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Identification of cardiovascular risk factors associated with bone marrow cell subsets in patients with STEMI: a biorepository evaluation from the CCTRN TIME and LateTIME clinical trials

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Abstract

Autologous bone marrow mononuclear cell (BM-MNC) therapy for patients with ST-segment elevation myocardial infarction (STEMI) has produced inconsistent results, possibly due to BM-MNC product heterogeneity. Patient-specific cardiovascular risk factors (CRFs) may contribute to variations in BM-MNC composition. We sought to identify associations between BM-MNC subset frequencies and specific CRFs in STEMI patients. Bone marrow was collected from 191 STEMI patients enrolled in the CCTRN TIME and LateTIME trials. Relationships between BM-MNC subsets and CRFs were determined with multivariate analyses. An assessment of CRFs showed that hyperlipidemia and hypertension were associated with a higher frequency of $CD11b^+$ cells ($P = 0.045$ and $P = 0.016$, respectively). In addition, we found that females had lower frequencies of $CD11b^+$ ($P = 0.018$) and $CD45^+CD14^+$ ($P = 0.028$) cells than males, age was inversely associated with the frequency of $CD45^+CD31^+$ cells ($P = 0.001$), smoking was associated with a decreased frequency of $CD45^+CD31^+$ cells ($P = 0.013$), glucose level was positively associated with the frequency of $CD45^+CD3^+$ cells, and creatinine level (an indicator of renal function) was inversely

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Compliance with ethical standards

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associated with the frequency of $CD45^+CD3^+$ cells ($P = 0.015$). In conclusion, the frequencies of monocytic, lymphocytic, and angiogenic BM-MNCs varied in relation to patients' CRFs. These phenotypic variations may affect cell therapy outcomes and might be an important consideration when selecting patients for and reviewing results from autologous cell therapy trials.

Keywords

Bone marrow mononuclear cells; Ischemic heart disease; Cardiovascular risk factors; ST-segment elevation myocardial infarction; Autologous cell therapy

Introduction

End-stage heart failure results in the deaths of more than 60,000 patients annually in the US. In the western world, the leading cause of heart failure is ischemic heart disease [59]. Several approaches have been proposed for the treatment of patients with ischemic heart disease (IHD), one of which is stem cell therapy. Although stem cell therapies have shown promise in preclinical studies as a treatment for IHD [22, 25, 33, 40], clinical trials have produced inconsistent results [1, 10, 13, 16, 20, 51, 52, 58]. Thus, efforts are underway to determine ways to optimize these therapies.

Patients with IHD have a wide range of cardiovascular risk factors (CRFs). In a study by Nauta et al. [32], 69.3% of patients with acute myocardial infarction (MI) had at least one CRF; from those, 39.2% had a single CRF, 21.7% had 2, and 8.4% had 3 or 4. These CRFs have been shown to correlate with changes in the frequencies of particular cell types in the blood. Recently, it was reported that hypertension is associated with an increase in the level of circulating $CD11b^+$ cells [38], advanced age and smoking are associated with a decrease in circulating $CD31^+$ leukocytes [14], age is inversely associated with the level of $CD34^+$ cells [30], and diabetes is associated with a decrease in circulating endothelial progenitor cells [26].

Changes in the cellular composition of the blood and bone marrow (BM) could affect the outcomes of IHD. For example, Cogle et al. [8] showed a negative correlation between the percentage of $CD11b^+$ cells in the BM and post-infarct left ventricular ejection fraction (LVEF) in patients with ST-segment elevation myocardial infarction (STEMI), regardless of whether they received BM mononuclear cells (BM-MNCs) or placebo. In addition, Schutt et al. [41] found that infarct size reduction after STEMI was greater in patients who had a higher percentage of $CD31^+$ mononuclear cells in the BM.

We hypothesized that a patient's CRFs may affect the frequencies of specific angiogenic, lymphocytic, monocytic, and hematopoietic cells within the BM, which could, in turn, impact the efficacy of autologous BM-MNC therapies. To test this hypothesis, we assessed the relationships between the frequencies of BM-MNCs expressing CD34, CD31, CD3, CD14, CD11b, CD19, CD45, and C-X-C chemokine motif receptor 4 (CXCR4) and 8 CRFs in patients with STEMI.

