presented as mean±SD, if not stated otherwise. Categorical variables were compared with the χ² test or Fisher’s exact test. Statistical comparisons between initial and follow-up data were performed in a nonparametric paired fashion using the Wilcoxon signed rank test. Nonparametric Mann–Whitney U and Kruskal–Wallis tests were used to compare continuous with categorical variables, as well as to compare the results between different groups. For survival analysis, a multivariable Cox proportional hazards regression model was used, taking into account the influence of other potential prognostic factors (in particular age, systolic blood pressure, diabetes, creatinine, functional class [New York Heart Association], mitral regurgitation, LV ejection fraction, and baseline serum levels of NT-proBNP and NT-proANP after logarithmic transformation). Survival curves were compared by the use of a 2-sided log-rank test. Kaplan–Meier curves were generated by stratifying according to the median value of the CFU capacity of the infused progenitor cells. The multivariable analysis was per-
formed using a stepwise linear regression model with a forward entry-stepping algorithm with the entry criteria probability of $F(0.05)$. Statistical significance was assumed if was $P\leq 0.05$. All reported probability values are 2-sided. Statistical analysis was performed using SPSS (version 14.0).

## Results

### Patient Characteristics and Risk Predictors

The patient characteristics are summarized in Tables 1 and 2. The mean time since the previous MI was 7 years (ranging from 4 months to 39 years). At inclusion into the study, NT-proBNP serum levels ranged from 42 to 55 456 pg/mL and NT-proANP from 177 to 36 620 fmol/mL. By univariate Cox regression analysis, NT-proBNP, NT-proANP, and creatinine serum levels, as well as severity of mitral regurgitation, age, LV ejection fraction, and functional class (New York Heart Association) at baseline were shown to have a statistically significant effect on mortality (Table 3). A step-wise multivariable Cox proportional hazards regression technique revealed that only elevated NT-proBNP levels ($P\leq 0.001$) and creatinine serum levels ($P\leq 0.001$) at baseline remained as significant independent predictors for death (Table 3).

### Procedure-Related Complications During Intracoronary Progenitor Cell Administration

There were no bleeding complications associated with bone marrow aspiration, and no major complications occurred during intracoronary administration of progenitor cells. Serum troponin T levels remained below the detection threshold

### TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>62±10</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Female sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114±23</td>
<td>68</td>
<td>11</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA class (1/2/3/4)</td>
<td>16/50/54/1</td>
<td>13/41/45/1</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>1.17±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>76</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>27</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Current or former smoking</td>
<td>89</td>
<td></td>
<td>74</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>99</td>
<td></td>
<td>82</td>
</tr>
<tr>
<td>Family history of coronary artery disease</td>
<td>65</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent of coronary artery disease</td>
<td>29/44/48</td>
<td>24/36/40</td>
<td></td>
</tr>
<tr>
<td>1-, 2-, 3-vessel disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery bypass grafting</td>
<td>41</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior/inferior/lateral</td>
<td>74/30/3</td>
<td>61/25/3</td>
<td></td>
</tr>
<tr>
<td>Anterior and inferior</td>
<td>13</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Age of last myocardial infarction, yr (median; range)</td>
<td>84±89 (60; 4–468)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target vessel (LAD/LCX/RCA)</td>
<td>86/12/23</td>
<td>71/10/19</td>
<td></td>
</tr>
<tr>
<td>Concomitant PCI</td>
<td>29</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Target vessel</td>
<td>22</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Nontarget vessel</td>
<td>10</td>
<td></td>
<td>8</td>
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<tr>
<td>Pacemaker/ICD, n</td>
<td>39</td>
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<td>32</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>13.4±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral regurgitation (low/moderate/severe/very severe)</td>
<td>72/35/12/2</td>
<td>60/29/10/2</td>
<td></td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending artery; LCX, left circumflex artery; RCA right coronary artery; PCI, percutaneous coronary intervention; ICD, implantable cardioverter defibrillator; LVEDP, LV end-diastolic pressure.

