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Brief Communication

Circulating CD34-positive cells have prognostic value for neurologic function in patients with past cerebral infarction

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Increasing evidence points to a role for circulating endothelial progenitors, including populations of CD34-positive (CD34$^+$) cells present in peripheral blood, in vascular homeostasis and neovascularization. In this report, circulating CD34$^+$ cells in individuals with a history of cerebral infarction were correlated with changes in neurologic function over a period of 1 year. Patients with decreased levels of CD34$^+$ cells displayed significant worsening in neurologic function, evaluated by the Barthel Index and Clinical Dementia Rating. These results support the hypothesis that levels of circulating CD34$^+$ cells have prognostic value for neural function, consistent with their potential role in maintaining cerebral circulation.
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Keywords: CD34; cerebral circulation; neurologic function

Introduction

Increasing evidence points to a role for circulating CD34-positive (CD34$^+$) cells in maintaining vascular homeostasis, both as a pool of endothelial progenitor cells (EPCs) and as a source of multiple growth/angiogenesis factors (Majka *et al*, 2001). Previously, we have shown accelerated neovascularization after administration of CD34$^+$ cells in an experimental model of stroke (Taguchi *et al*, 2004*b*), and observed a positive correlation between levels of circulating CD34$^+$ cells and neovascularization (Yoshihara *et al*, 2008) and regional blood flow (Taguchi *et al*, 2004*a*) in patients with chronic cerebral ischemia. In addition, we have delineated a contribution of circulating CD34$^+$ cells in support of neurologic function, presumably through their positive influence on the cerebral circulation in settings of ischemic stress (Taguchi *et al*, 2008). A role for circulating CD34$^+$ cells in vascular homeostasis has also been considered in other ischemic settings, such as myocardial (Okada *et al*, 2008) and peripheral vascular disease (Fadini *et al*, 2006*b*).

On the basis of these observations, we have hypothesized that circulating CD34$^+$ cells may contribute to the maintenance of neurologic function by enhancing cerebrovascular homeostasis in patients with a history of cerebral infarction. In this study, we have investigated the predictive value of the level of peripheral CD34$^+$ cells on neurologic function in patients with past cerebral infarction. Our results display a correlation between decreased levels of CD34$^+$ cells and diminished neurologic function over a study period of 1 year.

Methods

This study was approved by the institutional review board of the National Cardiovascular Center. All subjects provided written informed consent. A total of

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40 individuals with history of cerebral infarction (3 years or more from the last onset of stroke) were enrolled and followed for 1 year. Exclusion criteria included the following: patients who experienced a vascular event within 30 days of enrollment, patients with neurodegenerative diseases including Alzheimer's-type cognitive impairment, history of cerebral hemorrhage, cerebral infarction not classified according major causes (lacunar, atherothrombotic, or cardiogenic embolism), evidence of infection, malignant disease, and/or premenopausal women. On the day the first blood sample was obtained and 1 year after, all individuals were evaluated using the National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS), Barthel Index (BI), and Clinical Dementia Rating (CDR) by a single examiner masked to the experimental protocol and level of circulating CD34+ cells. Hypertension, hyperlipidemia, and diabetes mellitus were defined based on the need for oral anti-hypertensive, anti-hyperlipidemic, or oral anti-diabetic drug therapy (or insulin), respectively, prescribed by the primary care physician. Smoking was defined as a history of >2 years and/or smoking in the last year. Using a modification of the International Society of Hematotherapy and Graft Engineering (ISHAGE) Guidelines (Sutherland *et al*, 1996), the number of circulating CD34+ cells was quantified as described (Kikuchi-Taura *et al*, 2006) at the point of the entry and 1 year later. In brief, blood samples were incubated with phycoerythrin (PE)-labeled anti-CD34 antibody, fluorescein isothiocyanate (FITC)-labeled anti-CD45 antibody, 7-aminoactinomycin-D (7-AAD), and internal control (all of these reagents are in the Stem-Kit, BeckmanCoulter, Marseille, France). 7-AAD-positive dead cells and CD45-negative cells were excluded, and the number of cells forming a cluster characteristic of CD34+ cells (i.e., low side scatter and low-to-intermediate CD45 staining) was counted. The absolute number of CD34+ cells was calculated using the internal control. On the basis of our previous studies, the cumulative intraassay coefficient of variation of the measurement was 7.4% and test–retest intraclass correlation of the level of CD34+ cells is 0.88 (Taguchi *et al*, 2004*a*). For statistical analysis, JMP version 5.1J was used. Individual comparisons were performed using a Mann–Whitney's *U*-test, χ^2-test, or two-tailed unpaired Student's *t*-test. Pearson's correlation coefficient was used to evaluate the correlation of the levels of CD34+ cells between measurements. Mean ± s.e. is shown.

Results

To investigate the possible relationship between circulating CD34+ cells and changes in neurologic status over the 1-year-study period, individuals were divided into two groups according to the level of circulating CD34+ cells at the point of the entry. Baseline characteristics of the

Table 1 Baseline characteristic

	Total	*Group low*	*Group high*	P-*value for trend*
N	40	20	20	
At the point of entry				
No. of CD34+ cells (per μL)	0.65 ± 0.07	0.34 ± 0.03	0.93 ± 0.10	
Age (years)	73.1 ± 1.1	72.9 ± 1.4	73.4 ± 1.7	0.85
Male gender, *n* (%)	28 (70)	12 (60)	16 (80)	0.16
Time from last stroke (years)	4.5 ± 0.2	4.5 ± 0.3	4.6 ± 0.3	0.75
Etiology, n *(%)*				0.83
Lacuna	25 (63)	13 (65)	12 (60)	
Atherothrombotic	12 (30)	6 (30)	6 (30)	
Cardiogenic embolism	3 (8)	1 (5)	2 (10)	
Risk factor, n *(%)*				
Hypertension	24 (60)	12 (60)	12 (60)	1.00
Hyperlipidemia	15 (38)	8 (40)	7 (35)	0.74
Diabetes mellitus	6 (15)	4 (20)	2 (10)	0.37
Smoking	8 (20)	5 (25)	3 (15)	0.42
Other cardiovascular disease	9 (23)	3 (15)	6 (30)	0.26
Treatment, n *(%)*				
Ca-channel blockers	13 (33)	7 (35)	6 (30)	0.74
ARB	14 (35)	7 (35)	7 (35)	1.00
ACE inhibitor	3 (8)	2 (10)	1 (5)	0.54
Diuretic	2 (5)	1 (5)	1 (5)	1.00
Beta-blockers	0 (0)	0 (0)	0 (0)	NA
Aspirin	19 (48)	7 (35)	12 (60)	0.11
Ticlopidine	8 (20)	6 (30)	2 (10)	0.11
Statin	14 (35)	8 (40)	6 (30)	0.51
One year after				
No. of CD34+ cells (per μL)	0.69 ± 0.07	0.42 ± 0.05	0.97 ± 0.09	<0.001

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; NA, not available.

groups are shown in Table 1. Comparing these groups, there were no significant differences in age, gender, etiology of cerebral infraction, hypertension, hyperlipidemia, diabetes mellitus, smoking, and drug treatments. In univariate analysis, each cerebrovascular risk factor, including hypertension ($P=0.46$), hyperlipidemia ($P=0.35$), diabetes mellitus ($P=0.12$), and smoking ($P=0.35$), was not significantly correlated with a decrease in the number of circulating CD34+ cells. Treatment with a Ca-channel blocker ($P=0.73$), angiotensin-converting enzyme (ACE) inhibitor ($P=0.053$), angiotensin II receptor blocker (ARB) ($P=0.53$), diuretics ($P=0.52$), statins ($P=0.47$), aspirin ($P=0.86$), and/or ticlopidine ($P=0.80$) also did not correlate with a consistent difference in the number of circulating CD34+ cells. Each cerebrovascular risk factor and particular drug treatment was also not associated with a significant difference in neurologic function in 1 year, based on NIHSS, mRS, BI, and CDR (data not shown). At the point of entry, there were no significant differences in neurologic or cognitive function between groups (Figures 1A–1D). Compared with levels of circulating CD34+ cells in non-stroke control subjects presented in our previous report (0.81 ± 0.06 cells/μL; age, 74.2 ± 0.7; $n=32$) (Taguchi *et al*, 2008), the level of circulating CD34+ cells was significantly reduced in patients in the CD34+ cell low group in the current study ($P<0.001$). There was no significant difference between the level of circulating CD34+ cells in the CD34+ cell high group (in the current study) and the previously reported value ($P=0.20$; Taguchi *et al*, 2008). During the period of our observation, no patients had special exercise training, other than intensive rehabilitation in patients who had recurrent strokes.

