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Enhanced mobilization of the bone marrow–derived circulating progenitor cells by intracoronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction

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Abstract

Autologous bone marrow cell transplantation (BMCs-Tx) is a promising novel option for treatment of cardiovascular disease. We analysed in a randomized controlled study the influence of the intracoronary autologous freshly isolated BMCs-Tx on the mobilization of bone marrow–derived circulating progenitor cells (BM-CPCs) in patients with acute myocardial infarction (AMI). Sixty-two patients with AMI were randomized to either freshly isolated BMCs-Tx or to a control group without cell therapy. Peripheral blood (PB) concentrations of $CD34/45^+$- and $CD133/45^+$-circulating progenitor cells were measured by flow cytometry in 42 AMI patients with cell therapy as well as in 20 AMI patients without cell therapy as a control group on days 1, 3, 5, 7, 8 and 3, 6 as well as 12 months after AMI. Global ejection fraction (EF) and the size of infarct area were determined by left ventriculography. We observed in patients with freshly isolated BMCs-Tx at 3 and 12 months follow up a significant reduction of infarct size and increase of global EF as well as infarct wall movement velocity. The mobilization of $CD34/45^+$ and $CD133/45^+$ BM-CPCs significantly increased with a peak on day 7 as compared to baseline after AMI in both groups ($CD34/45^+$: $P < 0.001$, $CD133/45^+$: $P < 0.001$). Moreover, this significant mobilization of BM-CPCs existed 3, 6 and 12 months after cell therapy compared to day 1 after AMI. In control group, there were no significant differences of $CD34/45^+$ and $CD133/45^+$ BM-CPCs mobilization between day 1 and 3, 6 and 12 months after AMI. Intracoronary transplantation of autologous freshly isolated BMCs by use of point of care system in patients with AMI may enhance and prolong the mobilization of $CD34/45^+$ and $CD133/45^+$ BM-CPCs in PB and this might increase the regenerative potency after AMI.

Keywords: $CD34^+$ • $CD133^+$ • acute myocardial infarction • freshly isolated bone marrow cell transplantation • infarct size • ejection fraction

Introduction

Progenitor cells derived from bone marrow (BM) circulate in the PB and have been implicated in neoangiogenesis after tissue ischaemia has occurred [1–3]. These bone marrow–derived circulating progenitor cells (BM-CPCs) express unique surface markers, such as $CD34^+$ and the early haematopoietic cell marker $CD133^+$($AC133^+$) [4]. In addition, BM-CPCs are capable of proliferating and differentiating into endothelial cells and are therefore ideal candidates for vascular regeneration [5]. In animal models, bone marrow–derived stem/progenitor cell infusion improves cardiac function and neovascularization after myocardial infarction [6, 7]. In addition, recent clinical studies provide further evidence for a promising improvement of cardiac function after intracoronary infusion of BM-stem/progenitor

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cells in patients with AMI [8–14]. However, the role of BM-CPCs after cell therapy is less clear. It is unknown whether the mobilization of progenitor cells relates to regeneration of infarcted heart muscle after tissue ischaemia. In this prospective randomized control trial, we analysed the influence of intracoronary freshly isolated BMCs-Tx by use of point of care system on cardiac function and their relation with the mobilization of BM-CPCs in patients following AMI.

Materials and methods

Patient characteristics

In a prospective randomized controlled trial, 62 patients between 18–80 years of age were eligible for inclusion in this study if they had had an acute ST-elevation myocardial infarction on the electrocardiogram. Exclusion criteria were the presence of acutely decompensated heart failure (HF) with a New York Heart Association (NYHA) class of IV, infectious or inflammatory disease, active bleeding, surgery or trauma within 2 months, renal or liver dysfunction, thrombocytopenia, or anaemia, a severe comorbidity and alcohol or drug dependency, a history of other severe chronic diseases or cancer, or unwillingness to participate. The study conforms with the principles outlined in the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from each patient. All AMI patients were discharged with standard medication consisting of acetylsalicylic acid and clopidogrel, an angiotensin-converting enzyme inhibitor or AT-II blocker, a β-blocker and a statin.

Study protocol

In this study, 62 patients with AMI who met inclusion criteria were randomly allocated by cardiologist in a 2:1 ratio to either receive freshly isolated BMCs-Tx by use of point of care system, or to a control group with no stem cell therapy after successful coronary revascularization. All AMI patients were treated with heparin, a GIIb/IIIa antagonist, acetylsalicylic acid, and they underwent coronary angiography as well as left ventriculography. Coronary revascularization of infarct-related artery was initiated by balloon angioplasty with subsequent stent implantation. The 42 patients of the intervention group underwent freshly isolated BMCs-Tx by use of point of care system on day 7 after AMI, whereas 20 patients of the second group served a control group who received only coronary angiography and left ventriculography without any cell-based therapy. The primary end point of the study was the change in global EF as well as the size of infarcted area as measured by left ventriculography at baseline and after three as well as 12 months. Secondary end points were the mobilization of BM-CPCs on days 1, 3, 5, immediately pre and post on day 7, 8 and 3, 6 as well as 12 months after procedure. Functional status was assessed by NYHA classification as well as brain natriuretic peptide (BNP) level in PB in both groups (Fig. 1). All data were obtained by blinded expert readers unaware of patient group assignment.

Coronary angiography and left ventriculography

All patients in both groups underwent left heart catheterization, left ventriculography and coronary angiography. Cardiac function and infarct size were determined by left ventriculography. Cardiac function was evaluated by global EF and by auxotonic myocardial contractility index, evaluated by the wall movement velocity of the infarcted area. Global EF was measured with Quantcor software (Siemens, Erlangen/Germany). To quantify the size of infarct area we used the centreline method according to Sheehan [15] by plotting five axes perpendicular to the long axis of the heart in the main akinetic or dyskinetic segment of ventricular wall. Systolic and diastolic lengths were then measured by two independent observers, and the mean difference was divided by systolic duration in seconds. The follow-up was 3 and 12 months after the treatment. All haemodynamic investigations were obtained by two independent observers. All data were obtained by blinded expert readers unaware of patient group assignment.

Preparation and administrations of bone marrow cells (BMC)

Seven days after AMI, a total of 120 ml bone marrow was taken from the iliac crest after local anaesthesia and mononuclear cells were isolated freshly by use of point of care system (with using of Harvest Technologies GmbH, Munich, Germany) and identified including $CD34^+$ and $CD133^+$. The cell suspension consisted of a heterogeneous cell population including haematopoietic, mesenchymal and other progenitor cells.

After undergoing arterial puncture, all patients received 7500 to 10,000 Units of heparin. Cell transplantation was performed *via* the intracoronary administration route [16] using four to six fractional infusions of 3–5 ml of cell suspension. All cells were infused directly into the infarcted zone through the infarct-related artery *via* an angioplasty balloon catheter, which was inflated at a low pressure (4 atm) and was located within the previously stented coronary segments. This prevented back flow of cells and produced stop flow beyond the site of balloon inflation to facilitate high-pressure infiltration of cells into the infarcted zone with prolonged contact time for cellular migration. Three and 12 months after catheter-guided cell transplantation, all functional tests were repeated, including coronary angiography and left ventriculography. There were no procedural or cell-induced complications and there were no side effects in any patients.

