Circulating CD34-positive cell number is associated with brain natriuretic peptide level in type 2 diabetes patients

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Running title: Circulating CD34+ cells are associated with BNP

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Patients with type 2 diabetes often suffer from asymptomatic left ventricular (LV) injury, including increased LV mass, without apparent myocardial ischemia. The mechanisms underlying diabetic LV injury remain unclear; however, it has been suggested that endothelial dysfunction plays a role. Accumulating evidence indicates that bone marrow-derived endothelial progenitor cells (EPCs) contribute to neovascularization of ischemic tissue and endothelialization of denuded endothelium. Recent studies have shown that circulating bone marrow-derived immature cells, including CD34+ cells, contribute to the maintenance of the vasculature, both as a pool of EPCs, and as the source of growth/angiogenesis factors (1). We hypothesized that circulating CD34+ cells might be associated with LV dysfunction in patients with type 2 diabetes. Therefore, we studied the correlation between circulating CD34+ cell levels and plasma brain natriuretic peptide (BNP) levels, one of LV dysfunction markers, in type 2 diabetes patients.

RESEARCH DESIGN AND METHODS

The institutional review board of the National Cardiovascular Center approved this study and all subjects provided informed consent. We examined 26 patients with type 2 diabetes (12 males and 14 females, duration of diabetes: 16.1 ± 10.7 years) who were all over 60 years old (70.5 ± 6.4 years). Statin was given to 9 subjects. Angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) was given to 9 subjects. Thiazolidinediones (TZD) was given to 2 subjects. Subjects were excluded from the study if they had known cardiovascular disease or chronic renal failure (defined as serum creatinine ≥ 180 µmol/l). All study subjects showed neither hypokinesis by echocardiography or ECG change indicating myocardial ischemia. Systolic (SBP) and diastolic (DBP) blood pressure, as well as anthropometric parameters were determined. Blood samples were taken after 12-h fasting to measure circulating CD34+ cells, plasma BNP, fasting plasma glucose (FPG), and HbA1C. Circulating CD34+ cells were quantified by flow cytometry according to the manufacturer’s protocol (ProCOUNT, Becton Dickinson Biosciences) as previously reported (2). BNP was quantified by enzyme immunoassay (TOHSO, Japan). We further examined LV fractional shortening (LVFS), LV mass index (LVMi) (3), Peak flow velocity of the early filling wave (E), the late filling wave (A), and the E/A-wave ratio (E/A) by echocardiography. All echocardiograms were performed by several expert physicians who were blinded to CD34+ cell level.

All statistical analyses were performed using JMP version 5.1.1 software (SAS Institute Inc.). Data are expressed as means ± SD. Comparisons of number of CD34+ cells by gender were made using the two-tailed unpaired t test. Correlations between number of CD34+ cells and clinical parameters were assessed by univariate linear regression analysis and multiple regression analysis. LVMi and plasma BNP concentrations were analyzed after logarithmic transformation.

RESULTS

FPG levels, HbA1C levels, and BMIs in the study subjects were measured to be 9.5 ± 2.6 mmol/l, 9.2 ± 1.8 %, and 26.4 ± 4.3 kg/m², respectively. 88% of the patients had hypertension (SBP: 142 ± 18 mmHg, DBP: 75.7 ± 13.5 mmHg). Plasma BNP levels was measured to be 33 ± 41
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pg/ml. Although, it has been reported that the level of BNP ≥ 100 pg/ml has a sensitivity of 90% of diagnosing congestive heart failure (CHF) in patients with CHF symptom (4), none of subjects in this study, including subjects with ≥ 100 pg/ml of BNP, showed symptom of CHF. The level of circulating CD34+ cells was measured to be 0.76 ± 0.39 cells/µl, and there was no significant difference between genders. The range of LVMI was 73.3-187.1, and 11 subjects applied to the definition of LV hypertrophy (LVMI: 131 ≤ in men and 100 ≤ in women) (3). Plasma BNP levels had a significant inverse correlation with number of circulating CD34+ cells (FIG 1 A), whereas FPG, HbA1c, BMI, SBP, DBP, and age showed no significant correlations. There was a significant correlation between number of circulating CD34+ cells and LVMI by echocardiography (FIG 1 B). LVFS and E/A were not associated with circulating CD34+ cell numbers (LVFS: r = -0.07, P = 0.72, E/A: r = -0.11, P = 0.59). There was also a significant correlation between BNP levels and LVMI (r = 0.59, p = 0.001).

In multiple regression analysis, the level of CD34+ cells was an independent correlate of both BNP (β = -1.64, p = 0.017) and LVMI (β = -0.337, p = 0.031) in model including age, HbA1c, SBP, BMI, and medication (ACE/ARB, statin, and TZD).

CONCLUSIONS

In this study, circulating CD34+ cell numbers were found to significantly correlate with, one of LV dysfunction markers, plasma BNP levels. To the best of our knowledge, this is the first report that circulating bone marrow-derived cells are associated with diabetic LV abnormality. Circulating CD34+ cell numbers also significantly correlated with LVMI, whereas they did not correlate with LVFS (an LV systolic function marker) or E/A (an LV diastolic function marker). LV hypertrophy is a well-known predictor of cardiovascular events independent of coronary artery disease. The Framingham Heart Study identified an association between diabetes and increased LV wall thickness and mass (5). Although the precise mechanisms underlying the association between diabetes and LV hypertrophy remains unknown, our results suggest that reduced circulating CD34+ cell numbers may be involved in the progression of LV hypertrophy in diabetic patients. However, further investigations are necessary to demonstrate this hypothesis.

We measured the level of CD34+ cell in this study, but not the levels of circulating CD34+/kinase insert domain receptor (KDR)+ cells that are regarded as EPCs. Circulating CD34+ cell level are associated with ischemic stroke (6), and administration of CD34+ cell ameliorates cerebral ischemia in mice (7). These indicate that CD34+ cell may be involved in cardiovascular disease. Indeed, another recent report indicated that the levels of circulating CD34+ cells are more strongly correlated with cardiovascular risk than the levels of EPCs (8). Therefore, our results suggest that the measurement of CD34+ cells may be available marker for diabetic LV hypertrophy.

Our study had several limitations. First, the study was performed only by cross-sectional analysis; therefore, a prospective study is needed to clarify whether circulating CD34+ cell numbers predict LV injury in diabetic patients. Second, although systemic blood pressure did not significantly associate with CD34+ cell numbers, further investigation of normotensive diabetic patients is needed to exclude the possible effects of...
hypertension on circulating CD34\(^+\) cell numbers, as most of the subjects in this study were hypertensive. Despite this caveat, these results may be of practical use in elderly patients with type 2 diabetes, as hypertension is a very common comorbid condition in this population. In conclusion, reduced circulating CD34\(^+\) cell numbers are significantly associated with plasma BNP concentration and LVMI in elderly patients with type 2 diabetes. These results suggest that decreased circulating CD34\(^+\) cells may be involved in LV hypertrophy and that measurement of circulating CD34\(^+\) cell numbers may be useful for the identification of diabetic patients at high risk of LV injury.
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REFERENCES


**FIG 1.** Correlation between CD34+ cell numbers and plasma BNP levels (A), and correlation between CD34+ cell numbers and LVMI (B), in type 2 diabetes patients (n = 26)