### TABLE 2. Medication Use

<table>
<thead>
<tr>
<th>Medication</th>
<th>Baseline</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Antiplatelet therapy (aspirin or clopidogrel or both)</td>
<td>117</td>
<td>97</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>110</td>
<td>93</td>
</tr>
<tr>
<td>ACE inhibitor/ATRB</td>
<td>115</td>
<td>98</td>
</tr>
<tr>
<td>Spironolactone/eplerenone</td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td>Diuretics</td>
<td>106</td>
<td>90</td>
</tr>
<tr>
<td>Warfarin (Coumadin)</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>Statin</td>
<td>107</td>
<td>93</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; ARTB, angiotensin receptor blocker.
TABLE 3. Impact of Baseline Characteristics on Mortality

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>0.006</td>
<td>0.15</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.49</td>
<td>0.30</td>
</tr>
<tr>
<td>Baseline creatinine serum level</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>NYHA class</td>
<td>0.004</td>
<td>0.28</td>
</tr>
<tr>
<td>Severity of mitral regurgitation</td>
<td>0.005</td>
<td>0.33</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>0.012</td>
<td>0.68</td>
</tr>
<tr>
<td>Baseline NT-proBNP (log)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Baseline NT-proANP (log)</td>
<td>&lt;0.001</td>
<td>0.41</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association.

of 0.01 mg/dL in 88 of 121 patients 24 hours after the procedure. In 14 patients before and in 25 patients after intracoronary progenitor cell infusion, troponin T was slightly elevated. In 5 patients, troponin T levels increased to >0.1 mg/dL, but none of these patients experienced a significant increase of creatine kinase levels of at least 2 times above the upper normal limit or underwent significant ECG changes.

Effects of Transcoronary Progenitor Cell Administration on Natriuretic Peptide Serum Levels and LV Function

Patients not undergoing follow-up in our clinic (n=8), as well as patients with missing natriuretic peptide values as a result of lipemic sera (n=2), were not entered into the exploratory natriuretic peptide serum level analysis. In addition, patients with hemodynamically significant coronary stenoses at the time of follow-up (n=2), atrial flutter or primary diagnosis of atrial fibrillation (n=6), MI or death during 3 months follow-up (n=3), initiation of hemodialysis (n=1), diagnosis of carcinoma (n=1), termination of medical therapy (n=1), or subarachnoidal hemorrhage (n=1) were excluded, leaving a total of 96 patients (79%) for exploratory analysis.

Overall, in the entire study population, NT-proANP serum levels were significantly reduced from 6190±6407 fmol/mL to 4392±3770 fmol/mL (P<0.001) and NT-proBNP levels declined from 1444±2603 pg/mL to 1186±1380 pg/mL (P=0.24) 3 months after transcoronary progenitor cell administration.

In the 86 patients, in whom quantitative analyses of paired LV angiograms were available, LV ejection fraction significantly (P<0.001) increased from 39.9±11.4% to 41.7±11.9% and LV stroke volume was significantly (P<0.05) augmented from 29.5 mL/m² to 31.2 mL/m² 3 months after intracoronary BMC administration.

Importantly, analysis of only patients with a NT-proBNP serum levels of >450 pg/mL at baseline, which is the previously established threshold level for the definition of heart failure, demonstrated that NT-proBNP serum levels were significantly reduced from 2137±3063 pg/mL to 1688±1509 pg/mL (P=0.03; n=61) and NT-proANP serum levels were reduced from 8337±6995 fmol/mL to 5661±3604 fmol/mL (P=0.001; n=60) 3 months after intracoronary administration of BMCs (Figure 1). The most pronounced reduction in NT-proBNP serum levels was observed in patients in whom the increase in LV ejection fraction was paralleled by a decrease in end-diastolic volume (NT-proBNP from 2218±2998 pg/mL to 1341±1166 pg/mL, P=0.05, n=16), reflecting the close association of changes in NT-proBNP serum levels with parameters of LV remodeling. Thus, in patients with objective measures of postinfarction heart failure, as evidenced by elevated NT-proBNP serum levels, intracoronary administration of BMCs is associated with significant reductions in natriuretic peptide serum levels within 3 months.

Predictors of a Decrease in Natriuretic Peptide Levels Following Intracoronary Progenitor Cell Administration

To identify potential predictors for a decrease in natriuretic peptide serum levels following intracoronary progenitor cell administration, patients were defined as “natriuretic peptide responders” if their baseline NT-proBNP or NT-proANP serum levels were reduced by >10% (>2 times reproducibility value of the measurement assay) at 3 months follow-up. With respect to NT-proBNP, baseline creatinine levels, age of the previous MI, percutaneous coronary intervention procedures performed at baseline, and concomitant medical therapy were not significantly different between “BNP responders” (n=38) and “BNP nonresponders” (n=58). “BNP responders” showed trends toward a higher age (P=0.07), more severe mitral regurgitation (P=0.08), and better pres-
Table 4 Univariate Predictors of NT-proBNP Responses