Mobilization of $CD34/45^{\pm}$ and $CD133/45^{\pm}$ BM-CPCs

BM-CPCs were collected in PB for $CD34/45^+$ and $CD133/45^+$ in both groups and quantified by flow cytometry (EPICS-XL, Beckmann Coulter). Assessments in patients with BMCs transplantation ($n = 42$) were performed on days 1, 3, 5, immediately pre and post on day 7, 8 as well as 3, 6 and 12 months after intracoronary cell transplantation. Also for the control group without BMCs-Tx ($n = 20$), measurements of $CD34/45^+$ and $CD133/45^+$ were performed on days 1, 3, 5, 7, 8 as well as 3, 6 and 12 months after AMI. PB samples were analysed within 2 hrs.

Samples were stained with fluorescein isothiacyanate (FITC) conjugate of a $CD45^+$ antibody (clone J33; Coulter Immunotech, Marseille, France) that detects all isoforms and glycoforms of the CD45 family, phycoerythrin (PE) conjugate of a $CD34^+$ antibody (clone 581; Coulter Immunotech) that detects a class III epitope on all glycoforms of the $CD34^+$ antigen and PE conjugate of a $CD133/1^+$ (Miltenyi Biotec, Bergisch Gladbach, Germany).

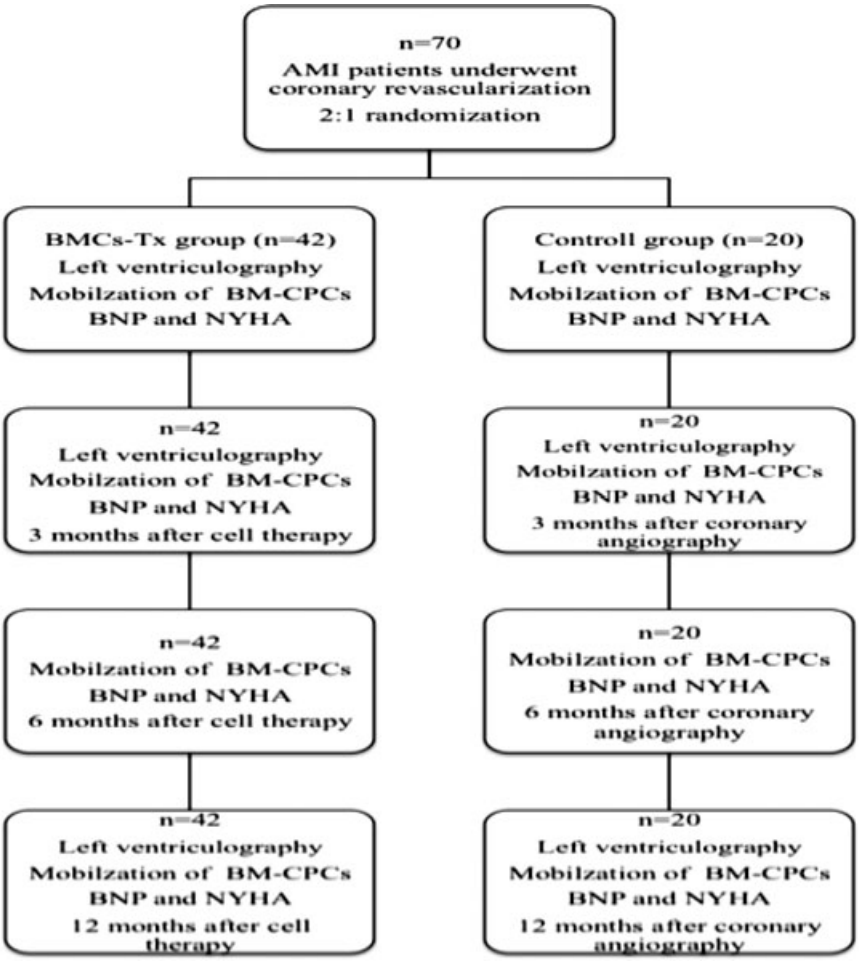

Fig. 1 Enrolment and follow-up analysis of trial.

Control samples were stained with $CD45^+$ FITC and an IgG1 PE (Coulter Immunotech) isotype.

Four each patient ethylenediaminetetraacetic acid (EDTA) blood samples were labelled with $CD34/45^+$, $CD133/45^+$ and IgG1/CD45. All tubes were incubated at room temperature in the dark. After incubation, cells were lysed with ammonium chloride, washed with phosphate-buffered saline (PBS). Samples were then stored on ice at 4°C in the dark for 20 min. and analysed by flow cytometry [17].

Samples were subjected to a two-dimensional side scatter-fluorescence dot plot analysis. After appropriate gating, the concentration of BM-CPCs with low cytoplasmic granularity (low side ward scatter) was quantified and expressed as concentration of cells per million white blood cells.

Safety parameters

To assess any inflammatory response and myocardial reaction after cell therapy, white blood cell count, the serum levels of C-reactive protein (CRP) and of cardiac enzyme (CK, CKMB, troponin) were determined immediately before as well as after treatment. Additional analysis was performed directly after transplantation and 3, 6 and 12 months later: BNP level in PB, ECG at rest, 24-hr Holter ECG and echocardiography.

Statistical analysis

Quantitative data are presented with mean ± S.D. and qualitative data are tabulated using absolute frequencies and/or percentages. Differences between therapy groups for qualitative variables are tested using Fisher's exact test due to small number of patients in therapy groups. Within differences of quantitative variables in each therapy, group are compared using the Wilcoxon test for depending samples, and differences between therapy groups of quantitative variables are compared with the Wilcoxon test for independent samples. Both of those nonparametric Wilcoxon tests are preferred due to the more likely expected non-normal distribution of the data. For all statistical tests, a result will be seen as statistically significant, if the corresponding two-sided *P*-value is smaller or equal to 0.05. Statistical analysis was performed with SPSS for Windows (Version 15.0).

Results

Baseline characteristics of the patients

We randomized 62 patients with AMI (2:1) after acute coronary revascularization in the study. Of these, 42 patients in first group received freshly isolated BMCs-Tx into the infarct-related coronary artery, whereas 20 patients in the second group received no intracoronary BMCs transplantation. There were no significant differences between the baseline characteristics and demographics of patients in both groups (Table 1).

