

# Circulating CD34-Positive Cell Number Is Associated With Brain Natriuretic Peptide Level in Type 2 Diabetic Patients

SADANORI OKADA, MD<sup>1</sup>  
 HISASHI MAKINO, MD, PHD<sup>1</sup>  
 AYAKO NAGUMO, MD<sup>1</sup>  
 TAKAKO SUGISAWA, MD, PHD<sup>1</sup>  
 MUNEYA FUJIMOTO, MD, PHD<sup>1</sup>  
 ICHIRO KISHIMOTO, MD, PHD<sup>1</sup>

YOSHIHIRO MIYAMOTO, MD, PHD<sup>1</sup>  
 AKIE KIKUCHI-TAURA<sup>2</sup>  
 TOSHIHIRO SOMA, MD<sup>2</sup>  
 AKIHIKO TAGUCHI, MD, PHD<sup>3</sup>  
 YASUNAO YOSHIMASA, MD, PHD<sup>1</sup>

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<sup>+</sup> 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<sup>+</sup> cells might be associated with LV dysfunction in patients with type 2 diabetes. Therefore, we studied the correlation between circulating CD34<sup>+</sup> cell levels and plasma brain natriuretic peptide (BNP) levels, an LV dysfunction marker, in type 2 diabetic 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 men and 14 women, duration of diabetes  $16.1 \pm 10.7$  years) who were over 60 years of age ( $70.5 \pm 6.4$  years). Statin was given to nine subjects. ACE inhibitor or angiotensin receptor blocker was given to nine subjects, and thiazolidinedione was given to two subjects. Subjects were excluded from the study if they had known cardiovascular disease or chronic renal failure (defined as serum creatinine  $\geq 180 \mu\text{mol/l}$ ). No study subject showed hypokinesia by echocardiography or electrocardiogram change, indicating myocardial ischemia. Systolic (SBP) and diastolic (DBP) blood pressure and anthropometric parameters were determined. Blood samples were taken after 12-h fasting to measure circulating CD34<sup>+</sup> cells, plasma BNP, fasting plasma glucose (FPG), and A1C. Circulating CD34<sup>+</sup> 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, Tokyo, Japan). We further examined LV fractional shortening (LVFS), LV mass index (LVMI) (3), and 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<sup>+</sup> cell level.

All statistical analyses were performed using JMP version 5.1.1 software (SAS Institute). Data are expressed as means  $\pm$  SD. Comparisons of number of CD34<sup>+</sup> cells by sex were made using the two-tailed unpaired *t* test. Correlations between number of CD34<sup>+</sup> 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, A1C levels, and BMIs in the study subjects were measured to be  $9.5 \pm 2.6 \text{ mmol/l}$ ,  $9.2 \pm 1.8\%$ , and  $26.4 \pm 4.3 \text{ kg/m}^2$ , respectively. A total of 88% of the patients had hypertension (SBP  $142 \pm 18 \text{ mmHg}$ , DBP  $75.7 \pm 13.5 \text{ mmHg}$ ). Plasma BNP levels were measured to be  $95 \pm 319 \text{ pg/ml}$ . Although it has been reported that the level of BNP  $\geq 100 \text{ pg/ml}$  has a sensitivity of 90% of diagnosing congestive heart failure (CHF) in patients with CHF symptoms (4), none of the subjects in this study, including subjects with  $\geq 100 \text{ pg/ml}$  of BNP, showed symptoms of CHF. The level of circulating CD34<sup>+</sup> cells was measured to be  $0.76 \pm 0.39 \text{ cells}/\mu\text{l}$ , and there was no significant difference between sexes. The range of LVMI was 73.3–340.2, and 11 subjects applied to the definition of LV hypertrophy (LVMI  $\leq 131$  in men and  $\leq 100$  in women) (3).

Plasma BNP levels had a significant inverse correlation with the number of circulating CD34<sup>+</sup> cells (Fig. 1A), whereas FPG, A1C, BMI, SBP, DBP, and age showed no significant correlations. There was a significant correlation between the number of circulating CD34<sup>+</sup> cells and LVMI by echocardiography (Fig. 1B). LVFS and E/A were not associated with circulating CD34<sup>+</sup> 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

From the <sup>1</sup>Department of Atherosclerosis, National Cardiovascular Center, Suita, Japan; the <sup>2</sup>Department of Hematology, Osaka Minami National Medical Center, Osaka, Japan; and the <sup>3</sup>Department of Cerebrovascular Disease, National Cardiovascular Center, Suita, Japan.

Address correspondence and reprint requests to Hisashi Makino, MD, Atherosclerosis, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan. E-mail: makinoh@hsp.ncvc.go.jp.

Received for publication 14 June 2007 and accepted in revised form 13 October 2007.