<table>
<thead>
<tr>
<th></th>
<th>BNP Nonresponder (N=58)</th>
<th>BNP Responder (N=38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr (mean±SD; median)</td>
<td>59±11; 61</td>
<td>63±11; 65</td>
<td>0.07</td>
</tr>
<tr>
<td>Age of previous MI, mo (mean±SD; median)</td>
<td>78±17; 60</td>
<td>85±102; 43</td>
<td>0.72</td>
</tr>
<tr>
<td>Baseline creatinine serum level, mg/dL (mean±SD; median)</td>
<td>1.10±0.4; 1.1</td>
<td>1.14±0.3; 1.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Severity of mitral regurgitation (mean±SD; median)</td>
<td>1.3±0.5; 1.0</td>
<td>1.6±0.7; 1.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Baseline ejection fraction (mean±SD; median)</td>
<td>38±11; 38</td>
<td>43±12; 44</td>
<td>0.08</td>
</tr>
<tr>
<td>Baseline NT-proBNP serum level, pg/mL (mean±SD; median)</td>
<td>876±893; 488</td>
<td>2311±3855; 817</td>
<td>0.03</td>
</tr>
<tr>
<td>CFU capacity, 10^6 (mean±SD; median)</td>
<td>0.56±0.4; 0.47</td>
<td>0.77±0.5; 0.76</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The results of the present study demonstrate that transcoronary transplantation of BMCs in patients with persistent LV dysfunction resulting from healed MI is associated with a decrease in NT-proBNP, which is inversely associated with baseline NT-proBNP serum levels. More specifically, when a decrease in NT-proBNP was used as an objective outcome parameter.

Functional Activity of Infused BMCs Interacts With Mortality During Follow-Up

The number of progenitor cells capable of forming colonies did not significantly correlate with any baseline clinical characteristic nor with individual LV functional parameters but showed a strong trend (P=0.07) towards being inversely associated with baseline NT-proBNP serum levels. Moreover, the number of infused progenitor cells capable of forming colonies did not predict changes in individual parameters of LV function, as assessed by quantitative angiography at 3 months follow-up. To determine a potential interaction between functionality of the infused BMCs and clinical outcome, Kaplan–Meier curves for all-cause mortality were constructed for the patient population divided according to the number of infused progenitor cells capable of forming colonies. There were no significant differences in baseline characteristics predicting mortality between the 2 groups of patients (supplemental Table II). However, as illustrated in Figure 2, patients receiving a higher number of colony-forming progenitor cells (greater than median) had a significantly better survival compared with patients receiving a lower number of colony-forming progenitor cells (less than or equal to median). Similar results were obtained when patients were divided into tertiles of the number of colony-forming progenitor cells received (P=0.003). Likewise, when a combined clinical end point of death and rehospitalization for heart failure was chosen, patients receiving higher numbers of colony-forming progenitor cells (greater than median) had a significantly better event-free survival (P=0.023). In addition, restricting the analysis to cardiac-related death gave similar results (P=0.047).

Because the CFU capacity showed a trend towards being associated with baseline NT-proBNP serum levels, we further stratified patients according to their baseline NT-proBNP. As illustrated in Figure 3, in patients with baseline NT-proBNP serum levels greater than median (735 pg/mL), the intracoronary infusion of a high number of colony-forming BMCs was associated with a complete abrogation of increased mortality during follow-up. Thus, the clinical benefit of intracoronary administration of functionally competent progenitor cells appears to be most significant in patients with established chronic postinfarction heart failure.

Discussion

The results of the present study demonstrate that transcoronary transplantation of BMCs in patients with persistent LV dysfunction resulting from healed MI is associated with a...
significant decrease in natriuretic peptide serum levels. The effects are most pronounced in patients with advanced stages of heart failure, as characterized by elevated NT-proBNP serum levels at baseline. Most importantly, intracoronary infusion of progenitor cells with a high functional capacity is associated with a significantly lower mortality during further follow-up.

Serum levels of natriuretic peptides are the most powerful independent markers of outcome in patients with chronic symptomatic heart failure.\textsuperscript{10,12,17,18} Moreover, serial measurements of natriuretic peptides were shown to serve as useful surrogate markers of ventricular remodeling and prognosticators for clinical risk stratification.\textsuperscript{11,19} Indeed, in the patient cohort of the present study, baseline NT-proBNP serum level was a powerful independent predictor of death, in addition to impaired renal function. Importantly, transcoronary transplantation of progenitor cells was associated with a significant and considerable reduction in natriuretic peptide serum levels specifically in those patients with elevated NT-proBNP levels at baseline. These data indicate that transcoronary BMC transplantation beneficially interferes with ventricular remodeling processes in patients at high risk for worse outcome in chronic postinfarction heart failure.