Table 1 Baseline clinical characteristics of the study population

	AMI with BMCs-Tx ($n = 42$)	AMI without BMCs-Tx ($n = 20$)	*P*
Age	61 ± 15	60 ± 11	NS
Male	28	14	NS
Cardiovascular risk factors (%)			
Hypertension	65	70	NS
Hyperlipidaemia	65	65	NS
Smoking	90	90	NS
Diabetes	25	20	NS
Positive family history of CAD	20	20	NS
No. of diseased vessels (1/2/3)	30/12/0	14/6/0	NS
Infarct-related vessel (LAD/LCX/RCA)	26/4/12	12/2/6	NS
PTCA/stent at the time of AMI	42/42	20/20	NS
Medication at discharge (%)			
Aspirin	100	100	NS
Clopidogrel	100	100	NS
ACE inhibitor or AT II blocker	100	100	NS
β-Blocker	100	100	NS
Aldosterone antagonist	25	25	NS
Statin	100	100	NS
Laboratory parameters			
CK U/l	1980 ± 815	1974 ± 800	NS

AMI: acute myocardial infarction; BMCs-Tx: bone marrow cell transplantation; PTCA: percutaneous transluminal coronary angioplasty; CK: creatine kinase; LAD: left anterior descending coronary artery; LCX: left circumflex artery; RCA: right coronary artery; NS: none significant.

Effect of BMCs transplantation

Left ventricular function, infarct size and infarct wall movement velocity

Global EF, left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), stroke volume index (SVI), infarct size and the wall movement velocity of the infarcted area were measured by left ventriculography in the first group at baseline and 3 as well as 12 months after freshly isolated BMCs-Tx as well as in the second group without BMCs-Tx at baseline and 3 as well as 12 months after acute coronary revascularization. There were no significant baseline differences in global EF, infarct size and infarct wall movement velocity between the two groups (Fig. 2A–C). Three and twelve months after cell therapy, we observed a significant increase of global EF and infarct wall movement velocity as compared to baseline, whereas there was no significant difference in control group. Furthermore, there was a significant decrease of infarct size after 3 as well as 12 months. Moreover, we found a significant increase of SVI and decrease of LVESV, whereas no significant change was observed in LVEDV 3 and 12 months after cell therapy (Table 2). In the control group, there were no significant changes in global EF, LVEDV, LVESV, SVI, infarct size and the wall movement velocity of the infarcted area 3 and 12 months after coronary angiography (Table 3). Moreover, we observed that the global EF and the wall movement velocity of the infracted area ($P < 0.001$) significantly increased 3 and 12 months after cell therapy compared to control group. Infarct size significantly decreased 3 and 12 months after freshly isolated BMCs-Tx as compared to control group without cell therapy (Fig. 2A–C).

Functional status and clinical safety parameters

To determine the functional status, we assessed NYHA classification in both groups by two independent and blinded physicians. We observed a significant improvement in NYHA classification 3, 6 and 12 months after intracoronary freshly isolated BMCs-Tx, whereas there was no significant difference in control group 3, 6 and 12 months after coronary angiography. Furthermore, we found a significant decrease of BNP level in PB 3, 6 and 12 months after freshly isolated BMCs-Tx with no significant difference observed in control group 3, 6 and 12 months after coronary angiography (Table 2 and 3). There were no significant differences of baseline NYHA classification and of BNP levels between both groups. The NYHA classification and BNP levels significantly decreased 3, 6 and 12 months after cell therapy compared to the control group (Fig. 3A and B).

ECG at rest, on exercise and 24-hr Holter ECG revealed no rhythm disturbances at any time point. There was no inflammatory

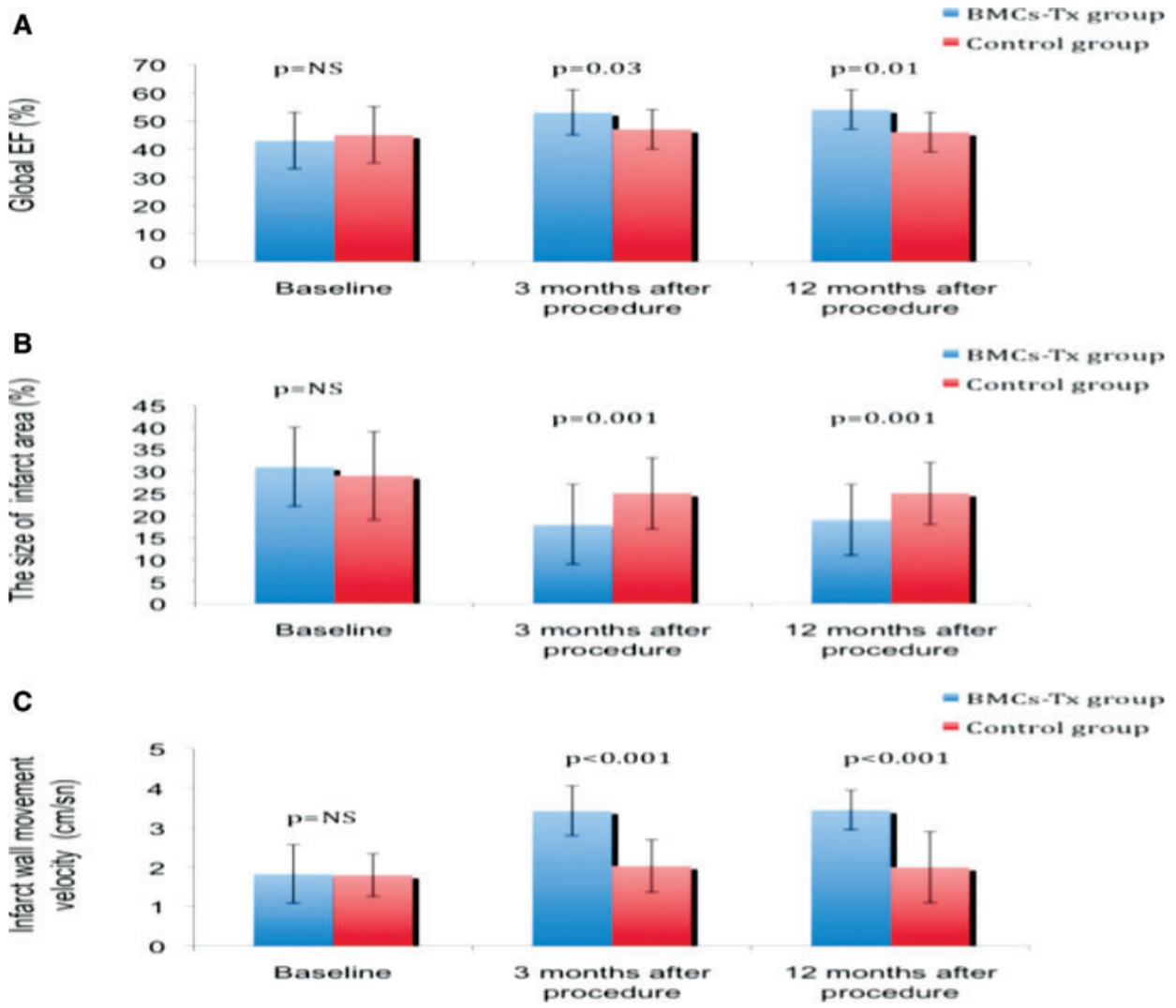

Fig. 2 Global EF, infarct size and the wall movement velocity of the infarcted area were measured by left ventriculography baseline, 3 and 12 months after procedure in both groups. There were no significant baseline differences in global EF, infarct size and in infarct wall movement velocity between the two groups. Global EF and infarct wall movement velocity significantly increased 3 and 12 months after cell therapy compared to control group. Furthermore, there was a significant decrease of infarct size 3 and 12 months after cell transplantation compared to control group without cell therapy. Moreover, no significant changes were observed in the control group at follow-up.

response or myocardial reaction (white blood cell count, CRP, cardiac enzyme) after cell therapy.