Methods

For this retrospective analysis, our study cohort comprised patients who participated in the Cardiovascular cell therapy research network (CCTRN) transplantation in myocardial infarction evaluation (TIME) and LateTIME trials and provided consent to have their remaining BM-MNC product further analyzed at the CCTRN Biorepository [51–54, 62]. The CCTRN TIME and LateTIME trials had similar inclusion criteria but differed in the timing of BM aspiration and cell delivery after STEMI. Institutional Review Boards at each clinical site approved the protocols for both trials, and all participants provided written informed consent.

Flow cytometry

BM-MNC samples collected at baseline (i.e., before treatment) were immunophenotyped via polychromatic flow cytometry to determine the frequency of hematopoietic, lymphocytic, monocytic, and angiogenic precursors in the BM, as previously described [51, 53]. Briefly, 1–5 million BM-MNCs incubated with antibodies against CD34, CD11b, CD31, CD45, CD3, CD14, CD19, and CXCR4 for 20 min at 4 °C in the dark, washed twice in 2.5% phosphate-buffered saline (PBS), and then resuspended to a final volume of 1 mL in 2.5% PBS for flow cytometry analysis. Samples were analyzed with an LSR II flow cytometer (Becton–Dickinson, Franklin Lakes, NJ, USA), and the data were analyzed with the FlowJo software (Tree Star, Inc., Ashland, OR, USA). All analyses were performed on gated lymphocytes or monocytes or using the International Society of Hematotherapy and Graft Engineering (ISHAGE) gating strategy [45]. Figures 1 and 2 show examples of the gating strategies used to determine the frequencies of specific lymphocyte, monocyte, and hematopoietic stem cell populations in the BM samples.

Statistical analyses

Patients from the TIME and LateTIME studies were combined into a single cohort for all analyses. Demographic data are shown as counts and percentages for dichotomous and polychotomous variables and as means and standard deviations for continuous variables. We calculated the associations between the frequencies of eight BM-MNC subsets (Table 1); specifically, each of the subsets was the dependent variable in a multiple regression model that contained one of eight CRFs (self-reported hypertension, hyperlipidemia, diabetes mellitus, and smoking, as well as sex, age, creatinine level, and blood glucose level) plus a dichotomous variable reflecting the study (TIME or LateTIME). In this exploratory analysis, no corrections made for multiplicity. All analyses conducted using SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Baseline clinical characteristics

The current study included 191 patients who provided consent for BM analysis by the CCTRN Biorepository. Demographic and CRF data for the current study cohort are reported in Table 2. The mean age of the population analyzed was 56 years (standard deviation, 11), and only 15% ($n = 28$) of the participants were female.

Associations between cardiovascular risk factors and BM-MNC subsets

A multivariable model was used to explore the relationships between eight CRFs (hyperlipidemia, hypertension, diabetes, sex, smoking, age, blood glucose level, and creatinine level) and the frequencies of particular BM-MNC subsets at study baseline, as determined by flow cytometry (Table 3). After adjusting for study, hyperlipidemia and hypertension were found to be positively associated with the frequency of $CD11b^+$ monocytes. Surprisingly, diabetes was not associated with changes in any of the cell types assessed. Women were found to have lower frequencies of $CD11b^+$ and $CD45^+CD14^+$ monocytes than men, and smokers had a lower frequency of $CD45^+CD31^+$ lymphocytes than non-smokers. Negative associations existed between age and the frequency of $CD45^+CD31^+$ lymphocytes (Fig. 3) and between creatinine level and the frequency of $CD45^+CD3^+$ cells. A positive association was found between glucose level and the frequency of $CD45^+CD3^+$ T-cell precursors. These data suggest that in our cohort of patients with STEMI, CRFs thought to influence IHD outcomes may have also affected the cellular composition of the BM, particularly the frequency of specific monocyte and lymphocyte subsets.

Discussion

Exploratory studies conducted in conjunction with the CCTRN TIME, LateTIME, and FOCUS trials have shown associations between specific BM-MNC subsets and clinical outcomes [35, 49, 51, 52]. It has been suggested that CRFs, such as age and sex, play an important role in a patient's response to cell therapy [47, 61]. However, to date, there has been no comprehensive assessment of how patient characteristics may alter BM composition in STEMI patients; thus, it is unknown whether or how the composition of autologous BM-MNC products may be altered by patients' CRFs.

