Impaired Mobilization of CD133\(^+\) Bone Marrow-Derived Circulating Progenitor Cells With an Increased Number of Diseased Coronary Arteries in Ischemic Heart Disease Patients With Diabetes

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Background: The influence of the number of diseased coronary arteries on the mobilization of CD133/45\(^+\) bone marrow-derived circulating progenitor cells (BM-CPCs) in peripheral blood (PB) in patients with ischemic heart disease (IHD) was analyzed.

Methods and Results: Mobilization of CD133/45\(^+\) BM-CPCs by flow cytometry was measured in 120 patients with coronary 1 vessel (IHD1, n=40), coronary 2 vessel (IHD2, n=40), and coronary 3 vessel disease (IHD3, n=40), and in a control group (n=40). The mobilization of CD133/45\(^+\) BM-CPCs was significantly reduced in patients with IHD compared to the control group (P<0.001). The mobilization of CD133/45\(^+\) BM-CPCs was impaired in patients with IHD3 compared to IHD1 (P<0.001) and to IHD2 (P<0.001). But there was no significant difference in mobilization of CD133/45\(^+\) BM-CPCs between the patients with IHD2 and IHD1 (P=0.35). Moreover, we found significantly reduced CD133/45\(^+\) cell mobilization in patients with a high SYNTAX-Score (SS) compared to a low SS (P<0.001) and an intermediate SS (P<0.001). In subgroup analyzes, we observed a significantly negative correlation between levels of hemoglobin A\(_1c\) and the mobilization of CD133/45\(^+\) BM-CPCs (P=0.001, r=−0.6).

Conclusions: The mobilization of CD133/45\(^+\) BM-CPCs in PB is impaired in patients with IHD. This impairment might augment with increased number of diseased coronary arteries. Moreover, mobilization of CD133/45\(^+\) BM-CPCs in ischemic tissue is further impaired by diabetes in patients with IHD. (Circ J 2011; 75: 2635–2641)

Key Words: CD133/45\(^+\); Diabetes; Ischemic heart disease; Mobilization

Circulating progenitor cells (CPCs) are primitive bone marrow cells (BMCs) that have the capacity to proliferate, migrate and differentiate into various mature cell types.\(^1\,2\) These bone marrow-derived CPCs (BM-CPCs) express unique surface markers, such as CD34\(^+\) and the early hematopoietic cell marker, CD133\(^+\) (AC133\(^+\)). During ischemia, populations of BM-CPCs are mobilized and recruited to ischemic areas, accelerating the neovascularization process.\(^3\) Previous studies demonstrate that cardiovascular risk factors (CVRFs) for coronary artery disease (CAD) correlate with a reduced number and functional activity of circulating endothelial progenitor cells.\(^4\) Moreover, diabetic patients showed impaired proangiogenic and colony-forming activity of CPCs.\(^5\,6\) However, it is unknown whether the mobilization of BM-CPCs relates to the number of diseased coronary arteries in patients with ischemic heart disease (IHD). In this study, we analyzed...
the mobilization of CD133/45+ BM-CPCs and their relationship with the number of diseased coronary arteries in patients with IHD.

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Methods

Study Protocol and Study Population

One hundred and twenty IHD patients between 18 and 80 years of age were eligible for inclusion in this study, if they had had a documented myocardial infarction within at least 6 months and had left ventricular dysfunction. All IHD patients underwent diagnostic cardiac catheterization. We selected a control group of 40 healthy subjects without overt heart disease and/or major CVRFs (diabetes, smoking, hypertension, hypercholesterolemia, and familial history), who had atypical chest pain, but no evidence of cardiac ischemia for enrollment in this study. Control subjects underwent a coronary angiography to rule out IHD within 24 h after admission. The patients were recruited during diagnostic cardiac catheterization by an interventional cardiologist and were allocated into the following 4 groups: (1) coronary 1 vessel (IHD1); (2) coronary 2 vessel (IHD2); (3) coronary 3 vessel disease (IHD3); and (4) control group of healthy subjects. A peripheral blood (PB) sample was taken during cardiac catheterization to measure the amount of CD133/45+ cells. Additionally, to accurately assess the severity of IHD-based diseased vessels, the SYNTAX-Score (SS) was calculated for all IHD patients, which was then divided into 3 groups: (1) low SS (0–22); (2) intermediate SS (23–32); and (3) high SS (>33). A CVRFs score, including age >40 years, protein cholesterol levels exceeding 130 mg/dl. Diabetes was defined as diabetes mellitus, and had left ventricular dysfunction. All IHD patients under went diagnostic coronary angiography and coronary angiography. Cardiac catheterization was performed according to the guidelines for coronary angiography from the American College of Cardiology and the American Heart Association.5 Cardiac function was determined by left ventriculography. Cardiac function was evaluated by global ejection fraction (EF). Global EF was measured with

Mobilization of CD133/45+ BM-CPCs

Ten-milliliter EDTA PB samples were taken during cardiac catheterization from all IHD patients and the control group. BM-CPCs were collected from PB for CD133/45+ in all groups and quantified by flow cytometry (EPICS-XL, Beckmann Coulter). PB samples were analyzed within 2 h.

