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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 ($P<0.001$, $R^2=0.68$).

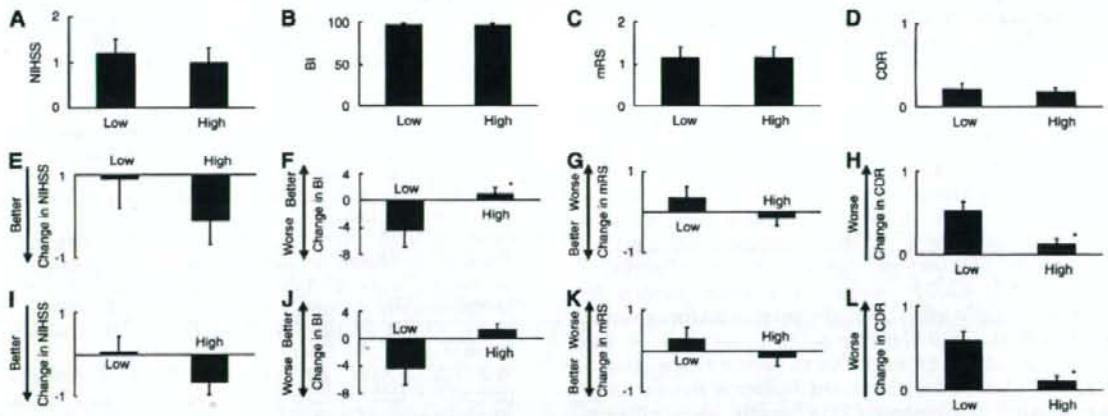

Figure 1 The level of circulating CD34+ cells and neurologic function in the study group after 1 year. (**A–D**) At the point of entry, there were no significant differences in the level of neurologic function, including NIHSS (**A**), BI (**B**), mRS (**C**), and CDR (**D**). (**E–H**) There was a trend 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.

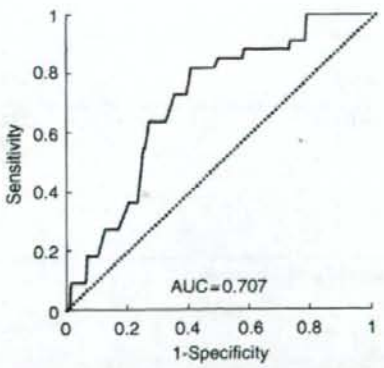

Figure 1 | ROC Curve Analysis. A ROC curve analysis was performed to determine a cutoff value for circulating CD34+ cell count. The result showed 0.37/μl to be the value (area under the curve = 0.707) to maximize the power of the CD34+ cell count in predicting a future CV event.

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).