Minute Myocardial Injury as Measured by High-Sensitive Troponin T Serum Levels Predicts the Response to Intracoronary Infusion of Bone Marrow-Derived Mononuclear Cells in Patients With Stable Chronic Post-Infarction Heart Failure

Insights From the TOPCARE-CHD Registry

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Rationale: **Cell-based therapies are a promising option in patients with chronic postinfarction heart failure (ischemic cardiomyopathy [ICM]). However, the responses after intracoronary infusion of autologous bone marrow–derived mononuclear cells (BMCs) are heterogeneous, which may be related to impaired cell retention in patients with ICM. Ischemic injury is associated with upregulation of prototypical chemoattractant cytokines mediating retention and homing of circulating cells. The development of ultrasensitive tests to measure high-sensitive troponin T (hs-TnT) serum levels revealed the presence of ongoing minute myocardial injury even in patients with stable ICM.**

Objective: **To test the hypothesis that serum levels of hs-TnT correlate with cell retention and determine the response to intracoronary BMC application in patients with ICM.**

Methods and Results: **About 157 patients with stable ICM and no substantial impairment of kidney function received intracoronary BMC administration. Immediately prior to cell application, hs-TnT levels to measure myocardial injury and NT-proBNP levels as marker of left ventricular wall stress were determined. Patients with elevated hs-TnT were older and had more severe heart failure. Importantly, only patients with elevated baseline hs-TnT≥15.19 pg/mL (upper tertile) demonstrated a significant (P=0.04) reduction in NT-proBNP serum levels (−250 [−1465; 33] pg/mL; relative reduction −24%) 4 months after BMC administration, whereas NT-proBNP levels remained unchanged in patients in the 2 lower hs-TnT tertiles. The absolute decrease in NT-proBNP at 4 months was inversely correlated with baseline hs-TnT (r=−0.27, P=0.001). Finally, retention of intracoronarily infused, ^{111}Indium-labeled cells within the heart was closely associated with hs-TnT levels in patients with chronic ischemic heart failure (P=0.0008, n=10, triple measurements).**

Conclusions: **The extent of ongoing myocardial injury as measured by serum levels of hs-TnT predicts the reduction of NT-proBNP serum levels at 4 months after intracoronary BMC administration in patients with ICM, suggesting that the beneficial effects of BMC application on LV remodeling and wall stress are confined to patients with ongoing minute myocardial injury.**

Clinical Trial Registration: **URL: www.clinicaltrials.gov. Unique identifier: NCT00962364. (*Circ Res*. 2017;120:1938-1946. DOI: 10.1161/CIRCRESAHA.116.309938.)**

Key Words: bone marrow ■ cell therapy ■ cytokines ■ heart failure ■ troponin T

Regenerative therapies are considered as a promising novel approach to improve heart function and prevent the development of end-stage heart failure.[1] Application of various cell types, including bone marrow–, heart-, or adipose tissue–derived cell populations, was shown to improve functional cardiac recovery after acute myocardial infarction.[2] In patients with chronic heart failure, the results of the individual trials are heterogeneous, although a recent meta-analysis suggested

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Novelty and Significance

What Is Known?

- Intracoronary cell therapy with autologous bone marrow–derived cells has limited and highly variable effects in patients with chronic postinfarction heart failure.

What New Information Does This Article Contribute?

- Serum levels of high-sensitive troponin T are frequently elevated and correlated with serum levels of NT-proBNP in patients with stable chronic ischemic heart failure.
- Serum levels of high-sensitive troponin T at baseline, a possible marker for ongoing minute myocardial injury, are associated with a reduction of NT-proBNP serum levels in patients with ischemic heart failure at 4 months after cell therapy.
- Reduced NT-proBNP levels at 4 months are associated with better survival than predicted with the use of the Seattle Heart Failure Score.
- Secondary analysis of the Indium trial data shows that serum troponin T levels are associated with myocardial radioactivity after intracoronary infusion of indium labeled bone marrow–derived cells in patients with ischemic heart failure, which could be indicative of improved retention of infused cells in patients with ongoing myocardial injury.

This retrospective analysis suggests that ischemic heart failure patients with ongoing minor myocardial damage might have a greater benefit from intracoronary cell therapy compared with patients with no ongoing myocardial damage.

Nonstandard Abbrevations and Acronyms

BMC	bone marrow–derived mononuclear cells
hs-TnT	high-sensitive troponin T
LV	left ventricular
NT-proBNP	N-terminal probrain natriuretic peptide
SDF-1	stromal cell–derived factor-1
SHFM	Seattle Heart Failure Model

an improved clinical outcome in the cell-treated patients.[3,4] The variable response to cell therapy in chronic heart failure may be attributed to the varying extent of cell retention in the heart in the absence of acute myocardial infarction.[5]

Bone marrow–derived mononuclear cells (BMCs) are attracted to the target tissue by chemoattractant cytokines, most notably by stromal cell–derived factor 1 (SDF-1). SDF-1 is transiently upregulated by tissue hypoxia and injury.[6] Thus, SDF-1 is profoundly increased after acute myocardial infarction,[7] but significantly reduced in chronic postischemic models. As a consequence, retention and homing of circulating repair cells are considerably lower in chronic postinfarction heart failure.[8]

