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# Increased Circulating Progenitor Cells in Alzheimer's Disease Patients with Moderate to Severe Dementia: Evidence for Vascular Repair and Tissue Regeneration?

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**Abstract**. Cerebrovascular dysfunction is a common finding in patients with Alzheimer's disease (AD) and may contribute to cognitive decline. Abundant evidence suggests that vascular and neuronal repair mechanisms are mediated by circulating progenitor cells *in vivo*. Whether $CD34^+$ and, specifically, $CD34^+/CD133^+$ progenitor cells are involved in the pathophysiology of AD is poorly understood so far. In the present study, peripheral blood concentrations of circulating $CD34^+/CD133^+$ and $CD34^+$ progenitor cells were measured in 45 AD patients and in 30 healthy elderly controls by flow cytometry. The severity of dementia was assessed by Mini-Mental Status Examination and Clinical Dementia Rating scale. AD patients were stratified into two groups showing mild ($n = 17$) and moderate to severe ($n = 28$) dementia. In the present study, AD patients with moderate to severe dementia, but not those with mild dementia, showed significantly increased circulating $CD34^+/CD133^+$ and $CD34^+$ progenitor cells compared to healthy elderly controls independent of cardiovascular risk factors and medication. In addition, the number of circulating $CD34^+/CD133^+$ progenitor cells in AD patients was significantly inversely correlated with cognitive function, age, and plasma levels of SDF-1, the most potent chemokine for progenitor cells. Our findings suggest a stage-dependent upregulation of circulating $CD34^+/CD133^+$ and $CD34^+$ progenitor cells in AD patients, which could take part in tissue healing processes of the brain in AD.

Keywords: Alzheimer's disease, CD133, CD34, dementia, SDF-1

## INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia in the elderly, which currently affects about 26 million people worldwide. Accumulating evidence from epidemiological, clinical, and experimental studies suggests that vascular risk factors and angiopathic mechanisms including arteriosclerotic microangiopathy and amyloid angiopathy are involved in the patho-

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Table 1
Differential baseline characteristics among AD patients with moderate to severe dementia, AD patients with mild dementia and healthy controls

| Characteristics | Moderate to severe AD ($n = 28$) | Mild AD ($n = 17$) | Healthy controls ($n = 30$) | P value |
|---|---|---|---|---|
| Female, n (%) | 16 (57.1) | 11 (64.7) | 13 (43.3) | 0.324[a] |
| Age (years), mean ± SD | 73.2 ± 9.3 | 73.8 ± 5.4 | 69.4 ± 7.2 | 0.097[b] |
| MMSE, mean ± SD | 16.4 ± 2.9 | 23.7 ± 1.9 | 28.5 ± 1.5 | $< 0.001$[b] |
| CVRFs, n (%) | | | | |
| Hypertension | 12 (42.8) | 7 (41.2) | 12 (40.0) | 0.977[a] |
| Hypercholesterinemia | 8 (28.6) | 10 (58.8) | 9 (30.0) | 0.116[a] |
| Diabetes mellitus | 1 (3.6) | 2 (11.8) | 2 (6.7) | 0.573[a] |
| Smoking | 1 (3.6) | 3 (17.6) | 1 (3.3) | 0.146[a] |
| Family History of CAD | 3 (10.7) | 0 (0) | 0 (0) | 0.085[a] |
| Medication, n (%) | | | | |
| Aspirin | 9 (32.1) | 7 (41.2) | 5 (16.7) | 0.248[a] |
| Statin | 6 (21.4) | 6 (35.3) | 5 (16.7) | 0.417[a] |
| Antihypertensives | 14 (50) | 6 (35.3) | 12 (40) | 0.329 |
| ChEI | 20 (71.4) | 15 (88.2) | 0 (0) | $< 0.001$[a] |

[a]chi-square test; [b]ANOVA; MMSE, Mini-Mental State Examination; ChEI, cholinesterase inhibitor; CVRFs, cardiovascular risk factors; CAD, coronary artery disease.

genesis of AD [1–3]. The amount of cerebrovascular degeneration with consecutive hypoperfusion of the brain seems to influence clinical manifestation and severity of cognitive decline in AD patients [4,5].

A growing body of evidence suggests that progenitor cells circulate in the blood and can play an important and perhaps the major role in repair and in formation of new blood vessels in pathologic conditions [6–11]. Moreover, increasing data support the role of $CD34^+$ cells in neuroregenerative procedures including differentiation to neural cells, neovascularization, and cognitive impairment after stroke [12–14]. The number of circulating progenitor cells is thought to be a marker of cerebrovascular function and repair capacity and decreases with age [15,16]. Thus, circulating progenitor cells may contribute to neural homeostasis, at least in part, through their role in maintaining cerebral microvascular function. Whether $CD34^+$ and in specific $CD34^+/CD133^+$ progenitor cells are involved in the pathophysiology of AD is poorly understood so far. The chemokine stromal cell-derived factor-1 (SDF-1; also called as CXCL12) is known to be responsible for both progenitor cell mobilization from the bone marrow to peripheral blood and homing to the sites of vascular and tissue injury [17–19].

Given the frequent finding of cerebrovascular dysfunction in AD patients and the potential role of progenitor cell mediated vascular repair mechanisms, we hypothesized that an increase in progenitor cells should be detectable in the peripheral blood during the course of AD. To test this hypothesis, we assessed the number of $CD34^+/CD133^+$ and $CD34^+$ progenitor cells in AD patients with different stages of dementia and age-matched healthy control subjects. In order to investigate the underlying mechanisms involved in the mobilization of progenitor cells in AD patients, we measured in addition plasma levels of SDF-1.