Samples were stained with a fluorescein isothiocyanate (FITC) conjugate of a CD45+ antibody (clone J33; Coulter/Immunotech, Marseille, France) that detects all isoforms and glycoforms of the CD45 family, and a phycoerythrin (PE) conjugate of a CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany). Control samples were stained with CD45+ FITC and an IgG1 PE (Coulter/Immunotech, Marseille, France) isotype.

For each patient, EDTA blood samples were labelled with CD133/45+ and IgG1/CD45. All tubes were incubated at room temperature in the dark. After incubation, cells were lysed with ammonium chloride, and then washed with phosphate-buffered saline. Samples were then stored on ice at 4°C in the dark for 20 min and analysed by flow cytometry.5,10

Samples were subjected to a 2D side-scatter fluorescence dot plot analysis. After appropriate gating, the concentration of BM-CPCs with low cytoplasmic granularity (low-side ward dot plot analysis. After appropriate gating, the concentration of BM-CPCs with low cytoplasmic granularity (low-side ward scatter) was quantified and expressed as a concentration of cells per million white blood cells.

Biochemical Measurements

PB was collected from the study population, and serum creatine kinase values (normal range: 24–195 U/L), inflammatory markers such as C-reactive protein (normal range <0.5 mg/dl), leukocytes (normal range: 4–12×10⁹/μl) and routine laboratory tests with hemoglobin A1c (HbA1c) were measured.

Statistical Analysis

Continuous data are presented as mean±SD. Comparison of the distributions of a continuous variable between 2 independent groups was performed using the 2-sided non-parametric Mann-Whitney U test. The type I error rate α was chosen as 5% and 2-sided P values equal or less than 0.05 were interpreted as statistically significant. Some qualitative baseline characteristics were compared using the Fisher’s exact test. The average age was presented as median, quantile (25/75) and range (minimum—maximum) for all groups.

Bivariate regression analysis was presented in a graphical form and Pearson’s correlation coefficient was obtained.

Multiple regression analyses with ANCOVA were performed for CD133/45+ BM-CPCs mobilization as a dependent variable, and severities of diseased coronary (1–3) as well as diabetes mellitus (DM) (yes/no) in all IHD patients were cofactors. To estimate independent predictors of CD133/45+ BM-CPCs mobilization, differences in the least squares means (LS-mean) and corresponding 95% confidence intervals (CI) were calculated.

Statistical significance was accepted if the corresponding 2-sided P value was lower or equal to 0.05. Statistical analysis was performed with SPSS for Windows (Version 15.0).
Results
Baseline Characteristics of the Patients and Healthy Control Subjects
We included 120 patients with IHD and 40 healthy subjects without heart disease in the study. The baseline characteristics of the study population are shown in Table. There were no significant differences in the total number of CVRFs and age between IHD groups. Additionally, individual CVRFs between IHD groups were compared. Interestingly, there was a significant difference in the number of DM patients in the IHD3 group compared to the IHD1 and IHD2 groups. In contrast, there was no significant difference of the number of DM patients between IHD1 and IHD2 groups. Also, no significant differences were observed in other baseline characteristics and demographics of patients between all IHD groups (Table).

Mobilization of CD133/45\(^+\) BM-CPCs
The mobilization of CD133/45\(^+\) BM-CPCs was measured by flow cytometry in 120 IHD patients as well as in 40 healthy subjects. The mobilization of CD133/45\(^+\) BM-CPCs was significantly reduced in IHD patients compared to healthy subjects (CD133/45\(^+\); P<0.001) (Figure 1). We observed a significant decrease of CD133/45\(^+\) mobilization in patients with...
Figure 2. Mobilization of CD133/45⁺ BM-CPCs was impaired in patients with IHD3 as compared to IHD1. But there was no significant difference in the mobilization of CD133⁺ BM-CPCs between the patients with IHD2 and IHD1. BM-CPCs, bone marrow-derived circulating progenitor cells; IHD, ischemic heart disease; IHD1, coronary 1 vessel disease; IHD2, patients with coronary 2 vessel disease; IHD3, patients with coronary 3 vessel disease.