Circulating levels of cardiac troponin T are the gold standard for assessing myocardial injury in patients with suspected acute myocardial infarction. However, the development of novel ultrasensitive assays to measure circulating high-sensitive troponin (hs-TnT) levels revealed the increased levels of circulating troponin also in patients with chronic postinfarction heart failure.[9] It is suggested that measurable levels of circulating hs-TnT reflect chronic sources of myocardial injury and increased wall stress with subclinical hypoxia even in the absence of flow-limiting stenosis in patients with chronic heart failure.[10,11]

Therefore, in this study, we wanted to test the hypothesis whether serum levels of hs-TnT determine the response to intracoronary application of BMCs in patients with chronic postinfarction heart failure.

Methods

Patients

Patients were eligible for inclusion into the present analysis, if they participated into an ongoing single-center registry to assess the effects of intracoronary administration of BMC for the treatment of chronic postischemic heart failure due to an at least 3-month-old myocardial infarction between 2003 and 2011. For inclusion into the present post hoc analysis, patients were required to have no significant impairment of renal function, defined as a baseline creatinine ≤1.5 mg/dL, and not to have non–ST-segment–elevation myocardial infarction as assessed by the absence of angina, lack of dynamic ECG changes and/or hs-TnT levels <100 pg/mL. General exclusion criteria for intracoronary cell therapy within the registry were the presence of acutely decompensated heart failure with New York Heart Association class IV, an acute ischemic event within 3 months prior to inclusion into the registry, a history of other severe chronic diseases, documented cancer within the preceding 5 years, or unwillingness to participate. A total 157 patients fulfilled these requirements.

The ethics review board of the Goethe University in Frankfurt, Germany, approved the protocol; the study is registered with www.clinicaltrials.gov (NCT00962364), and all patients gave written informed consent to participate in this registry. The study complies with the Declaration of Helsinki.

Preparation and Administration of BMCs

Bone marrow aspirate (50 mL) was obtained from the iliac crest under local anesthesia in the morning of the cell administration day. BMC were then isolated by Ficoll density-gradient centrifugation, as previously reported.[12,13] A mean of $175\pm104\times10^6$ cells were available for the intracoronary infusion procedure after processing. For cell administration, arterial puncture was followed by the administration of 5000 to 7500 U of heparin. BMC were infused into the coronary arterial vessel supplying the most dyskinetic left ventricular area by means of the stop-flow technique with an over-the-wire balloon catheter. In detail, the vessel was occluded for 3 minutes after intracoronary BMC application, followed by 3 minutes reflow. This cycle was repeated 3 times, thus resulting in a total of 9±1 minutes of vessel occlusion.

Measurement of Natriuretic Peptides and hs-TnT

To assess baseline hs-TnT and NT-proBNP serum levels, blood was collected for analysis from every patient before bone marrow aspiration at the day of cell therapy, as well as at 4 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 NT-proBNP using a 1-step enzyme-immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics). Reproducibility and precision of this assay is well below 5% even at high concentrations of NT-proBNP.[14] Hs-TnT was measured with the STNTHS ECLIA (Roche).

Calculation of the Seattle Heart Failure Model Score

The Seattle Heart Failure Model (SHFM is a validated risk prediction model based on routinely collected clinical variables, including age, sex,

pathogenesis of cardiomyopathy (ischemic origin), heart rate, systolic blood pressure, ejection fraction, medication (angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, aldosterone blocker, β-blocker, statins, as well as diuretic type and daily dose, and allopurinol) and laboratory values (serum sodium, total cholesterol, hemoglobin, percent lymphocytes, and uric acid).[15] In addition, the presence of any implantable device (pacemaker, implantable cardioverter-defibrillator, and cardiac resynchronization therapy) is included into the calculation of both, the SHFM Score and the SHFM-predicted mortality. However, NT-proBNP values are not included into the model.

Indium Pilot Study

To assess the extent and potential determinants of proangiogenic progenitor cell homing into the damaged myocardium after intracoronary cell infusion, the ^{111}Indium pilot trial was conducted between 2005 and 2006, and results were published in *Circulation*.[5] Briefly, 20 post–myocardial infarction patients were recruited into the trial, ^{111}In-oxine–labeled progenitor cells were infused using the stop-flow technique, and subsequent daily FDG-PET (^{18}F-fluorodeoxyglucose–positron emission tomography) imaging was performed. For the present subgroup analysis, only patients with a successful infarction revascularization procedure at least 14 days before Indium-labeled cell application were included into the analysis (median time from AMI 778 [interquartile range 43:4849] days). For details about further patient characteristics, cell isolation, cell labeling, and FDG-PET imaging procedures, we refer to the previous publication.[5] Frozen serum samples (stored at −80°C) from patients receiving Indium-labeled cells were used to measure hs-TnT and NT-proBNP serum levels for the present analyses.