In the current study, we looked for associations between specific BM-MNC subsets and eight CRFs in STEMI patients enrolled in either the TIME or LateTIME trials. Hypertension, hyperlipidemia, sex, smoking, age, glucose, and creatinine levels were found to be associated with differences in BM composition in these patients. These data suggest that each patient's BM composition differs according to the individual's CRF profile. Thus, these attributes may affect the composition of the autologous BM-MNC therapy received and the associated outcomes.

An inflammatory process that involves the transmigration and accumulation of both innate and adaptive immune cells into the interstitium of affected tissues may play a role in hypertension [28]. Furthermore, hypertension has been associated with atherosclerosis, endothelial dysfunction, and the accumulation of monocytes within the endothelium [17]. However, the effects of hypertension on human BM monocytes are not defined. In this study, hypertension was positively associated with the frequency of $CD11b^+$ cells in the BM. To our knowledge, our study is the first to describe an association between hypertension and the level of BM monocytes in humans. Increased expression of CD11b is suggested to stimulate the development of IHD by promoting myocyte oxidative injury and myocardial hypertrophy [12, 31]. Interestingly, Cogle et al. [8] found that an increased level of $CD11b^+$ cells in the BM of patients at 1–3 weeks after acute MI was associated with worse LVEF at the 6-month

follow-up. Our results suggest that hypertension may predispose an individual to increased levels of $CD11b^+$ cells and, thereby, to poor outcomes after STEMI.

Hyperlipidemia, a well-known CRF, is associated with plaque formation and vessel rupture [3] by increased endothelial permeability to LDL cholesterol. In our study, we found an association between the frequency of $CD11b^+$ cells and hyperlipidemia. Similarly, Serrano et al. [42] observed an increase in the circulating numbers of $CD11b^+$ cells in patients with untreated hypercholesterolemia. Furthermore, they observed decreased numbers of circulating $CD11b^+$ cells and LDL cholesterol in these patients after treatment. These findings suggest that this cell type plays an important role in the pathogenesis of atherosclerosis.

Increased age is a major contributor to endothelial dysfunction [5, 6, 11, 43, 44, 46] and an increased risk of developing IHD [39]. In our study, increased age was inversely associated with the frequency of $CD45^+CD31^+$ lymphocytes. This is similar to the findings of, both Hur et al. [19] and Ge et al. [14] who showed an inverse correlation between the level of circulating peripheral blood $CD31^+$ T cells and age. One possible explanation for this decrease in cell number with age is age-related apoptosis. Kushner et al. [27] showed that caspase-3, a critical downstream protein involved in the execution phase of the apoptotic pathway, is higher in $CD31^+$ T cells of middle-aged and older men than in those of younger men, supporting this correlation between age and apoptosis of $CD31^+$ cells. Interestingly, increased age has been shown to be associated with increases in cellular apoptosis more broadly and of course with the incidence of cardiovascular disease [28]. This loss of angiogenic lymphocytes with age, or immunosenescence of multiple cell types, suggests that the BM-MNC products from elderly patients may be less effective as a cell therapy. Another cell type shown to change in frequency with age is $CD34^+$ cells. Moresi et al. [30] found that the number of circulating $CD34^+$ cells significantly decreased with increasing age in a population of healthy individuals (age range 16–100 years old). Although our results showed a similar trend, the association was not statistically significant. The lack of significance may have been due to differences in the sample types assessed (blood vs bone marrow) or due to differences in the age range and health status of the respective study participants. Because the patients in our study had sustained an MI recently, the $CD34^+$ stem cells may have been released into circulation in response to the recent injury, thereby masking the effects of age on this cell population.