Figure 3. Mobilization of CD133/45⁺ BM-CPCs were significantly reduced in patients with a high SS as compared to patients with an intermediate and low SS. There was also a significant difference in CD133/45⁺ mobilization between patients with a low SS and those with an intermediate SS. BM-CPCs, bone marrow-derived circulating progenitor cells; SS, Syntax Score.

Figure 4. A significant inverse correlation was observed between the mobilization of CD133/45⁺ BM-CPCs and levels of HbA₁c in total of IHD patients with diabetes. IHD, ischemic heart disease; BM-CPCs, bone marrow-derived circulating progenitor cells; HbA₁c, hemoglobin A₁c.
IH3 compared to IH1 (CD133/45+: P<0.001) and IH2 (CD133/45+: P<0.001) patients. There was no significant difference of CD133/45+ mobilization between IH1 and IH2 patients (CD133/45+: P=0.35) (Figure 2).

We calculated for all IH patients the SS to accurately assess the severity of IH-based diseased vessels and then divided the patients into 3 groups: (1) low SS (0–22); (2) intermediate SS (23–32); and (3) high SS (>33). The mobilization of CD133/45+ BM-CPC was compared between the 3 SS-groups. We found the significantly reduced mobilization of CD133/45+ cells in patients with a high SS as compared to patients with intermediate (P<0.001) and low SS (P<0.001). There was also a significant difference in CD133/45+ mobilization between patients with low SS and intermediate SS (P=0.02) (Figure 3).

Relationship Between DM and Mobilization of CD133/45+ BM-CPCs in IH3 Patients

After we had observed that patients with IH3 had a significantly higher incidence of DM compared to patients with IH2 and IH1, we investigated in subgroup analyzes of the link between DM and mobilization of CD133/45+ BM-CPCs in IH patients. We observed a significant inverse correlation between levels of HbA1c and the mobilization of CD133/45+ BM-CPCs (P=0.001, r=−0.6) (Figure 4). To identify independent predictors of the mobilization of CD133/45+ BM-CPCs, step-wise multivariate regression with ANCOVA analyzes were performed. Multivariate analyzes showed severities of diseased coronary artery (LS-mean; 11.03, 95%CI; 1.54–20.53, P<0.001) and DM (LS-mean; 11.96, 95%CI; 5.56–20.36, P=0.006) as statistically significant independent predictors of the mobilization of CD133/45+ BM-CPCs.

Moreover, we compared the CD133/45+ BM-CPCs mobilization between the patients with good regulated DM (HbA1c: <7%) and poorly regulated DM (HbA1c: >7%). The mobilization of CD133/45+ cells was significantly reduced in patients with poorly regulated DM compared to well regulated DM (CD133/45+: P<0.001).

Discussion

In this controlled study, we examined the effect of the number of diseased coronary arteries on the mobilization of CD133/45+ BM-CPCs in patients with IH.