Statistical Analysis

If not stated otherwise, data are shown as median and interquartile range. Differences between groups were assessed by nonparametric tests (Wilcoxon Mann–Whitney *U* test and Jonckheere Terpstra test) and correlations are assessed as Spearman correlation. The χ^2 test was used to compare categorical variables. Association with binary end points was analyzed with multivariable logistic regression. Statistical comparisons between initial and follow-up data were performed using Wilcoxon paired sample test.

The calculation of estimated survival by the SHFM score is based on a parametric (exponential) survival model.[15] A likelihood ratio test is used to compare SHFM predicted and observed mortality as described previously.[16]

The association between hsTnT levels and the dynamics of Indium activity in the Indium pilot study was evaluated with a linear mixed effect regression analysis checking residuals for deviations from the Gaussian distribution with a Shapiro–Wilk test.

In general, statistical significance was assumed if $P<0.05$ and all reported *P* values are 2-sided. Statistical analysis was performed with SPSS (Version 23.0, SPSS Inc.) and R (Version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient Characteristics

The patient baseline characteristics are summarized in Table 1. Myocardial infarction had occurred at a mean of 7 years (median 58 months) before inclusion into the study. Baseline median left ventricular ejection fraction was 39% and medication was according to the current guidelines at inclusion into the study (Table 2). At baseline, NT-proBNP serum levels ranged from 30 to 20512 pg/mL. Likewise, baseline hs-TnT demonstrated a broad distribution in these stable and, with respect to clinical signs of myocardial ischemia, asymptomatic patients (Figure 1). Of note, only 31 patients (19.7%) had hs-TnT levels of ≤3 pg/mL, which is the lower detection limit of the test. Thus, more than 80% of the chronic postinfarction heart failure patients had detectable hs-TnT serum levels. Importantly, hs-TnT levels did not differ between patients undergoing concomitant percutaneous coronary intervention at the time of cell administration and those who did not (Figure 1).

Heart Failure and hs-TnT Levels

For further analyses, patients were divided into 3 groups according to the tertiles of hs-TnT serum levels. As shown in Figure 2 and Table 1, patients with higher hs-TnT levels did experience more severe heart failure, as evidenced by higher New York Heart Association class, higher SHFM score, and lower baseline left ventricular ejection fraction. This association was further confirmed by the fact that baseline NT-proBNP serum levels did closely correlate with hs-TnT (r=0.57, $P<0.001$). Likewise, elevated hs-TnT was also associated with impaired colony-forming unit (CFU) capacity of the isolated BMCs. Of note, total cell number and the number of hematopoietic subpopulations were not significantly different between the groups of hs-TnT tertiles (Table 3). By multivariable analysis including all univariate predictors, only NT-proBNP ($P<0.001$), patient age (P=0.01), and time from the last myocardial infarction (P=0.02) remained significant independent predictors of elevated hs-TnT serum levels.

Response to BMC Therapy

At 4 months follow-up (mean 120, median 119 days), patient–individual pairwise comparison of NT-proBNP values revealed that only patients within the highest hs-TnT tertile demonstrated a profound and significant reduction of NT-proBNP (Figure 3A). Thus, in the lowest hs-TnT tertile, 27 patients demonstrated a reduction and 26 patients demonstrated an increase in NT-proBNP serum levels at follow-up, compared with 38 patients with reduced and 14 patients with increased NT-proBNP serum levels in the highest hs-TnT tertile. In detail, the median absolute changes in NT-proBNP levels were −12 pg/mL [−52; 57] in the lowest hs-TnT tertile, −38 pg/mL [−266; 217] in the intermediate tertile and −250 pg/mL [−1465; 33] in the highest hs-TnTtertile (P for trend=0.004; Figure 3B). In addition, application of the guideline definition for abnormal hs-TnT (14 pg/mL), which triggers suspicion of myocardial necrosis in the emergency room setting, revealed a 22% relative reduction in NT-proBNP serum levels in response to BMC therapy in patients with hs-TnT> 99th percentile, whereas patients below this threshold demonstrated a nonsignificant relative 1% reduction. Taken together, elevated levels of circulating troponin T as a marker of minute myocardial injury at baseline correlate with responses to intracoronary BMC administration at 4 months as measured by changes in NT-proBNP serum levels as a marker of myocardial wall stress.

Assessment of mortality in our retrospective patient cohort included 153 patients (4 patients were lost-to follow-up at 3 years), and a total of 16 patients died during this period. We compared the observed mortality with the mortality predicted by the Seattle Heart Failure Model. As shown in Figure 4A, observed mortality was significantly better compared with estimated mortality in the NT-proBNP responder group (P=0.0023), whereas in the NT-proBNP nonresponder group (Figure 4B), observed and estimated mortality did not show a significant difference (P=0.30), which further corroborates our findings.