The mobilization of BM-CPCs

The mobilizations of BM-CPCs were analysed in both groups on days 1, 3, 5, 7 (in addition, immediately pre- and post-procedure in cell therapy group), 8 and 3, 6 as well as 12 months after AMI. Both mobilizations of BM-CPCs ($CD34^+$ and $CD133^+$) significantly increased with a peak on day 7 as compared to baseline after AMI in both groups. In contrast to BMCs-Tx group, the mobilizations $CD34/45^+$ and $CD133/45^+$ BM-CPCs significantly decreased on day 8 and 3, 6 as well as 12 months after AMI. The significant increase of both BM-CPCs mobilization on day 7 existed also 3, 6 as well as 12 months after AMI in the first group with BMCs-Tx (Tables 2 and 3, Figs 4 and 5) whereas there were no significant changes between immediately pre- and post intracoronary cell transplantation. There was a significant increase of $CD34/45^+$ mobilization 3, 6 and 12 months after intracoronary cell transplantation compared to control group (Fig. 6A). The mobilization of

Table 2 Cardiac function, clinical function status parameters and mobilization of BM-CPCs at baseline, on day 7 and 3, 6 as well as 12 months after AMI in the group with BMCs-Tx

	Baseline	On day 7 after AMI	3 months after BMCs-Tx	6 months after BMCs-Tx	12 months after BMCs-Tx
Global EF (%)	43 ± 10		53 ± 8*		54 ± 7*
The size of infarct area (%)	31 ± 10		18 ± 8†		19 ± 8†
Infarct wall movement velocity (cm/s)	1.83 ± 0.74		3.43 ± 0.63†		3.45 ± 0.5†
End-diastolic volume (LVEDV) (ml)	129 ± 31		147 ± 59‡		148 ± 52‡
End-systolic volume (LVESV) (ml)	78 ± 23		59 ± 25*		57 ± 22*
Stroke volume index (SVI) (ml/m^2)	30 ± 12		48 ± 10*		45 ± 9*
CD34/45$^+$ BM-CPCs	170 ± 80	451 ± 151†	401 ± 98†	405 ± 100†	390 ± 90†
CD133/45$^+$ BM-CPCs	41 ± 22	111 ± 30†	98 ± 28†	90 ± 22†	91 ± 26 *
BNP (pg/ml)	165 ± 92		70 ± 41†	65 ± 45†	68 ± 39†
NYHA classification	2.8 ± 0.7		1.6 ± 0.5†	1.5 ± 0.6†	1.6 ± 0.5†

Values are mean ± S.D. BM-CPCs: bone marrow–derived circulating progenitor cells; NYHA: New York Heart Association; BNP: B-type natriuretic peptide; Global EF: Global ejection fraction. There was no significant difference in baseline cardiac function, clinical function status parameters as well as mobilization of BM-CPCs between both groups at baseline.
*P = 0.01–0.001 (compared to baseline).
†$P < 0.001$ (compared to baseline).
‡P = non-significant (NS).

Table 3 Cardiac function, clinical function status parameters and mobilization of BM-CPCs at baseline on day 7 and 3, 6 as well as 12 months after AMI in control group without bone marrow cells transplantation

	Baseline	On day 7 after AMI	3 months after AMI	6 months after AMI	12 months after AMI
Global EF (%)	45 ± 10		47 ± 7†		46 ± 7†
The size of infarct area (%)	29 ± 9		25 ± 9†		25 ± 7†
Infarct wall movement velocity (cm/s)	1.80 ± 0.54		2.03 ± 1.06†		2.00 ± 0.9†
End-diastolic volume (LVEDV) (ml)	132 ± 29		134 ± 37†		134 ± 31†
End-systolic volume (LVESV) (ml)	73 ± 29		70 ± 20†		71 ± 27†
Stroke volume index (SVI) (ml/m^2)	32 ± 10		36 ± 11†		34 ± 9‡
CD34/45$^+$ BM-CPCs	178 ± 58	438 ± 152*	199 ± 45†	185 ± 50†	180 ± 46†
CD133/45$^+$ BM-CPCs	45 ± 22	124 ± 41*	50 ± 19†	45 ± 15†	48 ± 17†
BNP (pg/ml)	161 ± 93		130 ± 79†	128 ± 55†	130 ± 60†
NYHA classification	2.5 ± 0.9		2.0 ± 1.1†	2.0 ± 0.9†	2.1 ± 0.8†

Values are mean ± S.D. BM-CPCs: bone marrow--derived circulating progenitor cells; NYHA: New York Heart Association; BNP: B-type natriuretic peptide; Global EF: Global ejection fraction. There was no significant difference in baseline cardiac function, clinical function status parameters as well as mobilization of BM-CPCs between both groups at baseline.
*$P < 0.001$ (compared to baseline).
†P = non-significant (NS).