During the 12-month-study period, 5 patients had recurrent strokes (3 patients in the lower CD34+ and 2 in the higher CD34+ group, respectively; $P=0.63$ between groups). After 12 months, neurologic and cognitive functions of all patients were reexamined, and changes in each score were recorded. Although there was no significant difference in the NIHSS score between groups (Figure 1E, $P=0.28$), there was significant worsening in neurologic function, based on BI in patients with decreased levels of CD34+ cells versus the group with increased levels (Figure 1F, $P=0.04$). Similarly, a trend towards worsening of mRS occurred in patients with decreased levels of CD34+ cells versus the group with increased levels, although these results did not achieve statistical significance (Figure 1G, $P=0.65$). In terms of cognitive function, a significant worsening in the CDR score was observed in patients with decreased levels of CD34+ cells, compared with the higher CD34+ cell group (Figure 1H, $P=0.002$). It is notable that no individual in the highest quartile ($n=10$) for levels of CD34+ cells displayed worsening of the CDR or BI score over the 1-year-study period. In the analysis of the patients without a recurrent stroke, a similar trend was observed (Figures 1I–1L), although the change of BI did not achieve statistically significant ($P=0.08$). Analysis of the correlation coefficient of the levels of CD34+ cells between at the point of the entry and 1 year later revealed significant strong correlation in patients without recurrence ($P<0.001$, $R^2=0.68$).

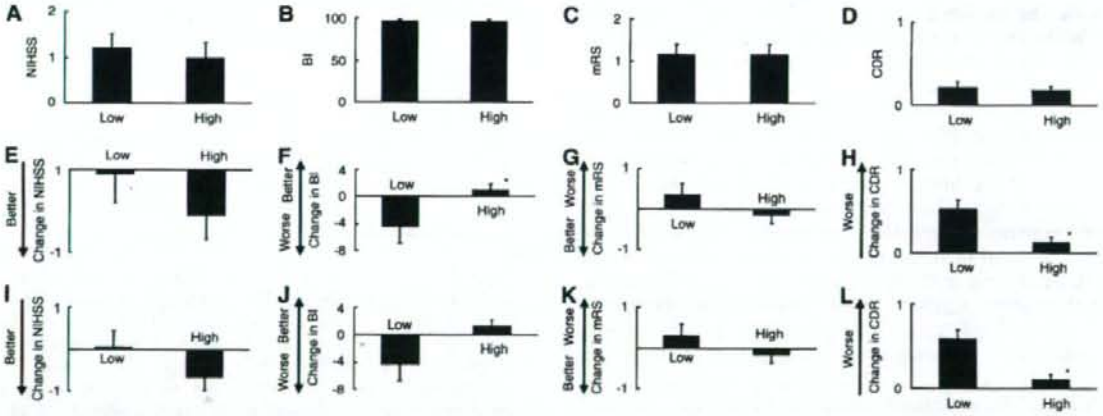

Figure 1 The level of circulating CD34+ cells and neurologic function in the study group after 1 year. (**A–D**) At the point of entry, there were no significant differences in the level of neurologic function, including NIHSS (**A**), BI (**B**), mRS (**C**), and CDR (**D**). (**E–H**) There was a trend suggesting accelerated worsening of neurologic function, evaluated by NIHSS, in patients with decreased levels of circulating CD34+ cells, although this did not achieve statistical significance (**E**). Compared with BI scores in patients with increased levels of circulating CD34+ cells, significant worsening was observed in patients with decreased levels of CD34+ cells (**F**). There was a trend of worsening of mRS in patients with decreased levels of circulating CD34+ cells, although this did not achieve statistical significance (**G**). Significantly poorer CDR scores were observed in patients with decreased levels of CD34+ cells, compared with those with increased levels of CD34+ cells (**H**). (**I–L**) Analysis of patients without recurrent strokes showed nonsignificant differences, but a similar trend was observed in changes in NIHSS (**I**), BI (**J**), and mRS (**K**). Poorer CDR scores were observed in patients with decreased levels of CD34+ cells, compared with those with increased levels of CD34+ cells (**L**), and this difference achieved statistical significance. *$P<0.05$ versus patients with decreased levels of circulating CD34+ cells.

Discussion

In this study, we have found that the level of circulating CD34+ cells has prognostic value for neural function in support of activities of daily living (BI) and cognitive function (CDR) in patients with a history of cerebral infarction. This result is potentially consistent with a role of CD34+ cells in maintenance of cerebral vasculature.

Similar to the correlation between mobilization of CD34+ cells and improved myocardial function after a coronary ischemic event (Wojakowski *et al*, 2006), mobilization of circulating CD34+ cells has been shown to correlate with functional recovery during the acute phase of cerebral infarction (Dunac *et al*, 2007; Yip *et al*, 2008). Our report herein shows a relationship between increased levels of CD34+ cells and improved functional outcome even in the extensive phase after stroke. These observations may reflect a close relationship between angiogenesis and neurogenesis under physiologic (Louissaint *et al*, 2002), as well as pathologic (Taguchi *et al*, 2004*b*) conditions.

The level of EPCs can be quantified using an assay for endothelial colony formation or fluorescence-activated cell-sorting analysis with multiple markers, including CD34 and kinase insert domain receptor (KDR) (Werner *et al*, 2005). Although the population of CD34+ cells is enriched in EPCs, it comprises multiple and heterogeneous subpopulations, indicating the possible advantage of selectively quantifying EPCs. However, measurement of EPCs is quite inexact, as large variations in their levels have been reported (i.e., by ~100-fold between reports) (Fadini *et al*, 2006*a*; Werner *et al*, 2005). Thus, there appears to be a need to standardize measurement of EPCs, in addition to a requirement for a relatively large blood volume to do the assay (for example, Loomans *et al* collected a 60 mL blood sample for EPC analysis) (Loomans *et al*, 2004). Our method for quantification of CD34+ cells is simple, reproducible (Kikuchi-Taura *et al*, 2006) and requires only 200 μL of peripheral blood. The latter method is suitable for screening a broad group of patients at risk for cerebrovascular disorders. Furthermore, CD34+ cells have been shown to secrete multiple growth/angiogenesis factors (Majka *et al*, 2001), contributing to maintenance of the microvasculature in addition to serving as a source of EPCs. These considerations indicate the value of quantitating peripheral CD34+ cells as a clinical biomarker in patients with vascular disease, not only as a substitute for quantifying EPCs.

In conclusion, our results indicate that circulating CD34+ cells in patients with cerebral ischemia have a positive impact on the course of disease, in terms of maintenance of neurologic function. In contrast, decreased levels of circulating CD34+ cells, possibly because of 'exhaustion' of the bone marrow or inability to mount an increase in cell counts, are associated with deterioration of neurologic status. Taken together with our previous results indicating that the level of circulating CD34+ cells can be correlated with cerebral blood flow and cerebral metabolic rate in patients with chronic cerebral hypoperfusion (Taguchi *et al*, 2004*a*), our present findings provide further support for a contribution of circulating CD34+ cells in maintenance of neurologic function in settings of ischemic stress. Although further basic and clinical studies will be required, we speculate that treatments with the goal of increasing levels of circulating CD34+ cells have the possibility of improving neurologic outcome in patients with impaired cerebral microcirculation.