Table 1. Patient Baseline Characteristics and Distribution of Heart Failure Characteristics Within the High-Sensitive Troponin T Tertiles

	Median [IQR]/n (%)	High-Sensitive Troponin T Levels (Tertiles)			*P* Value
		≤6.48; n=53	>6.48 and ≤15.19; n=52	>15.19; n=52	
Age, y	63 [55 to 70]	56 [45 to 63]	63 [56 to 68]	70 [61 to 74]	<0.001
Sex (male)	136 (87)	44/9	48/4	44/8	0.329
Heart rate, bpm	68 [61 to 77]	66 [60 to 72]	66 [60 to 76]	73 [66 to 83]	0.005
Systolic blood pressure, mm Hg	116 [100 to 132]	119 [100 to 130]	112 [104 to 131]	113 [100 to 138]	0.81
NYHA class (1/2/3/4)	28 (18)	2 [2 to 2]	2 [2 to 2]	3 [2 to 3]	0.002
	68 (43)				
	60 (38)				
	1(1)				
Hypertension	113 (72)	34	36	43	0.104
Hypercholesterolemia	127 (81)	43	41	43	0.637
Diabetes mellitus type 2	39 (25)	6	14	19	0.010
Current or former smoking	107 (68)	45	33	29	0.010
Positive family history of CAD	83 (53)	31	28	24	0.82
Pacemaker/implantable cardioverter defibrillator	18 (12)/30 (19)	4/5	8/7	6/18	0.452/0.002
Seattle Heart Failure Score	0.15 [–0.21 to 0.67]	0.01 [–0.29 to 0.22]	0.13 [–0.20 to 0.69]	0.49 [–0.02 to 0.96]	<0.001
Recent anterior infarction	80 (51)				
Time since last infarction, mo	58 [14 to 144]	54 [15 to 108]	72 [8 to 147]	91 [13 to 185]	0.27
Mitral regurgitation (grade)	1 [0.5 to 1.5]	1 [0 to 1]	1 [1 to 2]	1 [1 to 2]	0.010
Extent of CAD (1/2/3 vessel disease)	43 (27)	17 (32)	15 (29)	11 (21)	0.044
	48 (31)	20 (38)	16 (30)	12 (23)	
	66 (42)	16 (30)	21 (41)	29 (56)	
Concomitant PCI during cell therapy	45 (29)	13 (25)	16 (31)	16 (31)	0.716
LVEF, %	39 [30 to 48]	45 [37 to 49]	38 [28 to 46]	33 [23 to 44]	<0.001
LVEDP, mm Hg	14 [9 to 20]	11 [8 to 18]	14 [8 to 22]	17 [12 to 22]	0.005
Creatinine, mg/dL	1.05 [0.90 to 1.18]	1.0 [0.8 to 1.1]	1.1 [0.9 to 1.2]	1.1 [1.0 to 1.3]	<0.001
NT-proBNP, pg/mL	629 [303 to 1623]	300 [171 to 506]	732 [408 to 1371]	1611 [673 to 4251]	<0.001
High-sensitive Troponin T, pg/mL	10.2 [4.4 to 18.8]	3.0 [3.0 to 4.5]	10 [8 to 12]	25 [19 to 35]	<0.001

CAD indicates coronary artery disease; IQR, interquartile range; LVEF, left ventricular ejection fraction; LVEDP, left ventricular end-diastolic pressure; NT-proBNP, N-terminal probrain natriuretic peptide; NYHA, New York heart Failure Association Class; and PCI, percutaneous coronary intervention.

In addition, restricting the survival analysis to patients with NT-proBNP >1000 pg/mL (n=58), even this small cohort demonstrated a strong trend toward improved survival in patients with an NT-proBNP reduction at 4 months (Online Figure I).

Insights From the Indium Pilot Study

Finally, to gain some mechanistic insights, we investigated the correlation between hs-TnT and homing of applied cells, labeled with ^{111}Indium, in patients of our previously published Indium pilot trial.[5] As illustrated in Figure 5A, there was a positive and significant correlation between hs-TnT levels and ^{111}Indium activity in the heart reflecting retention of the ^{111}Indium-labeled cells after intracoronary cell application when assessed with a linear mixed effect regression (n=10, P<0.001). Of course, the strong decay of ^{111}Indium-activity of the labeled cells after 24 and 72 hours was significant, too, when compared with the quantifications after 1 hour (P=0.002 at 24 hours, P<0.001 after 72 hours). Nevertheless, in the same patient cohort, baseline hs-TnT serum levels were slightly, but not significantly correlated with the absolute reduction in NT-proBNP levels at 4 months (Figure 5B). Although the number of patients is low, these results strengthen the hypothesis that minute myocardial

Table 2. Patient Medication

Medication	Percentage
Antiplatelets	96
Angiotensin-converting enzyme inhibitor/Angiotensin-1 receptor blocker	99
β-blocker	93
Mineralocorticoid antagonist	47
Diuretics	85
Statin	91
Digitalis	37

injury, as evidenced by circulating hs-TnT, increases acute retention and homing of cells and, therefore, might facilitate improvement of overall cardiac function after intracoronary cell administration.

Discussion

This study demonstrates that elevated levels of circulating hs-TnT as indicator of minute myocardial injury in chronic postinfarction heart failure identify patients who respond to intracoronary application of BMC with a reduction in NT-proBNP levels at 4 months follow-up. Moreover, novel analyses from our previous study using Indium labeling of the cells disclosed a significant correlation between hs-TnT serum levels und acute retention of the cells in the heart after intracoronary administration.