Indeed, when alterations in NT-proBNP serum levels were correlated with angiographically derived changes in LV functional parameters, the most pronounced decrease in NT-proBNP was observed in patients with an increase in ejection fraction that was paralleled by a decrease in end-diastolic volume, reflecting a favorable LV remodeling process. Given that the effects were observed in the presence of full conventional pharmacological treatment, transcoronary transplantation of progenitor cells appears to be capable of modifying the clinical course of patients with advanced stages of chronic postinfarction heart failure. Indeed, when analyzing Kaplan–Meier survival curves, it becomes evident that those patients receiving functionally competent progenitor cells (as measured by their colony-forming capacity) exhibited a significantly lower mortality during further follow-up. Taken together, these data indicate that transcoronary transplantation of progenitor cells may emerge as a potentially valuable therapeutic approach to reduce mortality in patients with advanced chronic postinfarction heart failure.

The results of the present study demonstrate, for the first time to our knowledge, an association between functionality of the infused progenitor cells and clinical outcome in patients with chronic postinfarction heart failure, suggesting a cause-and-effect relationship. The association between CFU activity of the infused cells as a measure of functionality and clinical outcome does not come as a surprise. In fact, we have shown previously in a hindlimb ischemia model that the CFU activity of BMCs derived from patients with chronic ischemic heart disease closely correlates with neovascularization capacity as a functional measure.\textsuperscript{14} The clonogenic potential of progenitor cells is not only a characteristic feature of their biological properties, but also closely correlates with cell-autonomous production of cytokines important for homing and engraftment of hematopoietic progenitor cells.

Clearly, the present clinical study could not determine the cellular mechanisms that may have contributed to the beneficial effects of administrating progenitor cells with a higher CFU capacity on LV remodeling and clinical outcome. Experimentally, it is well established that the functional activity is a major determinant of homing of patient-derived progenitor cells into ischemic tissue.\textsuperscript{20–22} Moreover, in addition to patient-related differences, cell-isolation procedures may significantly affect the functionality of progenitor cells and, thus, determine the functional effects observed in patients after intracoronary administration. Indeed, we have previously demonstrated that even slight alterations in storage temperature, the choice of buffer solution, or the use of plasma from patients themselves during cell isolation and processing profoundly impairs progenitor cell functionality, as measured by the CFU capacity, migratory capacity toward their chemoattractants, and neovascularization capacity of cells infused into a hindlimb ischemia model.\textsuperscript{23} As such, the results of the present study provide further evidence for the pivotal role of preserving functionality of the cells during cell preparation to maximize their potential beneficial effects in clinical application.

The mechanisms contributing to the heterogeneous impairment in functionality of BMCs in patients with postinfarction heart failure are currently not fully understood. We have recently shown that, in addition to risk factors for coronary artery disease,\textsuperscript{14} patients with previous MI demonstrate a
functional exhaustion of BMCs capable of forming hematopoietic colonies compared with healthy controls, as well as compared with patients within 7 days of a first acute MI.\(^{24}\) Moreover, impaired functionality including reduced CFU capacity of BMCs is a characteristic feature of mice deficient in endothelial NO synthase.\(^{20}\) Impaired bioavailability of NO is a hallmark both in patients with coronary atherosclerosis as well as in patients with chronic heart failure. Importantly, the strong trend of CFU capacity inversely correlating with serum levels of NT-proBNP as a marker of severity of postinfarction heart failure observed in the present study suggests that heart failure itself may impair functionality of BMCs. As such, we cannot fully exclude that the improved clinical outcome observed in patients receiving a high number of progenitor cells capable of forming colonies may at least in part reflect less severe heart failure. Therefore, future studies aiming at improving functionality of BMCs before administration are necessary to conclusively demonstrate that the intrinsic functionality of the administered cells determines clinical outcome. Indeed, experimental studies have shown that increasing the expression of endothelial NO synthase with a transcriptional endothelial NO synthase enhancer before readministration of BMCs derived from patients with chronic heart failure is associated with a significant reduction in infarct size and improved cardiac function in an experimental model,\(^{21}\) indicating that improving cell functionality may indeed translate into increased recovery of cardiac function.