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Conflict of interest

We declare that we have no conflicts of interest.

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Low circulating CD34$^+$ cell count is associated with poor prognosis in chronic hemodialysis patients

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Circulating CD34-positive (CD34$^+$) cells, a population that includes endothelial progenitor cells, are believed to contribute to vascular homeostasis. Here we determine the prognostic value of CD34$^+$ cell measurements in 216 chronic hemodialysis patients. A total of 43 cardiovascular events and 13 deaths occurred over an average 23 months follow-up in this cohort. A cutoff number for circulating CD34$^+$ cells was determined by receiver operating characteristic curve analysis to maximize the power of the CD34$^+$ cell count in predicting future cardiovascular events. Based on this, 93 patients were categorized as having low and 123 patients as having high numbers of CD34$^+$ cells, determined by flow cytometry at the time of enrollment. Both cumulative cardiovascular event-free survival and all-cause survival were significantly less in the group of patients with low numbers of CD34$^+$ cells. By multivariate analyses, a low level of circulating CD34$^+$ cells was an independent and significant predictor for both cardiovascular events and all-cause mortality. Our study shows that a reduced number of circulating CD34$^+$ cells is significantly associated with vascular risks and all-cause mortality in patients on chronic hemodialysis. These cells may be a useful biomarker.

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KEYWORDS: dialysis; endothelial progenitor cells; cardiovascular disease; risk factors

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It is well known that cardiovascular disease (CVD) is the leading cause of death among chronic hemodialysis (HD) patients.[1] However, the traditional risk factors (including hypertension and increased low-density lipoprotein (LDL) cholesterol) and uremia-related risk factors (hemodynamic overload, abnormal calcium metabolism, and so on) do not fully explain the extent and severity of CVD observed among this population.[2–4]

Growing evidence suggests that bone-marrow-derived circulating progenitor cells, including CD34-positive (CD34$^+$) cells, contribute to vascular homeostasis in adults,[5,6] not only as a pool of endothelial progenitor cells (EPCs) but also as the source of growth/angiogenesis factors.[7] The level of EPCs has been shown to predict future events and deaths from CVD among patients with coronary artery disease (CAD).[8,9] We have also shown that a lower number of circulating CD34$^+$ cells is significantly associated with vascular risks.[10–12]

Several researchers have demonstrated that patients on dialysis had a lower EPC count than did control subjects.[13–16] However, there is no definite consensus concerning the absolute number of EPCs in HD patients and its relationship with the prognosis.

These observations prompted us to conduct the present study. We hypothesize that circulating CD34$^+$ cells accelerate the repair of the dysfunctional endothelium, and that a reduced number of these cells results in poor outcomes in chronic HD patients. In this study, we measured the levels of circulating CD34$^+$ cells and prospectively analyzed first CV (cardiovascular) events and deaths by any cause.

RESULTS

Relationship between CD34$^+$ cell count and baseline variables

Out of 216 chronic HD patients who participated in this study, none was lost to follow-up, and none received kidney transplants. The number of circulating CD34$^+$ cells ranged from 0.07 to 2.17/μl (median, 0.41/μl), with a mean (±s.d.)

of 0.49 ± 0.32/μl. The age of the patients was 65 ± 11 years (range, 35–94 years). A multivariate regression analysis revealed that factors positively associated with the $CD34^+$ cell count were gender (male), elevated white blood cell count, and high serum albumin, whereas the negatively associated factors were advanced age and smoking (Table 1).

Baseline clinical variables for the low/high $CD34^+$ groups

To further clarify the importance of $CD34^+$ cells, we then determined a cutoff value. A receiver operating characteristic (ROC) curve analysis showed 0.37/μl to be the value (area under the curve = 0.707) to maximize the power of circulating $CD34^+$ cell levels as a predictor of a CV event (Figure 1). The patients were categorized into two groups according to the cell count at the time of enrollment: the low $CD34^+$ group representing 93 patients with circulating $CD34^+$ cell counts less than 0.37/μl (a mean of 0.23 ± 0.08/μl) and the high $CD34^+$ group representing 123 patients with counts of 0.37/μl or greater (a mean of 0.69 ± 0.30/μl). The baseline characteristics are shown in Table 2. Patients in the low $CD34^+$ group were older (68 ± 9 years) than those in the high $CD34^+$ group (62 ± 11 years) ($P < 0.0001$). White blood cell counts were lower in the former group than in the latter. Body mass index and calcium-phosphate product (Ca × Pi) levels were also lower in the patients of the low $CD34^+$ group. Gender, duration of HD, smoking, incidence of diabetes, history of CVD, and the use of erythropoietin, were comparable between the two groups. Medications commonly used to decrease CVD, including statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium antagonists, β-blockers were also comparable (Table 2).

Table 1 | Relationship between $CD34^+$ cell count and baseline variables on multivariate regression analysis

	β	P-value
Male	0.197	**0.021**
Age	−0.157	**0.039**
Duration of HD	0.001	0.99
Diabetes	0.054	0.50
Hypertension	−0.079	0.24
Smoking	−0.294	**0.0001**
Body mass index	0.043	0.57
History of CVD	−0.035	0.61
Hemoglobin	−0.124	0.54
WBC	0.300	**<0.0001**
Albumin	0.148	**0.049**
HDL cholesterol	−0.036	0.61
LDL cholesterol	−0.058	0.39
Ca × Pi	0.092	0.19
Intact PTH	0.197	0.34
C-reactive protein	−0.002	0.97
Kt/V_{urea}	0.080	0.36

Ca × Pi, calcium-phosphate product; CVD, cardiovascular disease; HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; Kt/V_{urea}; urea clearance × time normalized by total body water; WBC, white blood cell. *P*-values <0.05 are shown in bold.

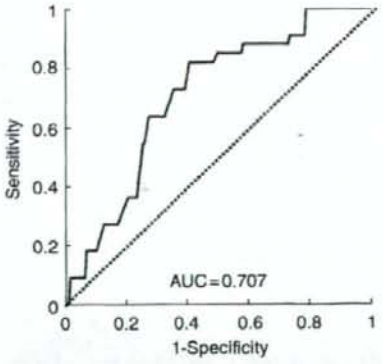

Figure 1 | ROC Curve Analysis. A ROC curve analysis was performed to determine a cutoff value for circulating $CD34^+$ cell count. The result showed 0.37/μl to be the value (area under the curve = 0.707) to maximize the power of the $CD34^+$ cell count in predicting a future CV event.

Incidence of CV events and all-cause deaths

Table 3 shows the incidence of outcomes. In the low $CD34^+$ group, a CV event occurred in 27 out of 93 patients (29%) and 5 patients died from CVD (5.4%). In the high $CD34^+$ group, a CV event occurred in 16 (13%) and only 1 patient died of CVD. Concerning death by any cause, 10 patients (10.8%) died in the low $CD34^+$ group, whereas three (2.4%) died in the high $CD34^+$ group (Table 3). The cumulative CV event-free survival was significantly lower in the low $CD34^+$ group (70.6%) than the high $CD34^+$ group (86.8%) ($P = 0.0034$; Figure 2). The cumulative all-cause survival was also lower in the low $CD34^+$ group (89.2%) than in the high $CD34^+$ group (97.5%) ($P = 0.012$; Figure 3).