In our cohort, detectable levels of serum hs-TnT were present in 80% of patients with stable postinfarction heart failure, which is comparable to the ValHeFT trial (Valsartan Heart Failure Trial), where 92% of patients had a detectable troponin T when using a high-sensitive assay.[9] Several mechanisms were shown to contribute to cardiac troponin T release in chronic stable heart failure, among them subendocardial ischemia leading to cardiomyocyte necrosis, damage by inflammatory cytokines and oxidative stress, and ongoing cardiomyocyte apoptosis.[17–20] Moreover, injured but viable cardiomyocytes with increased permeability of the plasma membrane were shown to experience troponin T leakage from the cytosolic pool of cardiac troponin.[21,22] Finally, it was suggested that the presence of microvascular dysfunction per se and diastolic load both contribute to cardiac troponin release in patients with nonischemic heart failure.[11] Microvascular dysfunction impairs myocardial perfusion and induces regional metabolic changes similar to recurrent or persistent ischemia.[23] Thus, it is tempting to speculate that the release of cardiac troponin is paralleled by increased tissue expression of SDF-1, which is profoundly upregulated during hypoxia and inflammatory tissue activation.[7] Unfortunately, to our knowledge, there are no data correlating tissue expression of SDF-1 with the release of cardiac troponin T in a chronic heart failure model. However, SDF-1 is the major chemoattractant-mediating recruitment of circulating bone marrow–derived cells into hypoxic or inflamed tissue.[6,8] Indeed, our studies using ^{111}Indium-labeled cell administration demonstrated a significant correlation between serum troponin T levels and acute retention of cells after intracoronary administration in patients with old myocardial infarction. Thus, it seems to be reasonable to postulate that increased levels of hs-TnT may be indicative of increased cell retention in the heart after intracoronary administration via so far unspecified mechanisms, which increase the stimulus for cell retention. Since time from the successful acute infarction reperfusion procedure was at least 14 days, we do not believe that microvascular obstruction just trapped the cells.

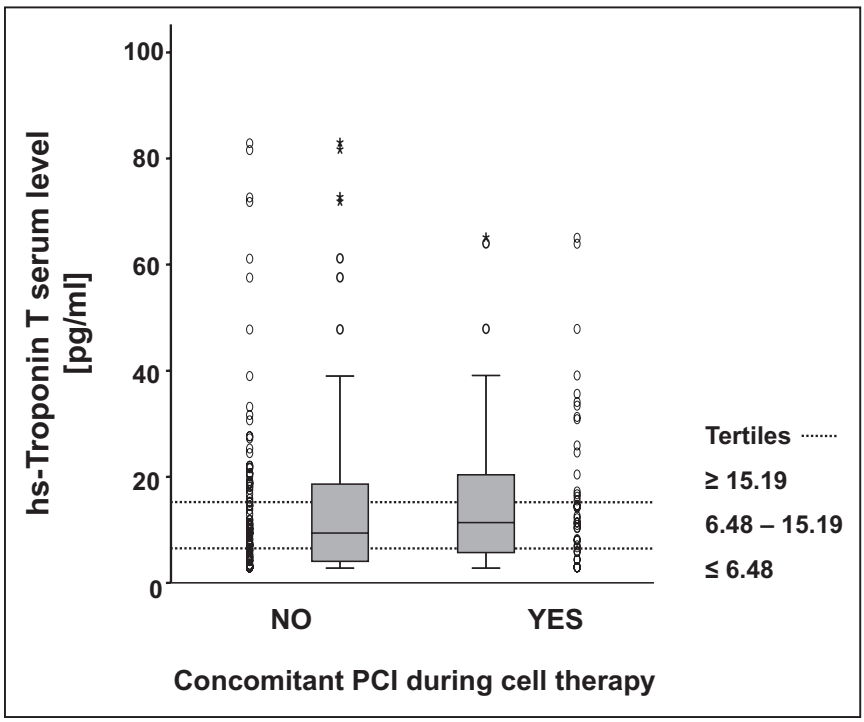

Figure 1. Distribution of high-sensitive troponin T serum values in the patient cohort. Boxplots indicate 25th and 75th percentile with the median value. Horizontal dotted lines indicate cut-off values for high-sensitive troponin T tertiles. PCI indicates percutaneous coronary intervention.