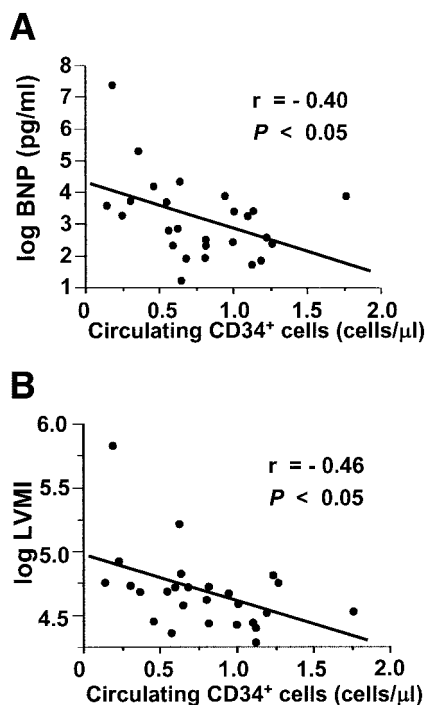
Published ahead of print at <http://care.diabetesjournals.org> on 24 October 2007. DOI: 10.2337/dc07-1125.

**Abbreviations:** BNP, brain natriuretic peptide; CHF, congestive heart failure; DBP, diastolic blood pressure; EPC, endothelial progenitor cell; FPG, fasting plasma glucose; LV, left ventricular; LVFS, LV fractional shortening; LVMI, LV mass index; SBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2008 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.



**Figure 1**—Correlation between CD34<sup>+</sup> cell numbers and plasma BNP levels (A) and correlation between CD34<sup>+</sup> cell numbers and LVMI (B) in type 2 diabetic patients (n = 26).

level of CD34<sup>+</sup> cells was an independent correlate of both BNP ( $\beta = -1.64$ ,  $P = 0.017$ ) and LVMI ( $\beta = -0.337$ ,  $P = 0.031$ ) in the model including age, A1C, SBP, BMI, and medication (ACE inhibitor/angiotensin receptor blocker, statin, and thiazolidinedione).

**CONCLUSIONS**— In this study, circulating CD34<sup>+</sup> cell number was found to significantly correlate with plasma BNP level, a marker of LV dysfunction. 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<sup>+</sup> 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 be-

tween diabetes and increased LV wall thickness and mass (5). Although the precise mechanisms underlying the association between diabetes and LV hypertrophy remain unknown, our results suggest that reduced circulating CD34<sup>+</sup> 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<sup>+</sup> cells in this study but not the levels of circulating CD34<sup>+</sup>/kinase insert domain receptor (KDR)<sup>+</sup> cells that are regarded as EPCs. Circulating CD34<sup>+</sup> cell levels are associated with ischemic stroke (6), and administration of CD34<sup>+</sup> cells ameliorates cerebral ischemia in mice (7). This indicates that CD34<sup>+</sup> cells may be involved in cardiovascular disease. Indeed, another recent report indicated that levels of circulating CD34<sup>+</sup> cells are more strongly correlated with cardiovascular risk than levels of EPCs (8). Therefore, our results suggest that measurement of CD34<sup>+</sup> cells may provide an indicator 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<sup>+</sup> cell numbers predict LV injury in diabetic patients. Second, although systemic blood pressure did not significantly associate with CD34<sup>+</sup> cell numbers, further investigation of normotensive diabetic patients is needed to exclude the possible effects of hypertension on circulating CD34<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> cells may be involved in LV hypertrophy and that measurement of circulating CD34<sup>+</sup> cell num-

bers may be useful for the identification of diabetic patients at high risk of LV injury.

## References

1. Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzakowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ: Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* 97:3075–3085, 2001
2. Kikuchi-Taura A, Soma T, Matsuyama T, Stern DM, Taguchi A: A new protocol for quantifying CD34(+) cells in peripheral blood of patients with cardiovascular disease. *Tex Heart Inst J* 33:427–429, 2006
3. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. *Circulation* 55:613–618, 1977
4. McCullough PA, Nowak RM, McCord J, Hollander JE, Herrmann HC, Steg PG, Duc P, Westheim A, Omland T, Knudsen CW, Storror AB, Abraham WT, Lamba S, Wu AHB, Perez A, Clopton P, Krishnaswamy P, Kazanegra R, Maisel AS, BNP multinational study investigators: B-type natriuretic peptide and clinical judgment in emergency diagnosis of heart failure: analysis from Breathing Not Property (BNP) Multinational Study. *Circulation* 106:416–422, 2002
5. Galderisi M, Anderson KM, Wilson PW, Levy D: Echocardiographic evidence for existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am J Cardiol* 68:85–89, 1991
6. Taguchi A, Matsuyama T, Moriwaki H, Hayashi T, Hayashida K, Nagatsuka K, Todo K, Mori K, Stern DM, Soma T, Naritomi H: Circulating CD34-positive cells provide an index of cerebrovascular function. *Circulation* 109:2972–2975, 2004
7. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T: Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* 114:330–338, 2004
8. Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A: Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. *Eur Heart J* 27:2247–2255, 2006