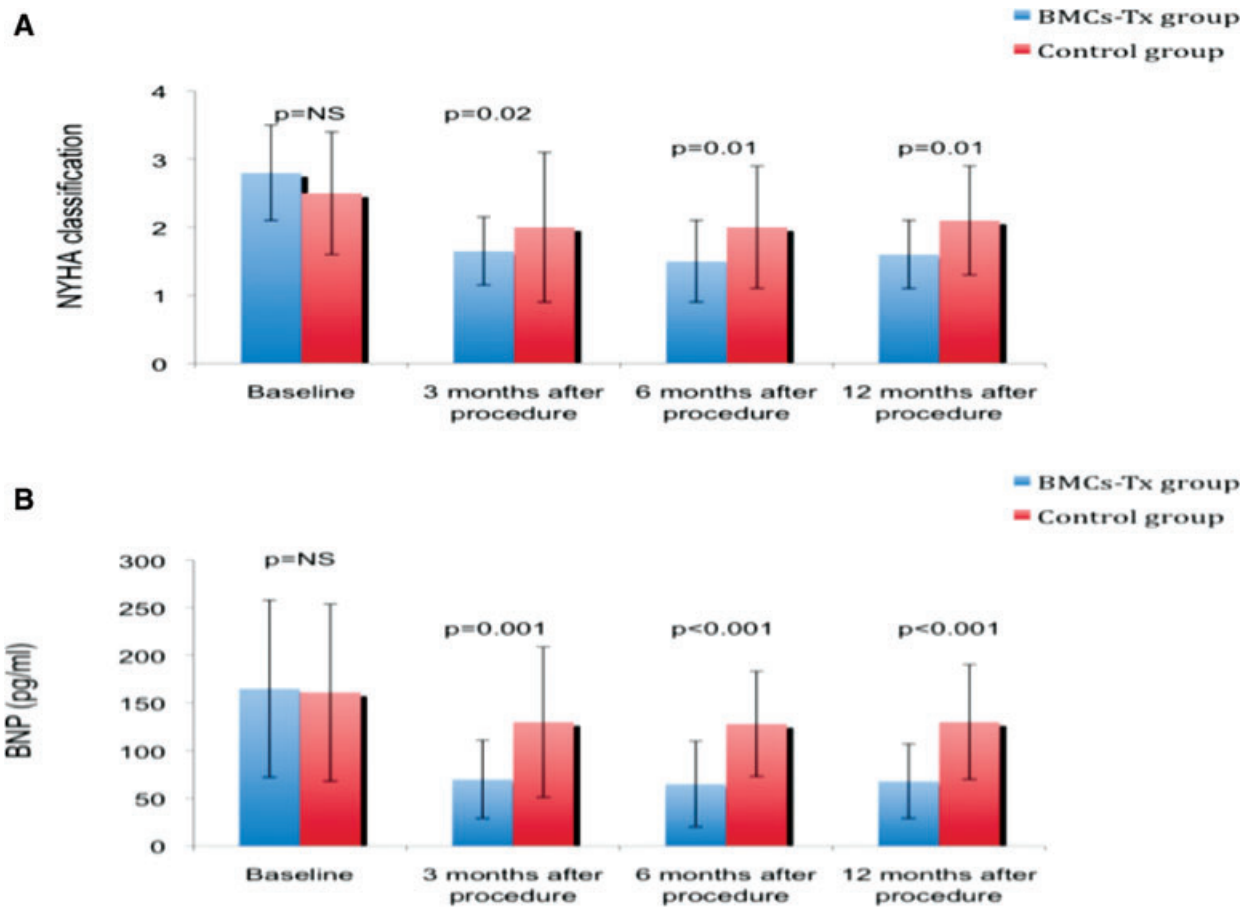

Fig. 3 NYHA classification and BNP levels in both groups. There were no significant differences of baseline NYHA classification and of BNP level between two groups; 3, 6 and 12 months after cell therapy there were a significant decrease of NYHA classification and of BNP level compared to control group without cell therapy. Moreover, no significant changes were observed in the control group at follow up.

$CD133/45^+$ showed the same pattern 3, 6 and 12 months after AMI with a significant increase in the cell therapy group compared to the control group without cell transplantation (Fig. 6B). In contrast to intracoronary cell therapy group with a significant increase of $CD34/45^+$ and $CD133/45^+$ mobilization between baseline and after 3, 6, 12 months follow-up, there was no difference in the control group between baseline and after 3, 6, 12 months follow up (Table 3, Fig. 5A and B).

Discussion

In this prospective randomized controlled study, we examined the influence of autologous intracoronary freshly isolated BMCs-Tx on the mobilization of BM-CPCs and left ventricular function in patients with AMI after 3, 6 and 12 months.

Despite widespread use of primary percutaneous coronary intervention for prompt reperfusion of the infracted myocardium, AMI is a major cause of chronic HF. The risk of chronic HF as well as mortality and morbidity are significantly increased in patients with reduced global EF after AMI. The use of stem cell-based therapy is becoming increasingly recognized as having the potential to salvage damaged myocardium and to promote endogenous repair of cardiac tissue, thus having the potential for the treatment of HF [18, 19]. In animal models, autologous infusion or injection of stem/progenitor cells derived from various sources was shown to enhance blood flow and neovascularization and improve heart function after myocardial infarction [20–22]. BMCs have been injected directly into the myocardium in patients with ischaemic heart disease and have been shown to have a beneficial effect on cardiac function [23–25]. Moreover, clinical pilot and randomized studies suggested that the intracoronary infusion of autologous bone marrow–derived stem/progenitor cells is safe and feasible as well as improve longstanding the recovery of left ventricular con-