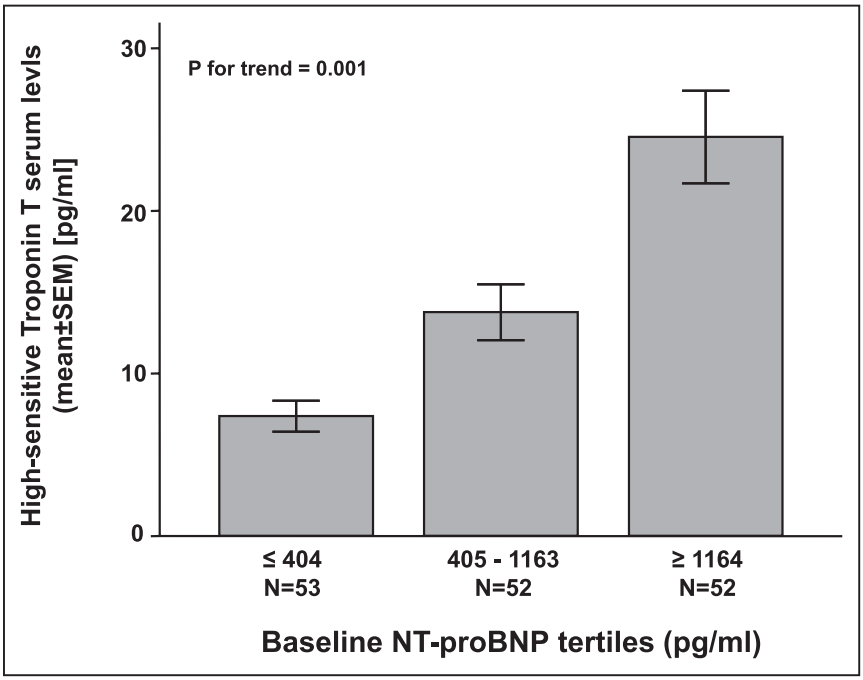

Figure 2. Baseline tertiles of N-terminal probrain natriuretic peptide (NT-proBNP) serum levels vs high-sensitive troponin T serum levels.

Importantly, both experimental and clinical studies documented that the extent of acute retention of administered cells determines functional improvement of the heart after cell administration.[24,25] This is corroborated by our data showing that acute cell retention slightly correlates with reductions in NT-proBNP levels 4 months after cell administration.

Finally, one other study investigated the role of cardiac troponin in the response to cell therapy, albeit in a different patient cohort and using intramyocardial injection of cells. In the ACT34-CMI trial (A Double-Blind, Prospective, Randomized, Placebo-Controlled Study to Determine the Tolerability, Efficacy, Safety, and Dose Range of Intramyocardial Injections of G-CSF Mobilized Auto-CD34$^+$ Cells for Reduction of Angina Episodes in Patients With Refractory Chronic Myocardial Ischemia),[26] patients with chronic refractory angina received intramyocardial injection of CD34$^+$ enriched autologous cells. Interestingly, in patients receiving cell therapy, periprocedural troponin elevation was associated with a significant longer time to major adverse cardiovascular endpoints (MACE) compared with patients without troponin elevation, whereas control patients with troponin elevation, but no cell therapy, had a significantly shorter time to MACE. Although this may point into a similar direction as our results, it may also be that simply better intramyocardial cell injections produced greater myocardial trauma and, thus, troponin elevations, which reduced MACE during further follow-up.

Finally, in this study, we do see a reduction of NT-proBNP serum levels despite significantly reduced CFU capacity as a marker for overall cell function in patients with elevated compared with low hs-TnT. At first glance, this observation seems to be contradictory to our previous report.[16] However, the CFU capacity of patients with low hs-TnT in the present cohort is significantly better (median 34) than the CFU capacity of patients from the larger registry cohort (median 20/23). A critical lower threshold for CFU capacity in autologous intracoronary bone marrow–derived cell therapy has not yet been established. This will be prospectively evaluated in the international randomized, currently recruiting REPEAT trial (Repetitive Progenitor Cell Therapy in Advanced Chronic Heart Failure Trial; NCT01693042).

However, there are some considerable limitations of the present analyses. The current analysis is a post hoc analysis of an ongoing registry and a pilot study, and, thus, our results should only be regarded as hypothesis generating. Prospective evaluation of baseline hs-TnT serum levels as a predictor of clinical response to intracoronary cell therapy in chronic heart failure is planned within the currently

Table 3. Cell Characteristics According to High-Sensitive Troponin T Tertiles

	High-Sensitive Troponin T Levels in Tertiles			*P* Value
	≤6.48, n=53	>6.48 and ≤15.19, n=52	>15.19, n=52	
No. of infused mononuclear cells (×10^6)	167 (114 to 241)	128 (85 to 200)	140 (87 to 237)	0.14
Colony-forming units	34 (19 to 43)	18 (11 to 32)±13	21 (13 to 32)	0.038
CD34$^+$ CD45$^+$ cells (% total MNC; n=65; assay 1)	0.55 (0.32 to 0.68) (n=32)	0.37 (0.18 to 0.59) (n=18)	0.50 (0.32 to 0.67) (n=17)	0.29
CD34$^+$ CD45$^+$ cells (% total MNC, (n=92; assay 2)	1.54 (0.73 to 2.38) (n=21)	1.04 (0.71 to 2.11) (n=36)	1.59 (0.80 to 2.81) (n=35)	0.78
SDF-1–induced migration	106 (70 to 173)	101 (63 to 143)	102 (70 to 159)	0.86