Furthermore, female sex has been found to be associated with a lower risk of death due to coronary artery disease after adjustment for CRFs and age [55]. In this study, female sex was associated with decreased levels of BM $CD11b^+$ and $CD45^+CD14^+$ monocytes. Sex steroids, including estrone, progesterone, and testosterone, can modulate the ability of monocytes and platelets to adhere to endothelial cells and, therefore, can either induce or inhibit the initiation and progression of vascular lesions [9]. Estrone, which is higher in women than in men, reduces the surface expression of CD11b and decreases monocyte adhesion to endothelial cells exposed to the pro-inflammatory agent lipopolysaccharide, suggesting that it may inhibit endothelial injury under inflammatory conditions [9]. Although we did not measure the serum levels in our cohort of patients, it is known that estrone production does

not stop after menopause [15]. Similar to our results, Heimbeck et al. [18] found that the level of circulating $CD45^{+}CD14^{+}$ monocytes is lower in women than in men.

Finally, cigarette smoking may cause apoptotic cell death and cellular senescence, and may inhibit repair functions [24]. We found that smoking was associated with a decrease in BM $CD45^{+}CD31^{+}$ lymphocytes. Similar to our findings, Ge et al. [14] found a negative association between smoking and the level of circulating $CD45^{+-}CD31^{dim}$ lymphocytes in healthy men and women. Further investigation of this cell type is warranted to determine its role in the prevention or repair of cardiac injury.

Study limitations

This study had several limitations. Before the TIME and LateTIME trials began, we chose the BM-MNC phenotypes and CRFs to assess based on the current knowledge of the factors that affect the repair process after acute MI, balanced by available fiscal resources. The fields of cardiovascular regeneration and cell therapy have evolved rapidly since the design of TIME and LateTIME studies; recent studies have revealed multiple cell types that may contribute to cardiovascular outcomes. Unfortunately, some of the cell populations that are now recognized as important in this field were either not understood 8 years ago or were too costly to evaluate in the first in-depth analysis, and thus were not included in the design of TIME and LateTIME. In particular, cell populations involved in pro-inflammatory responses, such as $CD14^{++}/CD16^{-}$ monocytes (“classical”, Mon1), $CD14^{+}/CD16^{++}$ monocytes (“non-classical”, Mon3), and $CD14^{++}/CD16^{+}$ monocytes (“intermediate”, Mon2), which are considered independent predictors of cardiovascular events [37], were not evaluated. We also did not assess the levels of T-cells subsets: $CD3^{+}CD4^{+}$ cells (T-helper cells), $CD3^{+}CD8^{+}$ cells (cytotoxic T cells), and other cell subsets now known to be involved in anti-inflammatory responses, (e.g., $CD4^{+}CD25^{+}CD127^{low}$ cells regulatory T cells) [56]. We identify this as a shortcoming of the study design. Unfortunately, because cell phenotypes can only be analyzed in fresh samples, we are not able to assess these newly recognized phenotypes. Despite this, we feel that our results provide a relatively comprehensive assessment of BM phenotypes in patients with STEMI. Another limitation was that the BM-MNC products were obtained from a cohort of patients with multiple risk factors, potentially making it difficult to discern associations between specific BM-MNC frequencies and individual CRFs. In addition, all the patients in this study had an STEMI event before the BM-MNCs were collected, which could have overshadowed other factors affecting the composition of the BM. Moreover, the timing between the STEMI event and BM-MNC collection varied among patients. Finally, because our study had a low number of patients in some demographic (e.g., females) and CRF groups (e.g., diabetes), the statistical power for these groups may have been too low to detect all associations between these factors and BM-MNC populations.

Conclusions

To our knowledge, this is the first study to show associations between specific CRFs and the frequencies of particular BM-MNC subsets, including monocytes and lymphocytes, in patients with STEMI. Because of the exploratory nature of this study, we cannot determine

whether the CRFs directly affected the BM composition or if other factors contributed to this association. Future studies will be necessary to assess whether a cause-and-effect relationship exists. Since the BM obtained from patients who had hyperlipidemia or hypertension who were advanced in age or who smoked showed changes that would be expected to be unfavorable for cardiac repair, our results suggest that the BM product from these patients may be less effective as a cell therapy than that from healthier individuals. If this is proven to be true, patients' CRFs may need to be considered when designing future autologous cell therapy studies and assessing clinical outcomes.