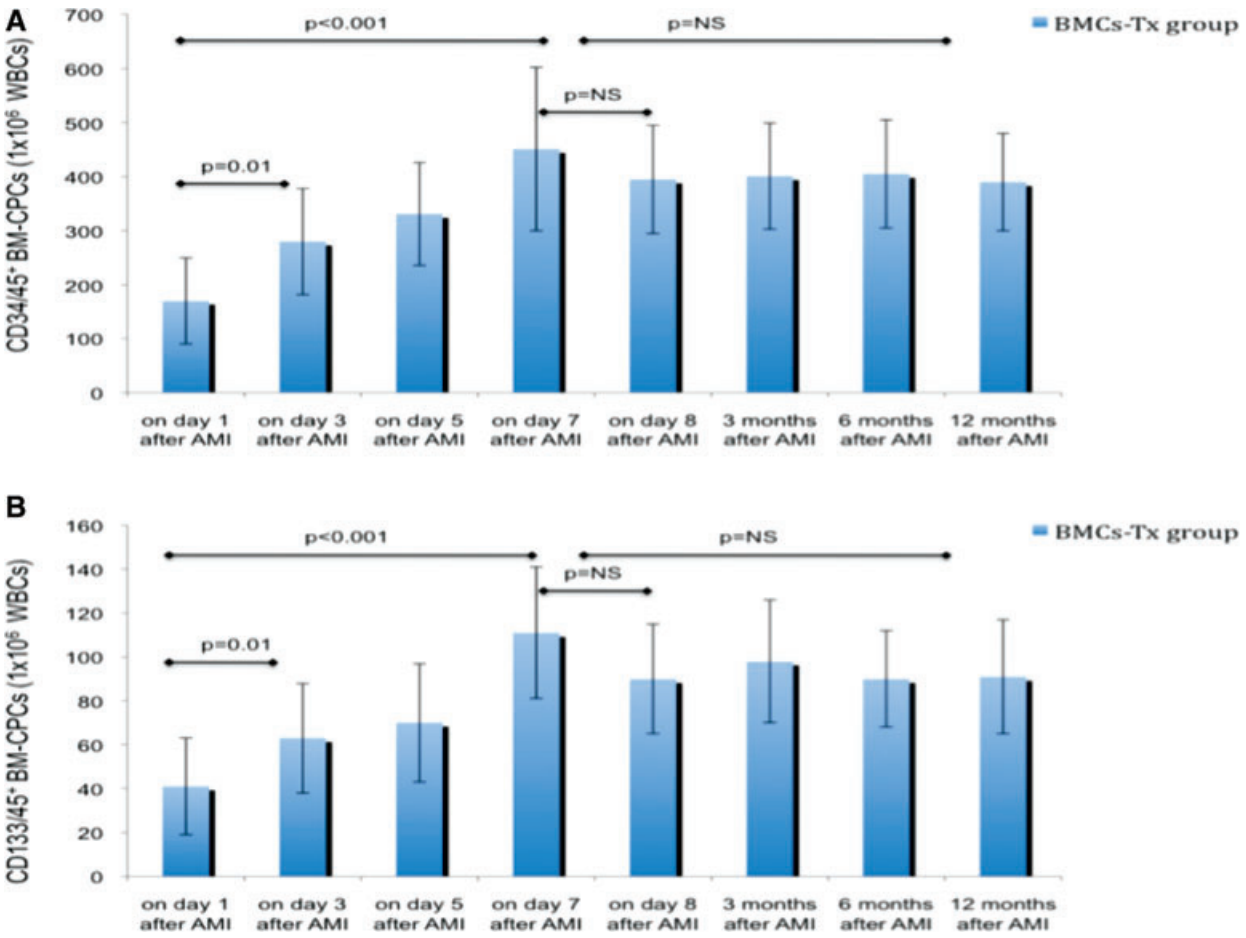

Fig. 4 The mobilization of CD34/45^{+} and CD133/45^{+} BM-CPCs increased significantly with a peak on day 7 after AMI. There was no significant difference between mobilization of both BM-CPCs on days 7, 8 as well as 3, 6 and 12 months after cell therapy. The significant increase of BM-CPCs after AMI exist also 3, 6 and 12 months after cell therapy as compared to day 1 after AMI.

tractility in patients with AMI [8–14]. The beneficial effects observed in most phase I/II studies were confirmed in the so far largest douple-blind, randomized multicentre REPAIR-AMI trial [12]. Only one larger study, the ASTAMI trial [26, 27] did not show any benefit on left ventricular functional parameters. The reason for the failure of the ASTAMI trial to show a benefit of cell therapy may have been the different cell isolation and storage protocol, which significantly affected the functional capacity of the cells [28]. Although in the REPAIR-AMI trial Ficoll gradient centrifugation was used for cell isolation, the negative clinical ASTAMI trial used a different, not yet validated, technique (LymphoPrep). Strikingly, the yield in total nucleated cells out of the same volume of 50 ml bone marrow aspirate was quite different. Although the Ficoll-based protocol, which was used for the isolation procedure in the REPAIR-AMI trial, provided threefold higher number of cells as compared to ASTAMI trial. Even more importantly, recent data also suggest that the number of haematopoietic colony-forming units and the SDF-1–induced migratory activity of recovered BMC based on the ASTAMI protocol are significantly lower compared to Ficoll protocol [28]. These data suggest that, although similar techniques were used, the functional activity and/or cellular composition of the obtained cellular product are quite different. Because most of the previous clinical trials involved BMCs isolated by Ficoll [8–14], this technique currently viewed as the gold standard. Our findings, that the infarct size reduced, whereas the global EF and regional infarct wall movement velocity increased 3 and 12 months after intracoronary cell therapy in patients with AMI, are in line with the data of previous published pilot and randomized clinical trials [8–14]. In addition, we observed improvement of the functional status (NYHA classification) and of BNP level 3, 6 as well as 12 months after cell therapy. Cell isolation procedures are crucial for the functional activity of the administered cellular product. In our trial, we chose to use a point of care system for the preparation of the

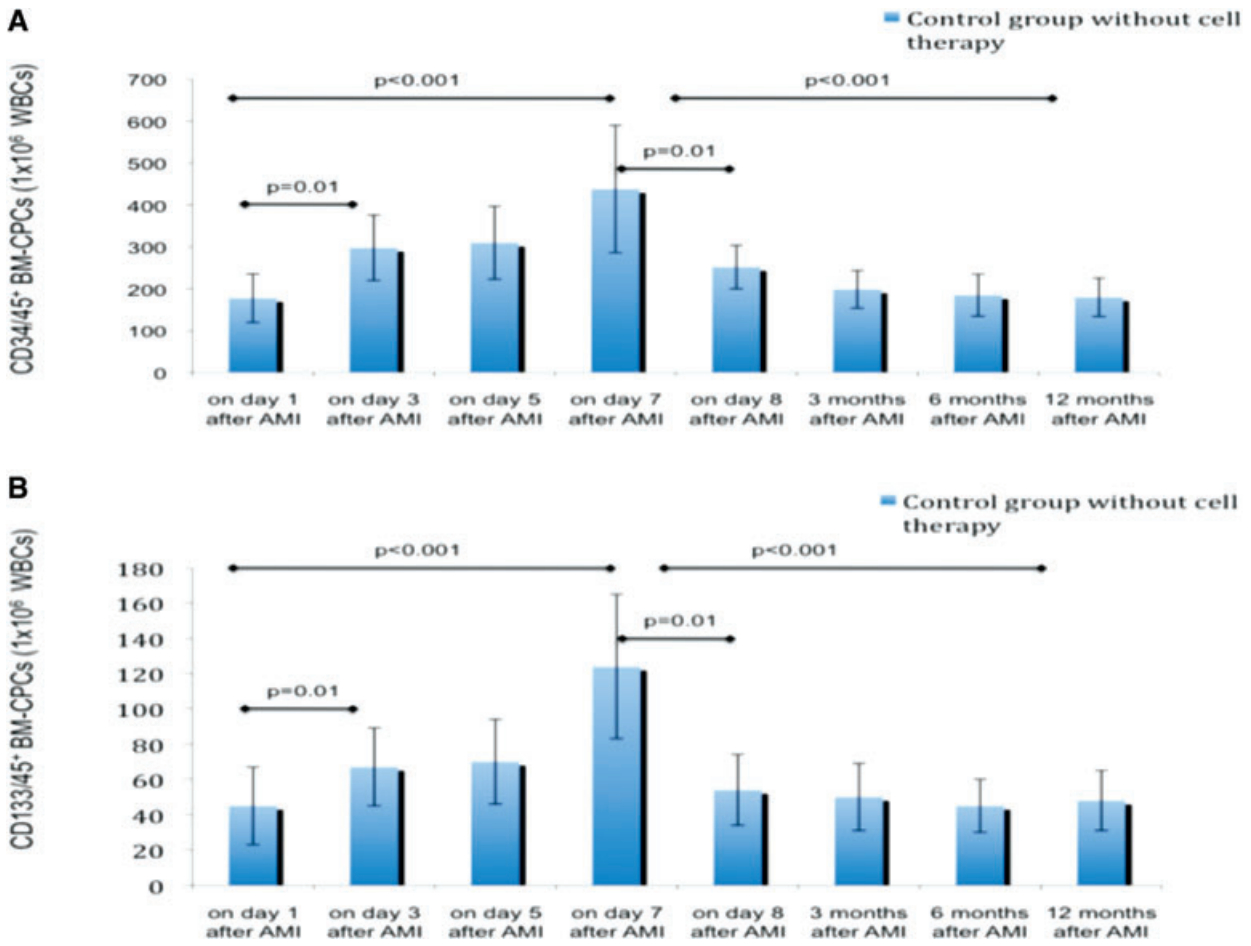

Fig. 5 The mobilization of CD34/45$^+$ und CD133/45$^+$ BM-CPCs increased significantly with a peak on day 7 after AMI, which significantly decreased on day 8 and 3, 6 and 12 months after AMI as compared to day 7. There was no significant difference between mobilization of both BM-CPCs on day 1 and on day 8 as well as 3, 6 and 12 months after AMI in patients without cell therapy.