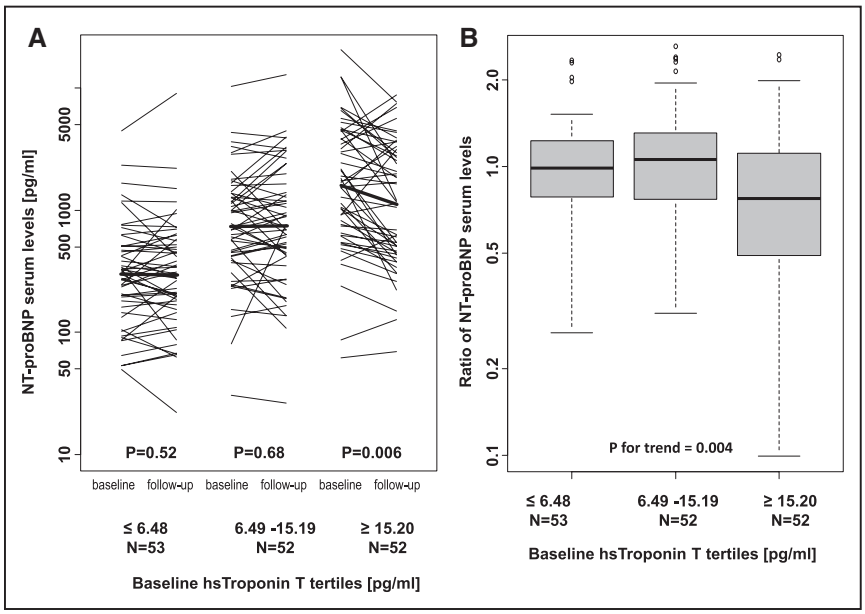

Figure 3. A, N-terminal probrain natriuretic peptide (NT-proBNP) serum levels at baseline and 4 months of follow-up according to baseline high-sensitive troponin T tertiles. **B**, Ratio of follow-up to baseline NT-proBNP serum levels within the high-sensitive troponin T tertiles. Boxplots depict 25th and 75th percentile with the median value.

recruiting REPEAT trial (NCT01693042). In addition, the efficacy of BMC therapy was assessed by serial measurements of NT-proBNP, which can only serve as a surrogate end point. However, it has been shown that increases in BNP serum levels at follow-up, compared with baseline values, are associated with an increased risk of adverse cardiovascular events, whereas a reduction corresponds to a reduced adverse event risk in patients with heart failure.[27] Moreover, the NT-proBNP analysis of the COPERNICUS trial (Carvedilol Prospective Randomized Cumulative Survival Trial) has demonstrated that substantial reductions of NT-proBNP serum levels were present only in the Carvedilol group, but not in the placebo group, and were associated with reduced mortality.[28,29] However, there is still some debate about intraindividual variations for NT-proBNP and BNP serum levels with repeated measurements in patients with stable chronic heart failure.[30–32] In addition, 20% of patients were in functional class 2, and almost 30% of patients had NT-proBNP serum levels close to normal values, which makes it even more difficult to show substantial reductions, and which might explain why the ratio of baseline and follow-up NT-proBNP serum levels is close to normal in a substantial number of patients. However, restricting the survival analysis to patients with substantially elevated NT-proBNP at baseline, this cohort demonstrates a survival benefit when the ratio was below 1 (Online Figure I), although there was no statistically significant difference in absolute mortality between NT-proBNP responders and nonresponders in the

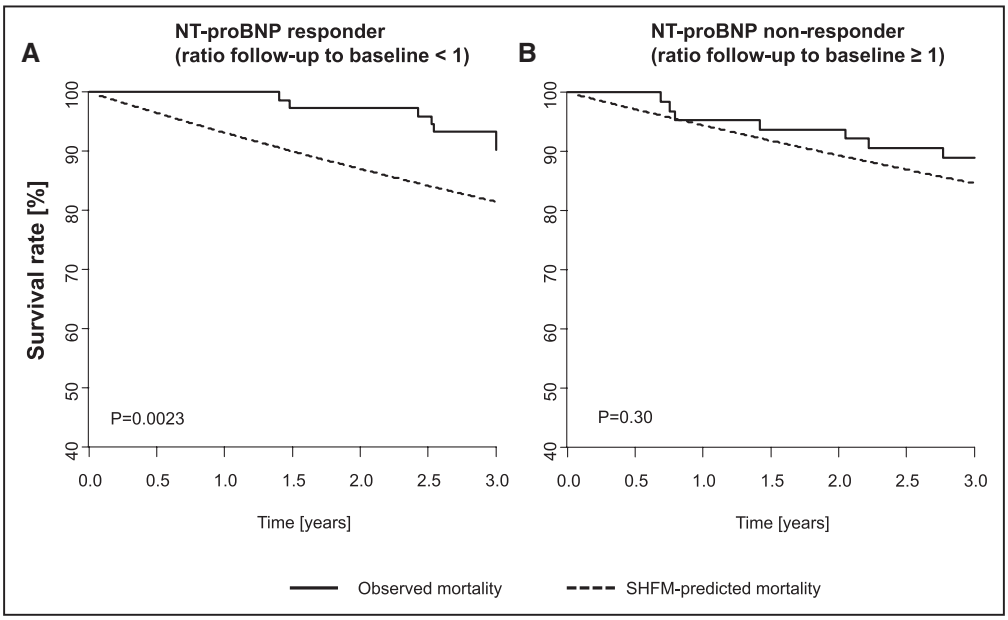

Figure 4. Parametric survival regression analysis and comparison between Seattle Heart Failure Model (SHFM)–predicted and observed mortality in the N-terminal probrain natriuretic peptide (NT-proBNP) responder (**A**) and NT-proBNP nonresponder group (**B**), defined by a ratio of follow-up to baseline NT-proBNP <1 or ≥1.