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Abbreviations

BM	Bone marrow
BM-MNC	Bone marrow mononuclear cell
CCTRN	Cardiovascular cell therapy research network
CRF	Cardiovascular risk factor
IHD	Ischemic heart disease
LVEF	Left ventricular ejection fraction
MI	Myocardial infarction
STEMI	ST-segment elevation myocardial infarction

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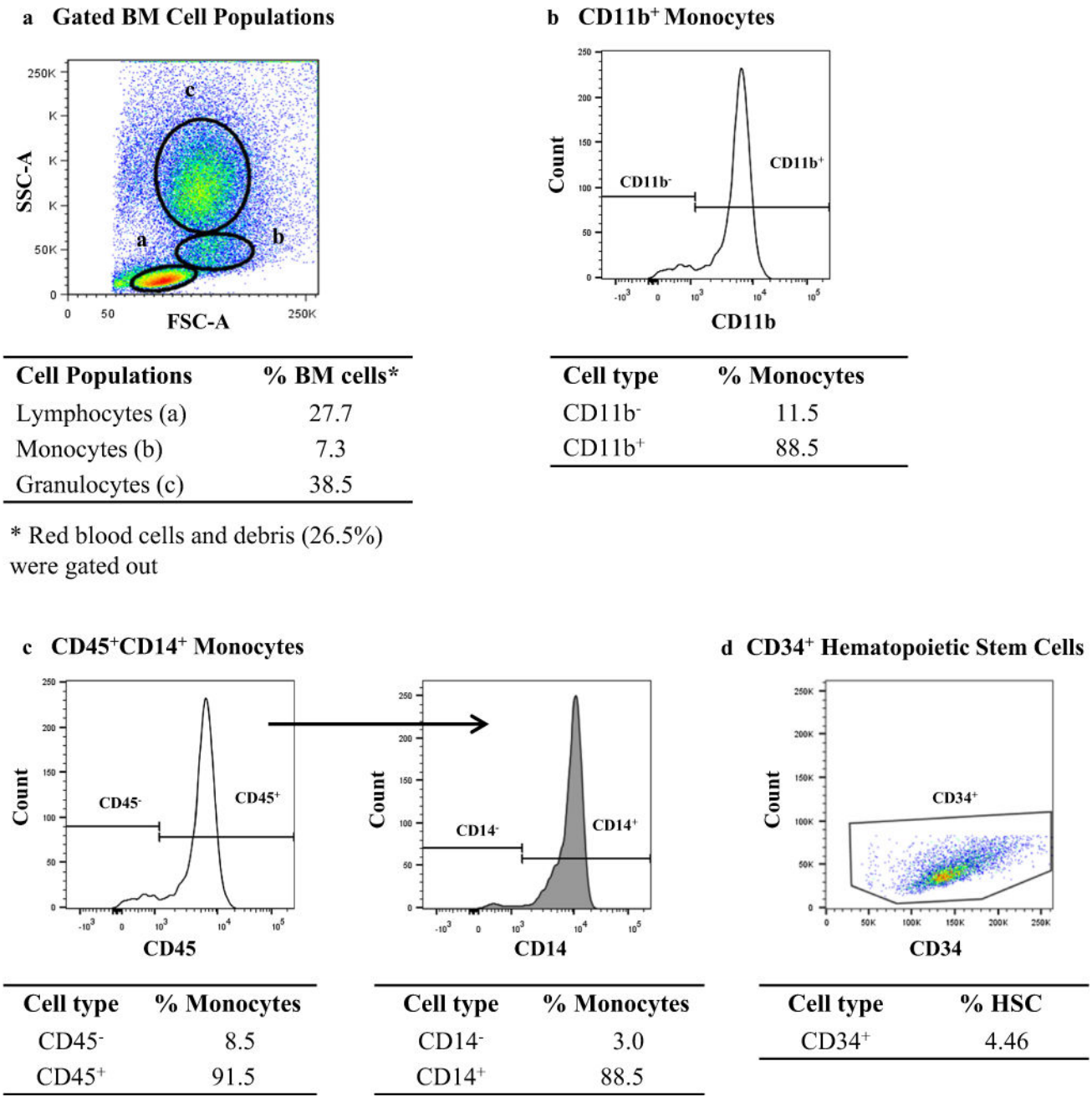


Cell Populations	% BM cells*
Lymphocytes (a)	27.7
Monocytes (b)	7.3
Granulocytes (c)	38.5