Finally, although the patient population of the present study was the largest reported and mean follow-up exceeded 19 months, the number of deaths during follow-up was still limited. Moreover, the lack of a randomized control group may be regarded as a major limitation of our study. As such, the data derived can only generate potentially important hypotheses for designing future clinical trials. However, these limitations may be outweighed by documenting an important association between the functionality of the infused cells and improved clinical outcome. Thus, it is extremely encouraging to note that a single intracoronary administration of functionally competent BMCs appears to translate into a significantly better survival during further follow-up. Given the excellent safety profile of transcoryonary administration of progenitor cells, the data of the present study may indeed provide a solid rationale to embark on a randomized controlled outcome trial aimed at establishing the effect of this novel therapeutic approach on morbidity and mortality in patients with advanced stages of postinfarction heart failure.

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**References**


Transcoronary Transplantation of Functionally Competent BMCs Is Associated With a Decrease in Natriuretic Peptide Serum Levels and Improved Survival of Patients With Chronic Postinfarction Heart Failure: Results of the TOPCARE-CHD Registry

Birgit Assmus, Ulrich Fischer-Rasokat, Jörg Honold, Florian H. Seeger, Stephan Fichtlscherer, Torsten Tonn, Erhard Seifried, Volker Schächinger, Stefanie Dimmeler and Andreas M. Zeiher

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### Online Table 1: Univariate predictors of NT-proANP responses

<table>
<thead>
<tr>
<th></th>
<th>ANP-non-responder N=41</th>
<th>ANP-responder N=54</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean±SD; median)</td>
<td>60 ± 10; 62</td>
<td>61 ± 11; 63</td>
<td>0.58</td>
</tr>
<tr>
<td>Age of previous MI (months; mean±SD; median)</td>
<td>87 ± 85; 57</td>
<td>78 ± 90; 48</td>
<td>0.38</td>
</tr>
<tr>
<td>Baseline creatinine serum level (mg/dl; mean±SD, median)</td>
<td>1.05 ± 0.3; 1.0</td>
<td>1.18 ± 0.4; 1.1</td>
<td>0.08</td>
</tr>
<tr>
<td>Severity of mitral regurgitation (mean±SD, median)</td>
<td>1.3 ± 0.5; 1.0</td>
<td>1.5 ± 0.7; 1.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Baseline ejection fraction (mean±SD, median)</td>
<td>38 ± 11; 38</td>
<td>43 ± 12; 44</td>
<td>0.11</td>
</tr>
<tr>
<td>Baseline NT-proBNP serum level (pg/ml, mean±SD, median)</td>
<td>1196 ± 2076; 629</td>
<td>1635 ± 2966; 678</td>
<td>0.71</td>
</tr>
<tr>
<td>Baseline NT-proANP serum level (fmol/ml, mean±SD, median)</td>
<td>3814 ± 2938; 2927</td>
<td>7840 ± 7612; 5719</td>
<td>0.003</td>
</tr>
<tr>
<td>Colony forming unit capacity (mean±SD, median; * 10^6)</td>
<td>0.63 ± 0.4; 0.54</td>
<td>0.66 ± 0.5; 0.50</td>
<td>0.76</td>
</tr>
</tbody>
</table>

### Online Table 2: Baseline predictors of mortality compared between patients with high or low colony forming unit capacity

<table>
<thead>
<tr>
<th></th>
<th>Low colony forming capacity</th>
<th>High colony forming capacity</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 11</td>
<td>60 ± 11</td>
<td>0.35</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>111 ± 23</td>
<td>117 ± 24</td>
<td>0.35</td>
</tr>
<tr>
<td>Diabetes mellitus (n; %)</td>
<td>13; 23</td>
<td>11; 20</td>
<td>0.82</td>
</tr>
<tr>
<td>Baseline creatinine serum level (mg/dl)</td>
<td>1.24 ± 0.4</td>
<td>1.12 ± 0.3</td>
<td>0.17</td>
</tr>
<tr>
<td>NYHA class</td>
<td>2.5 ± 0.7</td>
<td>2.3 ± 0.7</td>
<td>0.31</td>
</tr>
<tr>
<td>Severity of mitral regurgitation</td>
<td>1.4 ± 0.9</td>
<td>1.3 ± 0.9</td>
<td>0.80</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>38.0 ± 12</td>
<td>39.6 ± 11</td>
<td>0.33</td>
</tr>
<tr>
<td>Baseline NT-proBNP (pg/ml)</td>
<td>3024 ± 7963 median 951</td>
<td>1337 ± 2063 median 695</td>
<td>0.07</td>
</tr>
<tr>
<td>Baseline NT-proANP (fmol/ml)</td>
<td>8281 ± 8922 median 4598</td>
<td>5361 ± 4127 median 5146</td>
<td>0.44</td>
</tr>
</tbody>
</table>