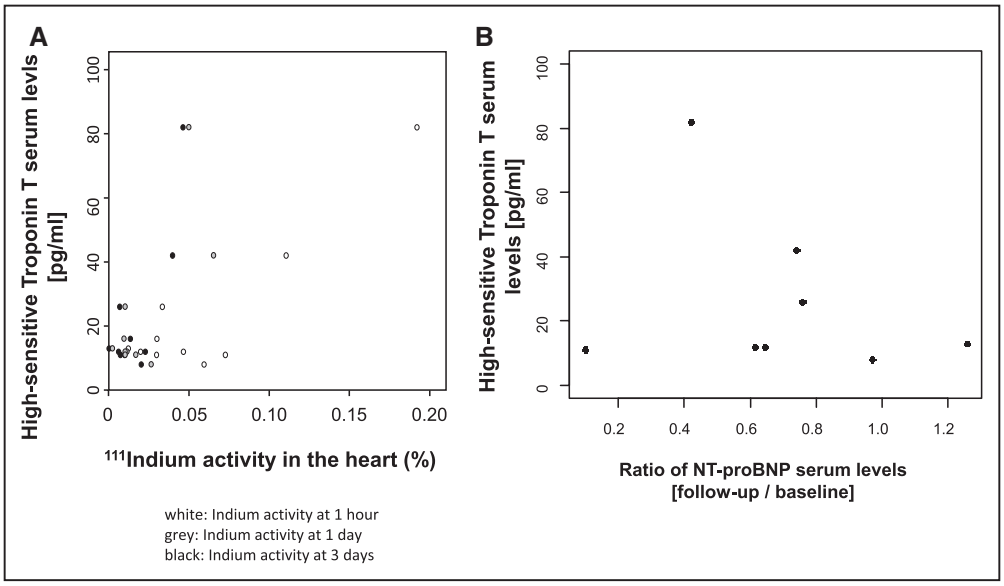

Figure 5. A, Scatterplot for the association between high-sensitive troponin T serum levels and ^{111}Indium activity within the heart after intracoronary infusion of labeled cells in patients with chronic postinfarction heart failure from the Indium pilot trial. All measurements of Indium activity are illustrated and the time of the quantification is indicated by symbol color. Linear mixed effect model showed a significant association (P=0.0008). **B**, Scatterplot for the association between high-sensitive troponin T serum levels and ratio of follow-up to baseline N-terminal probrain natriuretic peptide (NT-proBNP) serum levels in chronic postinfarction heart failure patients of the Indium pilot trial. The association was not significant.

entire, rather low-risk patient cohort. This observation might be mainly because of the limited number of patients included into the analysis, which by itself may not allow for the detection of significant differences in total number of deaths between the 2 groups. Therefore, we purposely compared observed mortality rates to mortality rates calculated by the SHFM score and demonstrated significant differences between responders and nonresponders.

Conclusions

Taken together, our data show that the extent of ongoing minute myocardial damage as measured by serum levels of circulating hs-TnT at the time of cell administration is correlated with the reduction of NT-proBNP serum levels at 4 months after intracoronary application of BMC in patients with stable chronic postinfarction heart failure. These data suggest that beneficial effects of BMC administration on left ventricular remodeling and wall stress are confined to patients with ongoing minute myocardial injury in stable chronic postinfarction heart failure, which will be prospectively evaluated in future trials, and may then serve as a tool to select patients for future cell therapy trials.

Perspectives

Competency in Medical Knowledge

Cell-based therapies are a promising option in patients with chronic postinfarction heart failure. However, the responses after intracoronary infusion of autologous BMCs are heterogeneous, which may be related to impaired cell retention in patients with chronic heart failure. Ischemic injury is associated with upregulation of prototypical chemoattractant cytokines mediating retention and homing of circulating cells. Using ultrasensitive tests to measure troponin T serum levels (hs-TnT), it was recently shown, that, even in patients with stable chronic postinfarction heart failure, there is ongoing minute myocardial injury.

Translational Outlook 1

Only patients with elevated levels of hs-TnT at baseline before BMC application demonstrated a significant reduction of NT-proBNP serum levels as a marker of reduced left ventricular wall stress at 4 months after cell therapy.

Translational Outlook 2

The correlation between baseline levels of hs-TnT and increased retention of labeled cells, which is associated with a reduction in NT-proBNP serum levels at 4 months after cell therapy, suggests that ongoing minute myocardial injury may be a predictor of a favorable response to BMC treatment. Further prospective evaluation of this effect is warranted.

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Supplemental figure I