Factors associated with CV events

Factors associated with CV events are shown in Table 4. In univariate analyses, the incidence of CV events was significantly associated with a level of circulating $CD34^+$ cells lower than 0.37/μl (hazard ratio (HR), 2.90; 95% CI, 1.45–5.81; $P = 0.0026$), advanced age (HR, 1.03; 95% CI, 1.01–1.06; $P = 0.021$), a history of CVD (HR, 7.85; 95% CI, 2.43–12.50; $P = 0.0045$), a low level of serum albumin (HR, 0.24; 95% CI, 0.08–0.67; $P = 0.0066$), or a high level of LDL cholesterol (HR, 1.02; 95% CI, 1.01–1.03; $P = 0.0048$). In a multivariate regression analysis, a level of circulating $CD34^+$ cells lower than 0.37/μl (HR, 2.23; 95% CI, 1.09–4.58; $P = 0.028$), a history of CVD (HR, 6.19; 95% CI, 1.63–9.90; $P = 0.014$), a low level of serum albumin (HR, 0.33; 95% CI, 0.11–0.99; $P = 0.049$), and a high level of LDL cholesterol (HR, 1.02; 95% CI, 1.01–1.03; $P = 0.011$) were identified as independent predictors of CV events among chronic HD patients (Table 4).

Factors associated with all-cause death

Factors associated with all-cause deaths are shown in Table 5. In univariate analyses, all-cause death was significantly associated with a level of circulating $CD34^+$ cells lower than

Table 2 | Baseline characteristics of the low/high CD34+ groups

	All patients (n=216)	Low CD34+ group (CD34+ <0.37/μl) (n=93)	High CD34+ group (CD34+ >0.37/μl) (n=123)	P-value
Male (%)	122 (56.4)	50 (53.7)	72 (58.5)	0.48
Age (years)	65 ± 11	68 ± 9	62 ± 11	**<0.0001**
Duration of HD (years)	8.1 ± 7.1	8.7 ± 7.7	7.8 ± 6.7	0.39
Diabetes (%)	105 (48.6)	44 (47.3)	61 (49.5)	0.73
Hypertension (%)	157 (72.7)	67 (72.0)	90 (74.3)	0.7
Smoking (%)	64 (29.6)	32 (34.7)	32 (26.0)	0.16
Body mass index	20.7 ± 3.2	19.9 ± 2.7	21.4 ± 3.4	**0.0008**
History of CVD (%)	94 (43.5)	46 (49.5)	48 (39.0)	0.12
CD34+ cells (/μl)	0.49 ± 0.32	0.69 ± 0.30	0.23 ± 0.08	**0.0001**
Hemoglobin (g/100 ml)	10.6 ± 1.1	10.3 ± 1.1	10.5 ± 1.3	0.11
WBC (10^3/μl)	5.9 ± 1.9	5.4 ± 1.6	6.4 ± 1.9	**<0.0001**
Albumin (mg/100 ml)	3.6 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	0.11
HDL cholesterol (mg/100 ml)	41 ± 13	42 ± 12	40 ± 14	0.3
LDL cholesterol (mg/100 ml)	77 ± 27	75 ± 27	76 ± 26	0.93
Ca × Pi	49.7 ± 11.8	47.2 ± 11.6	51.7 ± 11.7	**0.0062**
Intact PTH (ng/ml)	122 ± 114	116 ± 130	126 ± 101	0.52
C-reactive protein (mg/100 ml)	0.42 ± 0.86	0.45 ± 0.78	0.36 ± 0.83	0.41
KT/V_{urea}	1.46 ± 0.23	1.49 ± 0.24	1.44 ± 0.22	0.1
Erythropoietin (U/kg)	93 ± 66	99 ± 69	86 ± 64	0.5
Statins (%)	27 (12.5)	10 (10.8)	17 (13.8)	0.49
ARB (%)	87 (40.3)	36 (38.7)	51 (41.5)	0.68
ACEI (%)	38 (17.6)	15 (16.1)	23 (18.7)	0.62
Ca antagonist (%)	133 (61.6)	60 (64.5)	73 (59.4)	0.44
β-Blocker (%)	45 (20.8)	24 (25.8)	21 (17.1)	0.12

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca × Pi, calcium-phosphate product; CVD, cardiovascular disease; HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; KT/V_{urea}: urea clearance × time normalized by total body water; WBC, white blood cell. P-values <0.05 are shown in bold.

Table 3 | First cardiovascular events and all-cause death during follow-up period

	All patients (n=216)	Low CD34+ group (CD34+ <0.37/μl) (n=93)	High CD34+ group (CD34+ >0.37/μl) (n=123)
Total number of CV events (%)	43 (19.9)	27 (29.0)[a]	16 (13.0)
Nonfatal			
Coronary artery disease	27	16	11
PCI	25	15	10
CABG	2	1	1
Stroke	5	3	2
PAD	5	3	2
Fatal			
Congestive heart failure	3	3	0
Stroke	1	0	1
Myocardial infarction	1	1	0
Valve disease	1	1	0
Total number of death (%)	13 (6.0)	10 (10.8)[b]	3 (2.4)
Congestive heart failure	5	3	2
Stroke	3	2	1
Myocardial infarction	1	1	0
Valve disease	1	1	0
Infection	2	2	0
Ischemic colitis	1	1	0

CV, cardiovascular; CABG; coronary artery bypass graft; PAD, peripheral artery disease; PCI, percutaneous coronary intervention.
[a]P=0.0032 vs high CD34+ group.
[b]P=0.012 vs high CD34+ group.

0.37/μl, advanced age, a low body mass index, or a low level of serum albumin. In a multivariate regression analysis, a level of circulating CD34+ cells lower than 0.37/μl (HR, 5.02; 95% CI, 1.08–23.25; P = 0.040), advanced age (HR, 1.09; 95% CI, 1.02–1.15; P = 0.0082), and a low level of serum albumin (HR, 0.16; 95% CI, 0.01–0.44; P = 0.0018) were identified as independent predictors of all-cause death among chronic HD patients (Table 5).

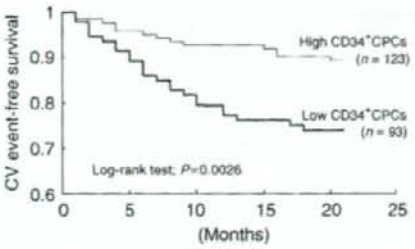

Figure 2 | Cumulative CV event-free survival in the low/high CD34$^+$ groups. Patients were categorized into the low CD34$^+$ group (CD34$^+$ cells $<0.37/\mu l$, $n=93$) or the high CD34$^+$ group (CD34$^+$ cells $>0.37/\mu l$, $n=123$) using the cutoff value determined by ROC curve analysis. CV event-free survival was defined as the period before the first CV event.

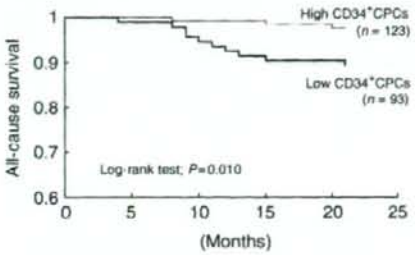

Figure 3 | Cumulative all-cause survival in the low/high CD34$^+$ groups. All-cause survival was analyzed for the low CD34$^+$ group (CD34$^+$ cells $<0.37/\mu l$, $n=93$) and the high CD34$^+$ group (CD34$^+$ cells $>0.37/\mu l$, $n=123$).

Stability of the CD34$^+$ cell count

At 3 years after enrollment, 20 HD patients were randomly selected from among the participants, and the second and the third measurements of CD34$^+$ cell count were conducted at an interval of 1 month. A good correlation was observed between these two additional measurements, which were then averaged for each of the 20 patients. When this value was compared to the first measurement conducted upon enrollment, a correlation was observed, suggesting that the CD34$^+$ cell count is stable over a 3-year period (Figure 4).