treating cell composition. We showed in pilot study that freshly isolated BMCs-Tx by use a point of care system is safe and feasible as well as may improve the cardiac function after AMI [29]. We demonstrated the same results in patients with AMI for the first time in randomized controlled study with intracoronary freshly isolated BMCs-Tx by use a point of care system with Harvest BMAC-system for the preparation of the treating cell composition, not Ficoll gradient separation as in other studies. The cellular composition of the concentrate, which was prepared by use a point of care system, differs from that prepared using the Ficoll method. The Ficoll composition contains predominantly mononuclear cells (lymphocytes, erythroblasts and monocytes) and very few granulocytes. The point of care system concentrates entire nucleated cell population with mononuclear cells and specific stem cell population (CD34$^+$ and CD133$^+$) as well as the platelets from the marrow aspirate (Table 4). Importantly, however, the point of care device provided advantage of significantly higher yield of isolated bone marrow cells compared to the Ficoll protocol. Thus, if the number of infused cells in *in vivo* neovascularization model was adjusted for this higher yield of bone marrow cells, the treatment effect was significantly greater compared to Ficoll BMCs, as assessed by limb perfusion measurement [30]. One obvious difference in the two compositions is the presence of significant numbers of granulocytes and platelets in the point of care system composition. Platelets and granulocytes have been shown to have a positive effect on the neovascular potential of the resulting concentrate. The presence of platelets within composition could be important because it has been shown that these platelet-derived mediators also potently enhance postnatal angiogenesis. Iba *et al.* demonstrated that implantation of mononuclear cells together with platelets into ischaemic limbs more effectively augments collateral vessel formation by supplying various angiogenic factors, in which VEGF played a key role [31]. Indeed, Massberg *et al.* provided compel ling evidence that platelets generate the critical sig-

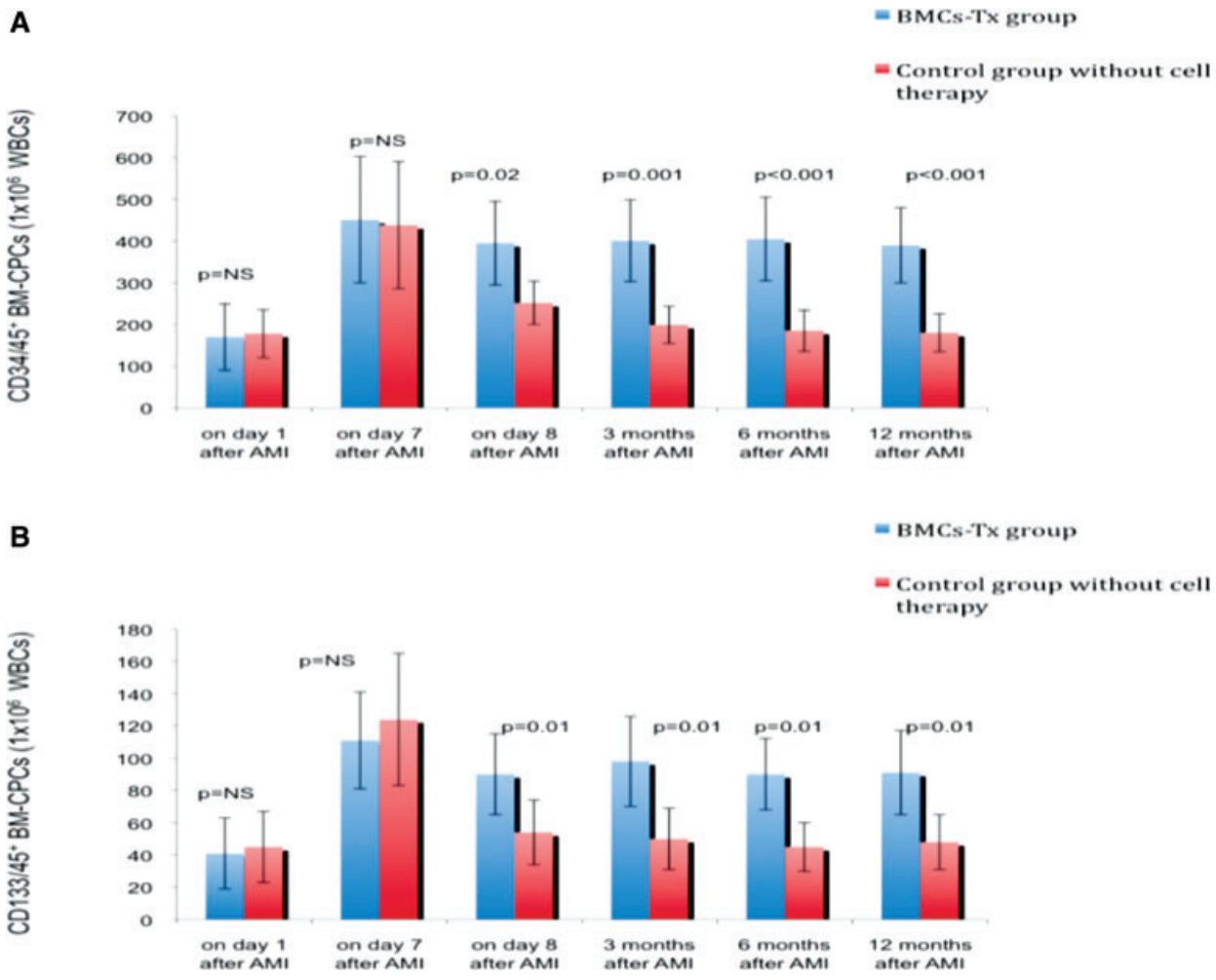

Fig. 6 There was significant increase of CD34/45$^+$ and CD133/45$^+$ BM-CPCs mobilization on days 7, 8 and 3, 6 and 12 months after AMI in BMCs-Tx group as compared control group without cell therapy, whereas there was no significant difference of BM-CPCs mobilization on days 1 and 7 after AMI between the BMCs-Tx group and control group without cell therapy.