CAD results from a chronic inflammatory disease of the vascular wall and leads to vessel occlusion and organ damage. Despite intense efforts to determine the pathogenesis of atherosclerosis, this process remains poorly understood. Reports suggest that risk factors and a genetic predisposition together induce inflammatory processes that lead to cell damage and impair regeneration within the vessel wall as resident endothelial cells infrequently proliferate. It has been postulated that there are other sources of vascular replenishment in response to continuous damage. CPCs derived from bone marrow circulate in the PB and have been implicated in neangiogenesis after tissue ischemia has occurred. Additionally, clinical trials indicate a beneficial effect of intracoronary infusion of BMPCs or CPCs on myocardial function in patients with acute myocardial infarction (AMI). BMPCs contain a complex assortment of progenitor cells, including hematopoietic stem cells, mesenchymal stem cells or stromal cells, and multipotent adult progenitor cells. BM-CPCs is another population of progenitor cell pool circulating within blood that has also been shown to have therapeutic potential. These cells were characterized by the expression of at least 2 hematopoietic stem cell markers (CD133+ or CD34+). BM-CPCs are capable of proliferating and differentiating into endothelial cells and are therefore ideal candidates for vascular regeneration. Experiments in animals show that the systemic application or mobilization of stem cells and progenitor cells beneficially influences the repair of endothelial cells after injury and the progression of atherosclerosis. CD34/45+ and CD133/45+ BM-CPCs can also be used as a predictive biomarker for cardiovascular risk, vascular function and the extent of cardiac repair. In a large clinical study, Hill et al reported that high-risk individuals have fewer BM-CPCs compared with their low-risk counterparts, whereas Werner et al identified a significant association between increasing numbers of BM-CPCs and decreased risk of major cardiovascular events and hospitalization in patients with CAD. BM-CPCs mobilization can also predict severe endothelial dysfunction in patients with coronary heart disease. Moreover, the transient increase in CD34/45+ and CD133/45+ BM-CPCs after regular symptom-limited (ischemic and/or subischemic) exercise training reached a maximum after regular exercise training for 3 weeks, but it did not persist up until 3 months after regular training and after AMI. Some reports suggested that the basal level of CD34+CD133+ and CD34+CD45+ cells using flow cytometry, as well as the number of CPC determined by a CFU assay, decreased in patients with CAD. However, it was unknown whether the mobilization of CD133+ BM-CPCs relates to the number of diseased coronary arteries in patients with IH. We demonstrated, in our study, that the mobilization of CD133+ BM-CPCs was significantly reduced in IH patients compared to healthy subjects. Additionally, we found that the mobilization of CD133+ BM-CPCs was impaired in patients with IH3 compared to patients with IH2 and IH1. To support this result, we calculated a SS for all IHD patients. We observed significantly reduced CD133+ BM-CPCs mobilization in patients with a high SS compared to patients with a low and intermediate SS, which might confirm the hypothesis that there are severities of the coronary artery that are associated with reduced mobilization of CD133+ BM-CPCs in IH patients. In patients with HF and preserved LVEF, diabetes is associated with a significantly increased risk of developing adverse HF outcomes. DM is associated with both an increased risk of atherosclerotic disease and poor outcomes after vascular occlusion. The clinical severity of vascular occlusive disease in diabetics has, in part, been attributed to impaired collateral vessel development. Extensive studies have shown that the numbers of circulating angiogenic cells are significantly lower in type II diabetes, and their angiogenic potential is also dramatically diminished. These cells display defective adhesion to the endothelium, reduced proliferation rate, and impaired ability to create new vascular structures. Although there was no significant difference in the total number of CVRFs between IHD groups, we compared individual CVRFs between IHD groups in our study. No significant differences of individual CVRFs incidence were observed between IHD groups, with the exception of DM, which was significantly higher in the IHD3 group compared to the IH2 and IH1 groups. On the basis of these findings, it is tempting to speculate that the decreased mobilization of CD133+ BM-CPCs by DM lead to progression of atherosclerosis and increase the number of diseased coronary arteries in patients with IHD. In line with this hypothesis, we analyzed the relationship between DM and mobilization of CD133+ BM-CPCs in all 3 IHD groups with DM. Furthermore, we demonstrated that the mobilization of CD133+ BM-CPCs inversely correlated with the level of HbA1c in IH patients with DM. Moreover, multivariate analyzes identified severities of the diseased
coronary artery and DM independent predictors of the mobilization of CD133/45+ BM-CPCs. Additionally we found that the CD133+ BM-CPCs mobilization in patients with poorly regulated DM was significantly reduced compared to patients with well regulated DM. In contrast, Arao et al. found no association between the basal level of CPC numbers and CVRFs such as diabetes. This conflict might arise because of the following: low numbers of CPCs in PB, which makes it difficult to isolate them; poor methodology in flow cytometry and cell culture techniques used to isolate CPCs; CPCs levels identified by different surface markers because of heterogeneity; and severity and type of vascular disease in individual patients. Recent studies have shown that the PPARγ agonist, pioglitazone, treatment increases the number and function of BM-CPCs in type 2 DM patients with CAD. Improved levels of HbA1c by pharmacological therapy might lead to an increase of CD133+ BM-CPCs mobilization and functional activity and thereby might enhance the vascular regeneration in IHD patients with DM. A limitation of this hypothesis is that HbA1c reflects a short-term glucose regulation condition. There was only the previous 1-year follow-up analyzes of HbA1c before CD133+ cell measurement in our study. In contrast, severities of the coronary artery reflect a long-term consequence of the process of atherosclerosis. Further study will be needed to validate the hypothesis with long-term follow-up HbA1c analyzes.

In the present study, we could demonstrate that CD133+ BM-CPCs mobilization was impaired in patients with IHD. This impairment correlates with an increased number of diseased coronary arteries. Moreover, the mobilization of CD133+ BM-CPCs is further impaired by DM in patients with IHD.

Acknowledgments and Disclosures
No conflicts of interest.

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