DISCUSSION

Recent studies have shown that a low number of EPCs is associated with a poor CV outcome among non-HD patients who had CAD.[8,9] The present study clearly demonstrated that a reduced number of CD34$^+$ cells in the peripheral blood was significantly associated with future CV events as well as all-cause deaths in chronic HD patients. Of importance is the fact that the absolute number of CD34$^+$ cells obtained from chronic HD patients was much lower ($0.49 \pm 0.32/\mu l$) than that obtained from patients with cerebrovascular disease ($1.1 \pm 0.31/\mu l$) or control subjects ($1.6 \pm 0.2/\mu l$).[11]

We measured the levels of CD34$^+$ cells but not the levels of EPCs, which are positive for both CD34 and kinase insert domain receptor. We have shown that circulating CD34$^+$ cell levels are associated with ischemic stroke,[11] brain natriuretic peptide level in type 2 diabetes patients,[10] and vascular risk associated with cognitive impairment.[12] We have also shown that administration of CD34$^+$ cell ameliorates cerebral

Table 4 | HR for cardiovascular events on Cox proportional hazard models

	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
CD34$^+$ cells $<0.37/\mu l$	2.90 (1.45–5.81)	**0.0026**	2.23 (1.09–4.58)	**0.028**
Male	0.87 (0.39–1.45)	0.4		
Age (years)	1.03 (1.01–1.06)	**0.021**	1.01 (0.97–1.04)	0.77
Duration of HD (years)	1.01 (0.95–1.04)	0.93		
Diabetes	1.76 (0.90–3.43)	0.099		
Hypertension	1.05 (0.54–2.17)	0.89		
Smoking	1.38 (0.74–2.59)	0.31		
Body mass index	0.90 (0.80–1.01)	0.075		
History of CVD	7.85 (2.43–12.50)	**0.0045**	6.19 (1.63–9.90)	**0.014**
Hemoglobin (g/100 ml)	0.93 (0.72–1.21)	0.57		
WBC ($10^3/\mu l$)	1.02 (0.87–1.19)	0.81		
Albumin (mg/100 ml)	0.24 (0.08–0.67)	**0.0066**	0.33 (0.11–0.99)	**0.049**
HDL cholesterol (mg/100 ml)	0.99 (0.97–1.02)	0.59		
LDL cholesterol (mg/100 ml)	1.02 (1.01–1.03)	**0.0048**	1.02 (1.01–1.03)	**0.011**
Ca × Pi	1.01 (0.92–1.11)	0.74		
Intact PTH (ng/ml)	1.00 (0.99–1.01)	0.37		
C-reactive protein (mg/100 ml)	1.18 (0.89–1.56)	0.26		
KT/V_{urea}	0.81 (0.22–2.98)	0.75		
Erythropoietin (U/kg)	1.00 (0.99–1.01)	0.34		
Statins	1.20 (0.46–3.23)	0.7		
ARB	0.96 (0.38–8.40)	0.93		
ACEI	1.47 (0.63–3.45)	0.37		
Ca antagonist	0.91 (0.57–2.11)	0.78		
β-Blocker	1.08 (0.42–2.02)	0.84		

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca × Pi, calcium-phosphate product; CVD, cardiovascular disease; HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; KT/V_{urea}: urea clearance × time normalized by total body water; WBC, white blood cell. Multivariate model includes variable with $P<0.05$ by univariate analysis. *P*-values <0.05 are shown in bold.

Table 5 | HR for all-cause mortality on Cox proportional hazard models

	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
CD34$^+$ cells < 0.37/μl	6.17 (1.34–18.57)	**0.019**	5.02 (1.08–23.25)	**0.04**
Male	0.92 (0.28–3.02)	0.88		
Age (years)	1.11 (1.04–1.18)	**0.0009**	1.09 (1.02–1.15)	**0.0082**
Duration of HD (years)	1.03 (0.94–1.10)	0.63		
Diabetes	1.86 (0.54–6.32)	0.32		
Hypertension	1.03 (0.27–3.86)	0.96		
Smoking	1.32 (0.43–4.04)	0.62		
Body mass index	0.73 (0.57–0.92)	**0.0087**	0.79 (0.62–1.01)	0.054
History of CVD	2.35 (0.72–7.69)	0.15		
Hemoglobin (g/100 ml)	0.86 (0.52–1.42)	0.56		
WBC (10^3/μl)	1.07 (0.83–1.37)	0.62		
Albumin (mg/100 ml)	0.19 (0.08–0.58)	**0.0006**	0.16 (0.01–0.44)	**0.0018**
HDL cholesterol	0.99 (0.97–1.04)	0.69		
LDL cholesterol	1.01 (0.99–1.03)	0.33		
Ca × Pi	1.02 (0.98–1.07)	0.75		
Intact PTH (ng/ml)	1.00 (0.99–1.02)	0.48		
C-reactive protein	1.04 (0.51–2.08)	0.91		
KT/V_{urea}	0.56 (0.07–4.78)	0.6		
Erythropoietin	1.01 (0.99–1.02)	0.34		
Statins	1.80 (0.38–8.40)	0.45		
ARB	0.32 (0.07–1.51)	0.66		
ACEI	0.60 (0.08–4.80)	0.63		
Ca antagonist	0.73 (0.46–4.08)	0.56		
β-Blocker	0.57 (0.17–1.87)	0.36		

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca × Pi, calcium-phosphate product; CVD, cardiovascular disease; HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; KT/V_{urea}, urea clearance × time normalized by total body water; WBC, white blood cell. Multivariate model includes variable with $P < 0.05$ by univariate analysis. *P*-values < 0.05 are shown in bold.

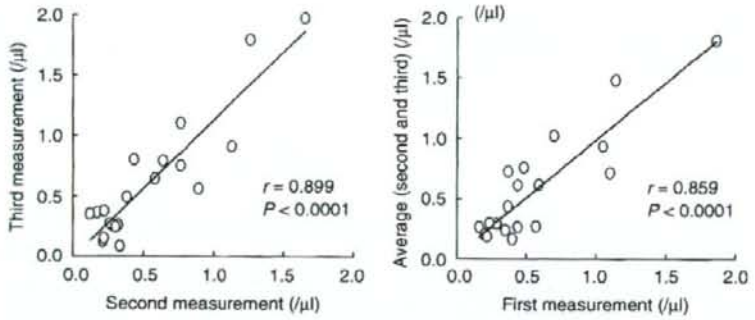

Figure 4 | Stability of CD34$^+$ Cell Count. Among 20 of the participants, CD34$^+$ cell counts were measured three times. The left graph shows the relationship between the second and the third measurements performed 3 years after enrollment, at an interval of 1 month. The right graph shows the relationship between the first measurement and the average of the second and the third measurements.

ischemia in mice.[17] In humans, injection of CD34$^+$ cells derived from peripheral blood improved the ischemia of the lower limbs.[18] These results support the hypothesis that circulating CD34$^+$ cells are involved in the pathogenesis of CVD. Indeed, a recent study by Fadini *et al.*[19] demonstrates that the levels of CD34$^+$ cells predict cardiovascular outcome more strongly than the levels of EPCs. In addition, previous studies have shown that our method for quantifying circulating CD34$^+$ cells is simple and reproducible.[10–12,20,21] Moreover, the present study demonstrated that the CD34$^+$ cell count is relatively stable over 3 years. Therefore, the measurement of CD34$^+$ cells would be useful for screening a high-risk population such as chronic HD patients.