Table 4 The cellular composition of bone marrow aspirate and bone marrow concentrate by use of point of care system in the group with BMCs-Tx

	Bone marrow aspirate (Pre-separation, 120 cm^3)	Bone marrow concentrate (Post-separation, 20 cm^3)
Total nucleated cells ($\times 10^6$ ml)	26 ± 10	96 ± 32
CD34$^+$ cells ($\times 10^6$ ml)	0.23 ± 0.09	0.97 ± 0.12
CD133$^+$ cells ($\times 10^6$ ml)	0.09 ± 0.006	0.38 ± 0.03
Platelet count ($\times 10^3$ /μl)	145 ± 25	692 ± 185
Viability of cells (%)	98 ± 1.5	

nal that recruits CD34$^+$ bone marrow cells and c-Kit$^+$ Sca-1$^+$ Lin- bone marrow–derived progenitor cells to sites of injury [32]. Therefore, these findings strongly support the notion that implanted platelets play a pivotal role in stem and progenitor recruitment and provide a rationale for the fact that point of care system produced functional *in vivo* results similar to or better than Ficoll. In our study despite higher number of platelets we observed no immediate peri-procedure as well as post-procedure adverse complications. In addition, unlike Ficoll isolation where cells are resuspended in a serum free medium, point of care system is always resuspended in the patient's own plasma. Thus, the isolated cells are not removed from their natural plasma microenvironment, which may be help to sustain the functionality of the cells. This has been further supported by experimental study of Hermann *et al*., who showed that the point of care system compo-

sition to be significantly more bioactive than the Ficoll composition. Intriguingly, however, due to the greater yield of cells generated by use a point of care system, the cellular product isolated from a given bone marrow aspirate by use a point of care device may actually translate into even greater therapeutic effects. In addition, practical aspects may also deserve consideration. As the concentration process by use of point of care system, everything can be accomplished in one session without adding excessive time to the overall procedure circumventing the previously mentioned disadvantages of the Ficoll isolation process. The point of care device represents time-efficient stand-alone technique for the isolation of autologous bone marrow cells suitable for cell therapy regimens in the rapidly growing field of regenerative medicine.

Several hypotheses have been proposed about, how intracoronary cell therapy improves myocardial function. Experimental studies addressing the capacity of transplanted bone marrow–derived stem cells to differentiate into the cardiomyogenic lineage yielded conflicting results. Recent well-conducted studies suggest that the BMCs do not transdifferentiate into cardiomyocytes but adopt mature haematopoietic characteristics. In contrast to embryonic stem cells, most adult stem or progenitor cells do not spontaneously differentiate into cardiomyocytes but rather require an adequate stimulus to do so. Another proposed mechanism is that cell therapy may increase angiogenesis and improve blood supply to ischemic regions, potentially aiding in the revascularization of hibernating myocardium and preventing cardiomyocyte apoptosis. In addition or alternatively, the local microenvironment plays an important role to induce cell fate changes by physical cell-to-cell interaction or by providing paracrine factors promoting tissue repair [33–37].

Cell-based therapy is a promising option for treatment of ischaemic disease. However, cell therapy is in its early stages, and various questions remain. For example, the mechanisms of action by which cells exhibit beneficial effects [38]. Currently, a variety of autologous adult progenitor cells are undergoing pre-clinical evaluation. BMCs are, at present, the most frequent source used clinically for cardiac repair [39]. BMC fractions include a heterogeneous mixture of cells with varying percentages of haematopoietic stem cells, BM-CPCs, mesenchymal stem cells and side population cells. BM-CPCs are another population of progenitor cells that has also been shown to have therapeutic potential. These cells were characterized by the expression of at least 2 haematopoietic stem cell markers ($CD133^+$ or $CD34^+$) [40]. BM-CPCs mobilize into the PB and contribute to neovascularization after tissue ischaemia in an animal model [41]. Previous study demonstrated that the mobilization and functional activity of BM-CPCs significantly increased after freshly isolated BMCs-Tx in patients with ischaemic heart disease [42, 43]. Our findings confirmed that the significant increase of both BM-CPCs mobilization with a peak on day 7 after AMI in both groups. In addition, we showed that the significant increase of $CD34/45^+$ and $CD133/45^+$ BM-CPCs mobilization on day 7 was also existed 3, 6 and 12 months after freshly isolated BMCs-Tx, whereas there was a significant decrease of BM-CPCs mobilization on day 7 and 3, 6 and 12 months after AMI in control group without cell therapy. On the basis of these findings, it is tempting to that this spontaneous mobilization of $CD34/45^+$ and $CD133/45^+$ BM-CPCs is a response to myocardial repair after AMI and this maximum effect of BM-CPCs mobilization may achieve on day 7 after AMI. Moreover, this response is mostly inadequate because of reduced mobilization of BM-CPCs by increased cardiovascular risk factors in patients with large myocardial infarction without cell transplantation [44]. Taken together, if this spontaneous mobilization of $CD34/45^+$ and $CD133/45^+$ BM-CPCs responsible myocardial repair, the extend of repair may more than limited in control group without cell therapy as compared to cell therapy group after AMI. The presence of immature circulating cells in the PB has been advocated as a marker of organism's regenerative capacity [45]. Experimental and clinical studies suggest that there is an evolving role for circulating progenitor cells in neoangiogenesis and rejuvenation of the endothelial monolayer [1, 46, 47]. Indeed, the mobilization of BM-CPCs is inversely correlated with endothelial function [48], which explains that the BM-CPCs may play an important role in endogenous repair mechanisms of the injured endothelial monolayer and thereby reduce atherosclerotic lesion formation [49]. The occurrence of a first major cardiovascular event (AMI, hospitalization, revascularization or death from cardiovascular causes) was associated with reduced BM-CPCs levels in patients with coronary artery disease [50]. Moreover, intracoronary administration of BMCs is associated with a significant reduction of major adverse cardiovascular events after AMI [51]. Previous studies demonstrate that patients with HF show endothelial dysfunction and in HF, nitric oxide production is diminished, whereas rate of endothelial apoptosis is increased [52]. Moreover, the impaired neovascularization in mice lacking eNOS is related to defect in progenitor cell mobilization from bone marrow [53]. Mechanistically, the improved perfusion capacity, which was demonstrated in the TOPECARE- and REPAIR-AMI trial in patients after cell therapy, may increase epicardial artery shear stress and stimulate the endothelium to release the NO, which may enhance the mobilization of BM-CPCs and exerts anti-atherosclerotic functions [54, 55].

The primary limitation of this study is the lack of placebo arm and the measurement of cardiac imaging. Moreover, for better understanding we added left ventricular volume data in our study, which was also investigated in several trials [8, 9, 12–14]. Therefore, a placebo-controlled study will be needed to validate the hypothesis.

In this study, we could demonstrate that intracoronary transplantation of autologous freshly isolated BMCs by use of point of care system improved global EF and reduced infarct size significantly in patients with AMI after 3 and 12 months. Moreover, we observed a significant mobilization of BM-CPCs even 3, 6 and 12 months after cell transplantation. This interesting observation could be implemented in future large-scale randomized studies, where the BM-CPCs mobilization after transplantation may serve as predictor for identifying AMI patients with greater benefit after cell therapy.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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