* Red blood cells and debris (26.5%) were gated out

Cell type	% Monocytes
CD11b$^-$	11.5
CD11b$^+$	88.5

Cell type	% Monocytes
CD45$^-$	8.5
CD45$^+$	91.5

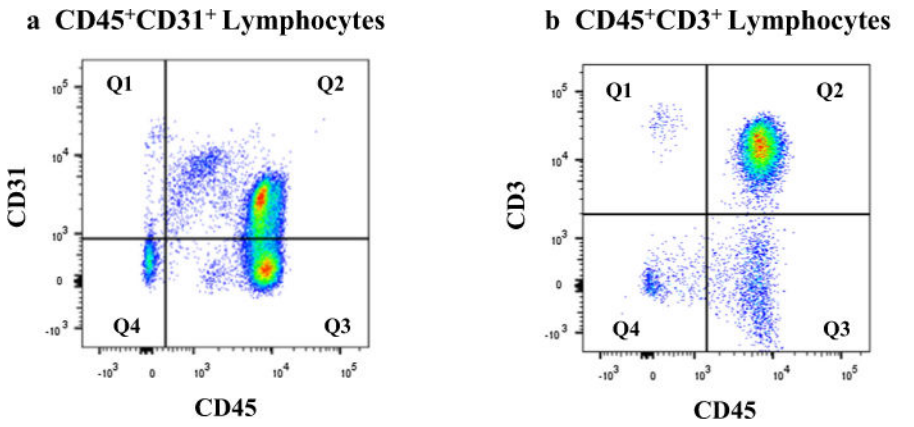
Cell type	% Monocytes
CD14$^-$	3.0
CD14$^+$	88.5

Cell type	% HSC
CD34$^+$	4.46

Fig. 1.

Gating strategy used for analyzing CD11b$^+$ and CD45^{+-}CD14$^+$ monocyte subsets and CD34$^+$ hematopoietic stem cells (HSC) in the bone marrow (BM). **a** Representative dot plot showing the gates used to identify bone marrow mononuclear cell (BM-MNC) populations based on forward scatter (FSC-A) and side scatter (SSC-A). **b** Representative histogram showing CD11b$^+$ cells within the monocyte gate. **c** Representative histogram showing the CD14$^+$ cells (*right panel*) gated from CD45$^+$ cells (*left panel*) within the monocyte gate. **d** Representative dot plot showing the CD45^{dim}CD31$^+$ SSC^{low} HSCs using the ISHAGE gating strategy (not shown). Percentages shown in **b** and **c** are based on the total monocyte

population. Percentage shown in **d** is based on the $CD45^+$ cells. All data presented are from a single patient



Quadrant	% Positivity
Q1	0.60
Q2	52.1
Q3	42.6
Q4	4.65

Quadrant	% Positivity
Q1	0.68
Q2	85.4
Q3	10.3
Q4	3.59

Fig. 2.

Gating strategy used for analyzing $CD45^+CD31^+$ and $CD45^+CD3^+$ lymphocyte subsets. **a** Representative dot plot showing $CD45^+CD31^+$ cells (*Q2*) within the lymphocyte gate. **b** Representative dot plot showing the $CD45^+CD3^+$ cells (*Q2*) within the lymphocyte gate. Percentages shown in **a** and **b** are based on the total lymphocyte population. All data presented are from a single patient