In the present study, we set the death from any cause, not the death from CVD, as the primary end point. We presumed that the number of CV deaths would be too small to draw a definite conclusion. Moreover, we thought that all-cause death may be more suitable for this study because it would be difficult to identify all deaths by CVD in chronic HD patients. In fact, both patients whose recorded cause of death was infection had suffered strokes, and one had severe peripheral vascular disease. Although these two patients did not die from CVD directly, their atherosclerotic vascular diseases likely contributed to their outcomes.

We tried to enroll all of the out patients undergoing HD in the clinic. Therefore, it is not likely that there was a selection bias.

The major limitation of the present study would be the sample size. It is known that cardiovascular events and all-cause mortality in Japanese are significantly lower than those among Caucasians and African Americans.[4,22] In the present study, only 13 (6%) out of 216 patients died during the follow-up period. To draw a more definite conclusion, a larger population would need to be studied. Moreover, a previous study has shown that HD patients who had an elevated number of circulating endothelial cells were at risk for a CV event probably because endothelial cells had become detached from the injured endothelium.[23] A study to clarify the relationship between circulating endothelial cells and CD34+ cells would be worth pursuing.

In summary, the present study demonstrates that a low level of circulating $CD34^+$ cells predicts both future CV events and all-cause deaths in chronic HD patients. Pending further studies, we propose that a single measurement of $CD34^+$ cells taken from the peripheral blood is useful in identifying chronic HD patients at high risk.

MATERIALS AND METHODS

Study population

All the outpatients who underwent maintenance HD therapy in Nagoya Kyoritsu Hospital were eligible for this study. Patients who experienced a vascular event within 30 days of measurements, and those with evidence of infection and/or malignant disease were excluded. A total of 216 chronic HD patients were enrolled in this study between March 2005 and May 2005. The study was performed according to the guidelines of the Declaration of Helsinki Principles, and all patients gave their informed written consent to participate in this study, which was approved by the local ethics committee.

Follow-up

Clinical follow-up was conducted until April 2007. The data for all participants were obtained from medical records kept during the clinical follow-up period. Patients were adequately managed with regular HD treatment three times a week, and routine screening tests for CVD were performed as described previously.[4] In brief, a standard electrocardiogram and chest X-ray were taken every month, and an echocardiogram and a treadmill exercise test were performed at least once a year. When a patient showed abnormal findings in these routine tests or symptoms of CAD during the follow-up period, coronary angiography was adequately and promptly performed.

Previous events, CV events, and causes of deaths

The classification of previous events was made on the basis of medical records and personal interviews. Causes of deaths were determined by examination of hospital records, autopsy reports, and medical files of the patients' general practitioners. CV events were defined as incidents requiring hospitalization due to CVD including CAD, stroke, and peripheral artery disease, or incidents requiring hospitalization for the purpose of percutaneous coronary intervention or coronary artery bypass graft. Deaths from CVD including CAD, congestive heart failure, stroke, arrhythmia, or valve disease were also defined as CV events. End points were the first CV event and all-cause death.

Preparation of blood samples and quantification of circulating $CD34^+$ cells in peripheral blood

Using a modification of the International Society of Hematotherapy and Graft Engineering guidelines,[18] the precise number of circulating $CD34^+$ cells was quantified as described (the cumulative intraassay coefficient of variation was about 7%).[11,19] Briefly, 0.2 ml of heparinized peripheral blood drawn from the arterial–venous shunt vessel before starting HD was incubated with antibodies to CD34 and CD45, and 7AAD (Stem count kit; Beckman Coulter, Fullerton, CA, USA) followed by lysis of red blood cells. After adding internal controls (Stem count kit; Beckman Coulter), samples were concentrated by centrifugation and analyzed by Coulter CYTOMICS FC500 and XL System II software (Beckman Coulter). Using this method, $CD34^+$ cells were clearly observed as a discrete population of $CD34^+/CD45^{mild}/7AAD^-$ cells.

Additional measurements of circulating $CD34^+$ cells. To examine the stability of the $CD34^+$ cell count, additional measurements were performed. In May 2008, 20 patients were randomly selected from among the participants. The first measurement had been performed upon enrollment. A second blood sample was taken in May 2008, and a third sample was taken 1 month later. The association between the second and the third measurements was analyzed. Then, these two values were averaged for each of the 20 patients, and compared to the first measurement.

Statistical analyses

First, the relationship between the $CD34^+$ count and other baseline clinical valuables was studied by a multivariable regression analysis. Next, a cutoff number of circulating $CD34^+$ cells (0.37/μl) was determined by ROC analysis (area under the curve = 0.707) to maximize the power of the $CD34^+$ cell count in predicting future CV events. The patients were then categorized into two groups according to the cell count at the time of enrollment; a low $CD34^+$ group ($CD34^+$ cells $<0.37/\mu l$, $n=93$) or a high $CD34^+$ group ($CD34^+$ cells $>0.37/\mu l$, $n=123$). The cumulative survival rates in each group were estimated by the Kaplan–Meier method, and the differences in survival rates between groups were evaluated by log-rank (Mantle–Cox) method. Student's *t*-test was used for comparison of quantitative data between the groups. HRs and confidence intervals were calculated for each factor by a Cox univariate analysis, and prognostic factors to predict cardiac events or all-cause death were determined. All the prognostic valuables with $P<0.25$ were entered into a Cox multivariable analysis to determine independent predictors. All the analyses were performed using a software program, StatView 5.0 (SAS Institute, Cary, NC, USA). Data were expressed as the mean value ± s.d. Differences were considered significant when *P*-value was <0.05.

DISCLOSURE

All the authors declared no competing interests.

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Brief Communication

Circulating CD34-positive cells provide a marker of vascular risk associated with cognitive impairment

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Maintenance of uninterrupted cerebral circulation is critical for neural homeostasis. The level of circulating CD34-positive (CD34$^+$) cells has been suggested as an index of cerebrovascular health, although its relationship with cognitive function has not yet been defined. In a group of individuals with cognitive impairment, the level of circulating CD34$^+$ cells was quantified and correlated with clinical diagnoses. Compared with normal subjects, a significant decrease in circulating CD34$^+$ cells was observed in patients with vascular-type cognitive impairment, although no significant change was observed in patients with Alzheimer's-type cognitive impairment who had no evidence of cerebral ischemia. The level of cognitive impairment was inversely correlated with numbers of circulating CD34$^+$ cells in patients with vascular-type cognitive impairment, but not Alzheimer's type. We propose that the level of circulating CD34$^+$ cells provides a marker of vascular risk associated with cognitive impairment, and that differences in the pathobiology of Alzheimer's- and vascular-type cognitive impairment may be mirrored in levels of circulating CD34$^+$ cells in these patient populations.

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Introduction

Maintaining integrity of the cerebral circulation has a critical role in neural homeostasis. Although analysis of risk factors for cerebrovascular disease has certainly provided insights into mechanisms of vascular disease, it is still difficult to predict accurately the contribution of vascular dysfunction in the long-term outcome of acute vascular insufficiency or in chronic neurodegenerative disorders. For example, in Alzheimer's disease (Casserly and Topol, 2004; Vagnucci and Li, 2003), assessment of a possible vascular component in the pathogenesis of neuronal degeneration is often ambiguous during a patient's lifetime.

Repair of the cerebral microcirculation has traditionally been assigned to ongoing replacement of damaged cerebral endothelium from outgrowth of preexisting vasculature. However, recent studies have identified circulating bone marrow-derived immature cells, including CD34-positive (CD34$^+$) cells, as contributors in maintenance of the vasculature; they have the potential to serve as a pool of endothelial progenitor cells (Asahara *et al*, 1997) and as a source of growth/angiogenesis factors (Majka *et al*, 2001). In a previous study, we have shown that circulating CD34$^+$ cells provide an index of cerebrovascular function (Taguchi *et al*, 2004*a*). We have also found that in a model of experimental cerebral ischemia, intravenous administration of CD34$^+$ cells improved neurologic function, at least in part, by restoring cerebral microcirculation in the ischemic area (Taguchi *et al*, 2004*b*).