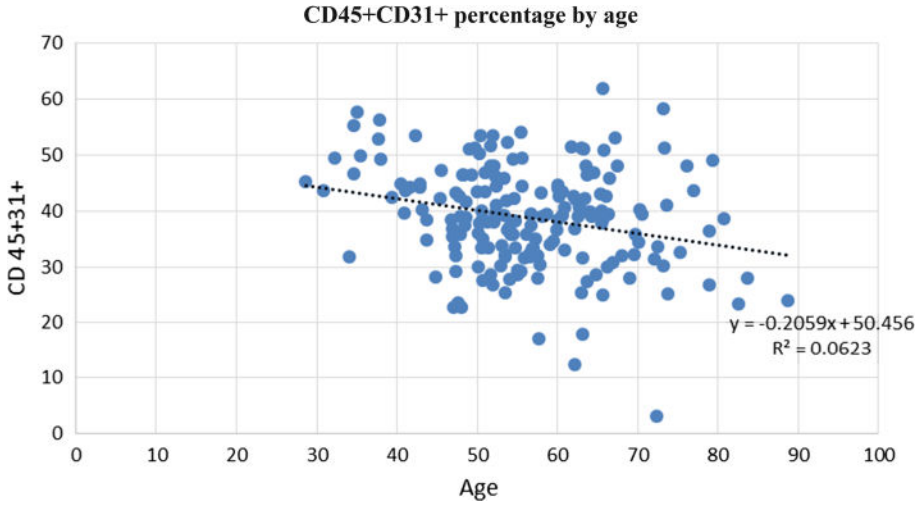


Fig. 3.

Association between the frequency of $CD45^{+}CD31^{+}$ lymphocytes and age. *Dot plot* showing that the percentage of $CD45^{+}CD31^{+}$ cells in the bone marrow decreased with age

Table 1

Identification of bone marrow cell subsets according to phenotype

Phenotype	BM-MCSs Type	Involvement in CVD
$CD34^+$	Hematopoietic stem cells	Angiogenesis and attenuation of negative left ventricular remodeling [21, 22, 35, 50]
$CD11b^+$	Monocytes	Worsening of LVEF after AMI [8]
$CD34^+CD31^+$	Angiogenic cells	Angiogenesis in ischemic vascular disease and reduction in infarct size [36, 41, 60]
$CD45^+CD31^+$	Lymphocytes (T cell)	Angiogenesis and vasculogenesis [23]
$CD45^+CD3^+$	Lymphocytes (T-cell precursor)	Immunoregulatory and cytotoxic effects [2, 4]
$CD45^+CD14^+$	Monocytes	Vascular inflammation in atherosclerosis [29, 57]
$CD45^+CD19^+$	Lymphocytes (B cell)	Protective immunity during atherosclerosis [7]
$CD19^+CXCR4^+$	Lymphocytes (B cell)	Improvement in heart function [34, 48, 49]

AMI acute myocardial infarction, *LVEF* left ventricular ejection fraction

Table 2

Baseline demographics and cardiovascular risk factors of patients in the study cohort

Characteristics	
Demographics	
Age, mean (SD), years	56 (11)
Female, *n* (%)	28 (15%)
Cardiovascular risk factors	
Diabetes, *n* (%)	39 (20%)
Hypertension, *n* (%)	107 (56%)
Hyperlipidemia, *n* (%)	130 (68%)
Total cholesterol, mean (SD), mg/dL	167 (48)
HDL cholesterol, mean (SD), mg/dL	37 (12)
LDL cholesterol, mean (SD), mg/dL, (*n* = 189)	102 (45)
Total/HDL cholesterol ratio, mean (SD), (*n* = 190)	4.6 (1.7)
Creatinine, mean (SD), mg/dL	0.9 (0.2)
Smoking, *n* (%)	115 (60%)
Systolic blood pressure, [a]mean (SD), mmHg	113 (14)

n = 191 unless otherwise noted

HDL high-density lipoprotein, *LDL* low-density lipoprotein

[a]At initial discharge

Table 3

Associations between cardiovascular risk factors and BM-MNC subsets

Phenotype	Hyperlipidemia		Hypertension		Diabetes	
	Effect size (95% CI)	***P* value**	**Effect size (95% CI)**	***P* value**	**Effect size (95% CI)**	***P* value**
[a]$CD34^+$	−0.25 (−1.16 to 0.65)	0.581	−0.72 (−1.57 to 0.12)	0.093	0.37 (−0.68 to 1.41)	0.49
[b]$CD11b^+$	4.14 (0.10 to 8.18)	0.045	4.65 (0.86 to 8.49)	0.016	−2.71 (−7.41 to 2.00)	0.258
[a]$CD34^+CD31^+$	−0.29 (−1.17 to 0.59)	0.511	−0.66 (−1.48 to 0.17)	0.117	0.41 (−0.60 to 1.42)	0.429
$CD45^+CD31^+$	0.62 (−2.19 to 3.43)	0.666	−1.28 (−3.92 to 1.36)	0.342	−2.42 (−5.63 to 0.79)	0.138
$CD45^+CD3^+$	−0.87 (−4.26 to 2.52)	0.612	1.77 (−1.41 to 4.95)	0.273	−0.43 (−4.36 to 3.49)	0.828
[b]$CD45^+CD14^+$	2.01 (−2.51 to 6.53)	0.381	4.18 (−0.05 to 8.42)	0.053	0.11 (−5.13 to 5.34)	0.968
$CD45^+CD19^+$	0.27 (−1.18 to 1.72)	0.712	0.33 (−1.03 to 1.69)	0.634	0.11 (−1.57 to 1.78)	0.901
$CD19^+CXCR4^+$	0.25 (−1.03 to 1.52)	0.702	0.32 (−0.87 to 1.52)	0.594	0.37 (−1.10 to 1.84)	0.619