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These results lead us to propose that circulating immature vascular progenitor cells contribute to neural homeostasis, at least in part, through their role in maintaining cerebral microvascular function. Using a recently developed method that allows precise measurement of the CD34+ cell population in peripheral blood (Kikuchi-Taura *et al*, 2006), we have evaluated the level of circulating CD34+ cells in patients with impaired neurologic function of diverse etiologies. Our goal has been to determine if there is relationship between levels of CD34+ cells, impaired neural function, and vascular integrity.

Materials and methods

This study was approved by Institutional Review Boards of the respective institutions (National Cardiovascular Center, Hyogo College of Medicine, Hoshigaoka Koseinenkin Hospital, and Osaka Minami National Medical Center). All subjects provided informed consent. Individuals with Mini Mental State Examination Score (MMSE) <24 and Clinical Dementia Rating (CDR) ≥ 0.5 were enrolled in this study and defined as having impaired cognitive function. In the view of history, evaluation of symptoms, and results of brain imaging studies (magnetic resonance imaging and single photon-computed tomography), patients with cognitive impairment were divided into two groups by neurologists blinded to the experimental protocol: vascular-type cognitive impairment or Alzheimer's-type cognitive impairment, according to the criteria of *Diagnostic and Statistical Manual of Mental Disorders* (4th ed, DSM-4) (American Psychiatric Association, 1994). To exclude the contribution of vascular element in patients with Alzheimer's-type cognitive impairment, patients' coexistent Alzheimer's-type cognitive impairment and cerebral infarction, observed by magnetic resonance imaging, were excluded from this study. In addition, patients with cognitive impairment diagnosed as neither of the Alzheimer's type nor vascular type were excluded. A total of 95 individuals, including 32 age-matched control subjects with no history of vascular disease, no neuronal deficiency, and no cognitive impairment, were enrolled. In addition, individuals excluded from the study included: premenopausal women, patients who experienced a vascular event within 30 days of measurements, history of cerebral hemorrhage, and evidence of infection or malignant disease. Using a modification of the International Society of Hematotherapy and Graft Engineering (ISHAGE) Guidelines (Sutherland *et al*, 1996), the number of circulating CD34+ cells was quantified as described (Kikuchi-Taura *et al*, 2006). In brief, blood samples were incubated with phycoerythrin-labeled anti-CD34 antibody, fluorescein isothiocyanate-labeled anti-CD45 antibody, 7-aminoactinomycin-D, and internal control (all of these reagents are from the Stem-Kit, Beckman Coulter, Marseille, France). 7-Aminoactinomycin-D-positive dead cells and CD45-negative cells were excluded, and the number of cells forming a cluster with characteristic CD34+ cells (i.e., low side scatter and low-to-intermediate CD45 staining) was counted. The absolute number of CD34+ cells was calculated using the internal control. In this study, we used a single measurement at the time of entry into the study, on the basis of our previous observation that the level of circulating CD34+ cells is relatively stable (Taguchi *et al*, 2004*a*). For statistical analysis, JMP version 5.1J (SAS Institute Inc, Co, NC, USA) was used. Individual comparisons were performed using a two-tailed, unpaired Students' *t*-test. Statistical comparisons among groups were determined using analysis of variance. Mean $\pm$ s.e. is shown.

Results

Baseline characteristics of the groups are shown in Table 1. In univariate analysis of control subjects, each cerebrovascular risk factor and other treatment showed no significant difference with the number of circulating CD34+ cells (data not shown).

To investigate a possible relationship between circulating CD34+ cells and cognition, the level of circulating CD34+ cells was compared among these groups. Representative fluorescence-activated cell sorting images are shown in Figure 1A (vascular-type) and 1B (Alzheimer's-type). Analysis of variance revealed a significant decrease of CD34+ cells in patients with vascular-type cognitive impairment compared with Alzheimer's-type cognitive impairment ($P<0.001$) and normal subjects ($P<0.001$, Figure 1C).

To investigate further a possible association of circulating CD34+ cells with cognitive impairment, patients with vascular-type impaired cognition were divided into two groups according to their CDR (mild: CDR $=0.5$, $n=22$, mean age $=75.2\pm1.6$ years; moderate-severe: CDR ≥ 1, $n=18$, mean age $=75.3\pm1.5$ years) or MMSE (mild: MMSE ≥ 20, $n=25$, mean age $=74.2\pm1.4$ years; moderate-severe: MMSE <20, $n=15$, mean age $=77.1\pm1.5$ years). The results showed a significant decrease in the level of circulating CD34+ cells in moderate-severe group, based on stratification by either CDR (Figure 1D, $P=0.01$) or MMSE (Figure 1E, $P=0.03$) in patients with vascular-type cognitive impairment. Similar analysis was applied to patients with Alzheimer's-type impaired cognition. They were divided into two groups according to CDR (mild: $n=8$, mean age $=73.0\pm4.7$ years; moderate-severe: $n=15$, mean age $=77.5\pm1.9$ years) or MMSE (mild: $n=12$, mean age $=74.1\pm3.0$ years; moderate-severe: $n=11$, mean age $=77.8\pm2.9$ years). However, in contrast to patients with vascular-type impaired cognition, there was no significant difference observed in patients with Alzheimer's-type cognitive impaired, based on CDR (Figure 1F, $P=0.86$) or MMSE (Figure 1G, $P=0.60$).

Discussion

Our results are consistent with a contribution of circulating CD34+ cells in support of cognitive function, presumably through their positive homeostatic influence on the cerebral circulation in

Table 1 Baseline characteristics

	Total	Cognitive impairment		Control	P-value for trend
		Vascular-type	Alzheimer's-type		
n	95	40	23	32	
Age, years	74.9±0.6	75.3±1.1	75.9±2.1	74.2±0.7	0.53
Male gender, *n* (%)	57 (60)	27 (68)	12 (52)	18 (56)	0.46
Risk factor, n (%)					
Hypertension	41 (43)	21 (53)	9 (39)	11 (34)	0.28
Hyperlipidemia	29 (31)	14 (35)	5 (22)	10 (31)	0.53
Diabetes mellitus	9 (9)	5 (13)	1 (4)	3 (9)	0.57
Smoking	20 (21)	10 (25)	6(26)	4 (13)	0.34
Treatment, n (%)					
Ca-channel blocker	30 (32)	15 (38)	6 (26)	9 (28)	0.56
β-Blocker	2 (2)	1 (3)	0 (0)	1 (3)	0.71
ACE inhibitor	4 (4)	3 (8)	1 (4)	0 (0)	0.29
ARB	8 (8)	3 (8)	3 (13)	2 (6)	0.65
Diuretics	6 (6)	2 (5)	1 (4)	3(9)	0.68
Statin	29 (31)	14 (35)	5 (22)	10 (31)	0.54
Aspirin	28 (29)	23 (58)	1 (4)	4 (13)	<0.01
Ticlopidine	11(12)	9 (23)	0 (0)	2 (6)	0.01

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

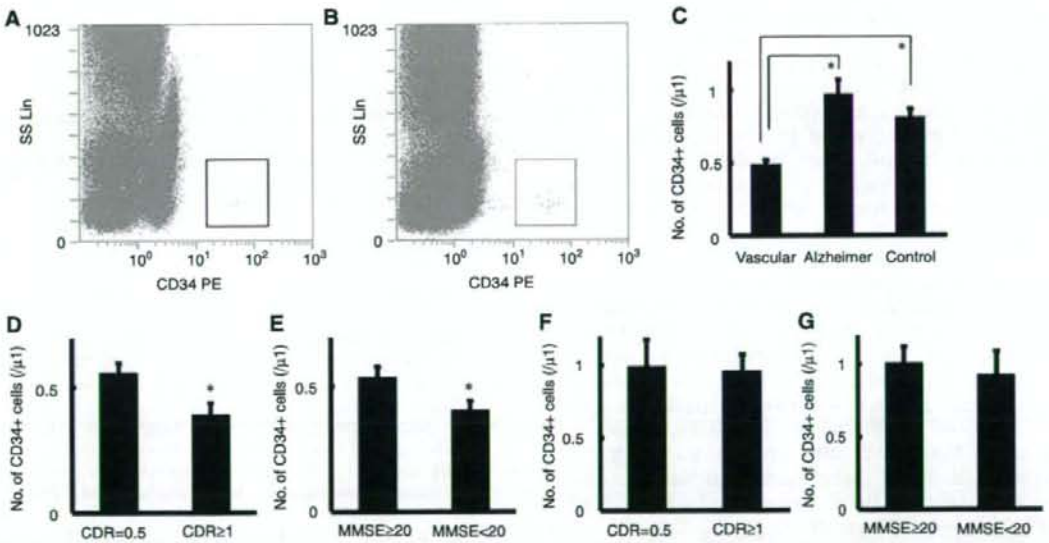

Figure 1 Levels of circulating CD34+ cells and cognitive impairment. (**A** and **B**) After exclusion of 7-AAD-positive dead cells and CD45-negative cells (non-leukocyte), CD34+ cells cluster at low side scatter were clearly observed (**A**, vascular-type; **B**, Alzheimer's-type). (**C**) Analysis of variance revealed a significant decrease in circulating CD34+ cells in patients with vascular-type cognitive impairment compared with normal subjects and individuals with Alzheimer's-type cognitive impairment. In contrast, no significant change in circulating CD34+ cells was observed in patients with Alzheimer's-type cognitive impairment compared with control subjects. (**D** and **E**) In the group of patients with vascular-type cognitive impairment, the level of circulating CD34+ cells was significantly reduced in patients with more severe cognitive impairment compared with the more mildly affected group (**D**, CDR; **E**, MMSE). (**F** and **G**) In contrast, no significant difference was observed in patients with Alzheimer's-type cognitive impairment based on assessment of cognition (**F**, CDR; **G**, MMSE). SS Lin, side-scatter linear scale. *$P < 0.05$.

settings of ischemic stress. Further, these observations suggest a basic difference between the pathobiology of dementia in Alzheimer's disease (without associated cerebral ischemia) and declining cognitive function in patients with ischemic cerebrovascular disorders.

Late onset, sporadic Alzheimer's disease is a heterogeneous disorder (Casserly and Topol, 2004) and the contribution of a vascular factor is still controversial. In contrast to vascular-type cognitive impairment, no significant change (at most, a mild increase) in the level of circulating CD34+ cells was observed in patients with Alzheimer's-type cognitive impairment who had no cerebral ischemia. Consistent with a CD34+ cell-independent mechanism of cognitive decline in Alzheimer's-type impaired cognition, there was no correlation between circulating CD34+ cells and the level of CDR or MMSE. These results suggest that the level of CD34+ cells in the peripheral circulation might provide a useful means of separating dementia with a vascular etiology from dementia associated with nonvascular causes. This is not inconsistent with a previous report indicating decreased levels of CD34+ cells in patients with early Alzheimer's disease that did not exclude patients with coexisting cerebral ischemia (Maler *et al*, 2006). Our findings could have implications for treatment, especially as more modalities become available for patients with declining cognitive function.

The level of circulating endothelial progenitor cells, identified based on positivity for CD34 and kinase insert domain receptor (CD34+/KDR+ cells), has been correlated with cardiovascular risk factors (Vasa *et al*, 2001) and cardiovascular outcomes (Schmidt-Lucke *et al*, 2005; Werner *et al*, 2005). However, large variations in the levels of CD34+/KDR+ cells in the latter reports (by ~100-fold between reports; Fadini *et al*, 2006; Werner *et al*, 2005) indicate the need to standardize this measurement. In contrast, in our study, although there was no strong correlation between levels of CD34+ cells and established cardiovascular risk factors and other treatments, probably because of the heterogeneity of our control subjects, the results indicate a close relationship between the overall CD34+ pool and the cognitive impairment with cerebral ischemia. Previous reports have indicated a positive correlation between mobilization of CD34+ cells and improved functional outcome in stroke patients (Dunac *et al*, 2007). Accelerated functional recovery after experimental stroke, because of administration of CD34+ cells (Shyu *et al*, 2006; Taguchi *et al*, 2004*b*), suggests the possible contribution of CD34+ cells in maintenance of brain function during cerebral circulation. Our method for quantification of CD34+ cells is simple, reproducible (Kikuchi-Taura *et al*, 2006), and suitable for screening a broad group of patients at risk for cerebrovascular disorders.

In conclusion, our results indicate that the level of circulating CD34+ cells provides a marker of vascular risk associated with cognitive impairment. Furthermore, differences in the pathobiology of Alzheimer's- and vascular-type cognitive impairment may be mirrored in levels of circulating CD34+ cells in these patient populations.

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Conflict of interest

The authors state no conflict of interest.

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Pioglitazone treatment stimulates circulating CD34-positive cells in type 2 diabetes patients

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ABSTRACT

Circulating bone marrow derived immature cells, including CD34-positive ($CD34^+$) cells, contribute to maintenance of the vasculature, not only as a pool of endothelial progenitor cells (EPCs), but also as a source of growth/angiogenesis factor. We hypothesized that the thiazolidineone compound pioglitazone could stimulate the circulating $CD34^+$ cells in diabetic patients. Thirty-four patients with type 2 diabetes received 15–30 mg pioglitazone for 24 weeks. The number of circulating $CD34^+$ cells significantly increased at 12 and continued this effect for 24 weeks (1.08 ± 0.39, 1.34 ± 0.34 and 1.32 ± 0.28 cells/μl at 0, 12 and 24 weeks, respectively). The change of $CD34^+$ cell levels ($\Delta CD34^+$ cells) between 0 and 12 weeks was significantly correlated with the change of high sensitive C reactive protein levels (Δhs-CRP) and change in adiponectin levels (Δadiponectin) ($r = -0.412$, $r = 0.359$, respectively). Our study demonstrated that pioglitazone treatment increased circulating $CD34^+$ cells, suggesting that this effect may at least partly contribute to the anti-atherosclerotic action of pioglitazone.

1. Introduction

Endothelial dysfunction plays a pivotal role in the progression of the atherosclerosis. Circulating EPCs contribute to the maintenance of vascular homeostasis and repair. They also play an important role in the maintenance of vascular endothelial function [1,2]. In diabetic patients, both a decrease in number and function of circulating EPCs are reported, suggesting that circulating EPCs participate in diabetic vascular complications [3].

Recent studies have identified circulating bone marrow derived immature cells, including $CD34^+$ cells, contribute to maintenance of the vasculature, not only as a pool of EPCs, but also as a source of growth/angiogenesis factor [4]. In fact, one recent report indicates that circulating $CD34^+$ cells are more strongly correlated with cardiovascular risk than circulating $CD34^+$/kinase insert domain receptor $(KDR)^+$ cells generally regarded as EPCs [5]. We have also reported that circulating $CD34^+$ cell levels are associated with cerebral infarction [6]. These findings indicate that persistent stimulation of $CD34^+$ cells may be a useful method to repair endothelial injury and microcirculation, and to suppress the progression of atherosclerotic disease at least theoretically. Recent experimental and clinical studies demonstrate that thiazolidinediones, peroxisome-proliferator-activated receptor γ (PPARγ) agonists, has the effects on the prevention of atherosclerosis including the maintenance of vascular endothelial function [7–9]. Therefore, we hypothesized that the thiazolidineone

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