Phenotype	Sex (female)		Age		Smoking	
	Effect size (95% CI)	***P* value**	**Effect size (95% CI)**	***P* value**	**Effect size (95% CI)**	***P* value**
[a]$CD34^+$	0.13 (−1.06 to 1.32)	0.833	−0.03 (0.07 to 0.01)	0.094	−0.52 (−1.38 to 0.35)	0.241
[b]$CD11b^+$	−6.40 (−11.70 to 1.10)	0.018	0.09 (−0.08 to 0.27)	0.299	1.07 (−2.84 to 4.97)	0.591
[a]$CD34^+CD31^+$	0.12 (−1.05 to 1.29)	0.842	−0.03 (−0.07 to 0.00)	0.088	−0.42 (−1.26 to 0.12)	0.328
$CD45^+CD31^+$	1.49 (−2.24 to 5.22)	0.431	−0.20 (−0.32 to −0.09)	0.001	−3.40 (−6.06 to 0.74)	0.013
$CD45^+CD3^+$	3.98 (−0.45 to 8.42)	0.078	−0.10 (−0.24 to 0.05)	0.178	2.55 (−0.66 to 5.77)	0.119
[b]$CD45^+CD14^+$	−6.61 (−12.49 to 0.72)	0.028	0.00 (−0.19 to 0.20)	0.968	1.92 (−2.42 to 6.27)	0.383
$CD45^+CD19^+$	−0.92 (−2.82 to 0.99)	0.343	−0.02 (−0.08 to 0.04)	0.561	1.33 (−0.04 to 2.7)	0.057
$CD19^+CXCR4^+$	−0.45 (−2.13 to 1.22)	0.594	0.00 (−0.05 to 0.06)	0.952	0.78 (−0.42 to 1.99)	0.203

Phenotype	Creatinine		Glucose	
	Effect size (95% CI)	***P* value**	**Effect size (95% CI)**	***P* value**
[a]$CD34^+$	1.00 (−0.90 to 2.89)	0.301	0.00 (−0.01 to 0.00)	0.399
[b]$CD11b^+$	6.65 (−1.9 to 15.19)	0.127	0.02 (−0.02 to 0.05)	0.356
[a]$CD34^+CD31^+$	0.89 (−0.95 to 2.73)	0.339	0.00 (−0.01 to 0.01)	0.545

Phenotype	Creatinine		Glucose	
	Effect size (95% CI)	***P* value**	**Effect size (95% CI)**	***P* value**
$CD45^+CD31^+$	−0.76 (−6.67 to 5.15)	0.8	0.01 (−0.02 to 0.03)	0.541
$CD45^+CD3^+$	−8.76 (−15.79 to 1.72)	0.015	0.03 (0.00 to 0.06)	0.031
[b]$CD45^+CD14^+$	4.90 (−4.59 to 14.39)	0.31	0.02 (−0.02 to 0.06)	0.294
$CD45^+CD19^+$	0.67 (−2.38 to 3.73)	0.663	0.00 (−0.02 to 0.01)	0.466
$CD19^+CXCR4^+$	1.19 (−1.48 to 3.86)	0.38	0.00 (−0.01 to 0.01)	0.854

The reported effect size is the model coefficient from a multiple linear regression model with the cell type as the independent variable and the cardiovascular risk factor as the dependent variable. The reported *P* values are adjusted for study (TIME or LateTIME). All cells were analyzed within the lymphocyte gate unless otherwise specified

CI confidence interval

[a]Analyzed using ISHAGE gating

[b]Analyzed within the monocyte gate