## MATERIALS AND METHODS

### *Subjects*

Forty-five AD patients from our Memory clinic and thirty age-matched healthy elderly controls were included in this study (Table 1). All AD patients fulfilled the ICD-10, DSM-IV, and NINCDS-ADRDA [20] criteria for probable AD. The severity of dementia was assessed by the Mini-Mental Status Examination (MMSE) [21] and the Clinical Dementia Rating scale (CDR) [22]. The AD patients were stratified into two groups showing mild (CDR=1; $n = 17$), and moderate to severe (CDR $\geqslant 2$; $n = 28$) dementia. The control subjects had a normal cognitive status according to clinical examination. In AD patients, a computed tomography or magnetic resonance imaging (MRI) were also performed to validate the diagnosis of AD. Routine exclusion criteria for AD patients and control subjects were a current or a history of depression or psychosis, alcohol or substance abuse, or use of psychoactive medications. The regional ethical committee approved the study and written informed consent was obtained from each individual.

*Sample collection*

Blood was obtained mornings (9:00–10:00am; in the fasting state). Venous blood was collected in EDTA tubes (for plasma levels of SDF-1) and into 3.8% citrate plasma tubes (for peripheral blood mononuclear cell isolation).

*Peripheral blood mononuclear cell isolation and flow cytometry*

Mononuclear cells were isolated using a Ficoll density gradient (Biocoll, Biochrom, Berlin, Germany) according to standard protocols as previously described [23]. For FACS analysis, mononuclear cells were resuspended in 100 $\mu$l of PBS. Immunofluorescence cell staining was performed in duplicate with the use of the fluorescent conjugated antibodies CD34–FITC (10 $\mu$l; Becton Dickinson, San Jose, USA; clone 8G12) and CD133-APC (10 $\mu$l; Becton Dickinson, San Jose, USA; clone AC133). IgG1–FITC (BD Biosciences Pharmingen, USA; clone MOPC-21) and IgG1-APC (BD Biosciences Pharmingen, USA; clone MOPC-21) antibodies served as a negative isotype control. Cell fluorescence was measured immediately after staining, and data were analyzed with the help of CellQuest software (FACSCalibur, Becton Dickinson, Heidelberg, Germany). Units of all measured components are absolute cell counts obtained after the measurement of 250,000 events in a lymphocyte gate.

*Enzyme-linked immunosorbent assay* (*ELISA*)

Plasma levels of SDF-1 were measured using a commercially available ELISA kit according to the manufacturer's guidelines (R&D Systems, Minneapolis, USA). EDTA plasma probes were centrifuged for 15 min at 10,000 g within 30 min of collection. Probes were aliquoted and stored at $-20\,^{\circ}$C before analysis. The lower detection limit of this assay is 18 pg/ml.

*Data presentation and statistical analysis*

The data are presented as mean $\pm$ SD. All statistical analyses were carried out using the statistical analysis software package SPSS 14.0® (Munich, Germany). For comparison of quantitative variables between diagnostic groups one-way ANOVA with a subsequent Scheffé post hoc analysis were used. For comparison of categorical variables the chi-square test was used. After controlling for Gaussian distribution with Kolmogorov-Smirnov test, correlations between variables were determined using Pearson test. Significance for the results was set at $P < 0.05$.

## RESULTS

The demographic and clinical parameters of the AD patients with mild or moderate to severe dementia and of the healthy control group are presented in the Table 1.

*Number of CD34$^+$/CD133$^+$ progenitor cells in AD patients*

AD patients with moderate to severe dementia, but not with mild dementia, presented with significantly increased circulating CD34$^+$/CD133$^+$ progenitor cells in comparison to healthy elderly controls (moderate to severe AD vs. mild AD vs. healthy elderly controls: mean $\pm$ SD: 71.8 $\pm$ 47.3 vs. 52.2 $\pm$ 19.5 vs. 42.2 $\pm$ 21.5; $P = 0.006$) independent of cardiovascular risk factors and medication (Table 2). Performing a Scheffé post hoc analysis, a significant increase was observed between AD patients with moderate to severe dementia and healthy controls (P=0.007), while no significant difference was observed between AD patients with mild dementia and healthy controls ($P = 0.635$; Fig. 1).

In a similar manner the number of CD34$^+$ cells was significantly increased in AD patients with moderate to severe dementia, but not in those with mild dementia, compared with healthy elderly controls (moderate to severe AD vs. mild AD vs. healthy elderly controls: mean $\pm$ SD: 222.5 $\pm$ 106.1 vs. 177.3 $\pm$ 70.1 vs. 163 $\pm$ 80.2; $P = 0.039$) independent of cardiovascular risk factors and medication (data not shown). Performing a Scheffé post hoc analysis, a significant increase was observed in AD patients with moderate to severe AD vs. healthy controls ($P = 0.046$), while no significant difference was observed in AD patients with mild dementia vs. healthy controls ($P = 0.875$; Fig. 1).

AD patients treated with a cholinesterase inhibitor (ChEI; $n = 35$) and those without ($n = 10$) showed no significant differences as regards the CD34$^+$ and CD34$^+$/CD133$^+$ cell number (data not shown). CD34$^+$ cell number showed a significant positive correlation with the number of CD34$^+$/CD133$^+$ progenitor cells in AD patients ($r = 0.648$, $P < 0.001$) and in healthy controls ($r = 0.677$, $P < 0.001$).

*Association of cognitive status with the number of CD34$^+$/CD133$^+$progenitor cells in AD patients*

A significant inverse correlation between cognitive status, as measured by MMSE score, and the number of circulating or CD34$^+$ progenitor cells was observed within the AD group ($n = 45$, or $r = -0.34$, $P =$

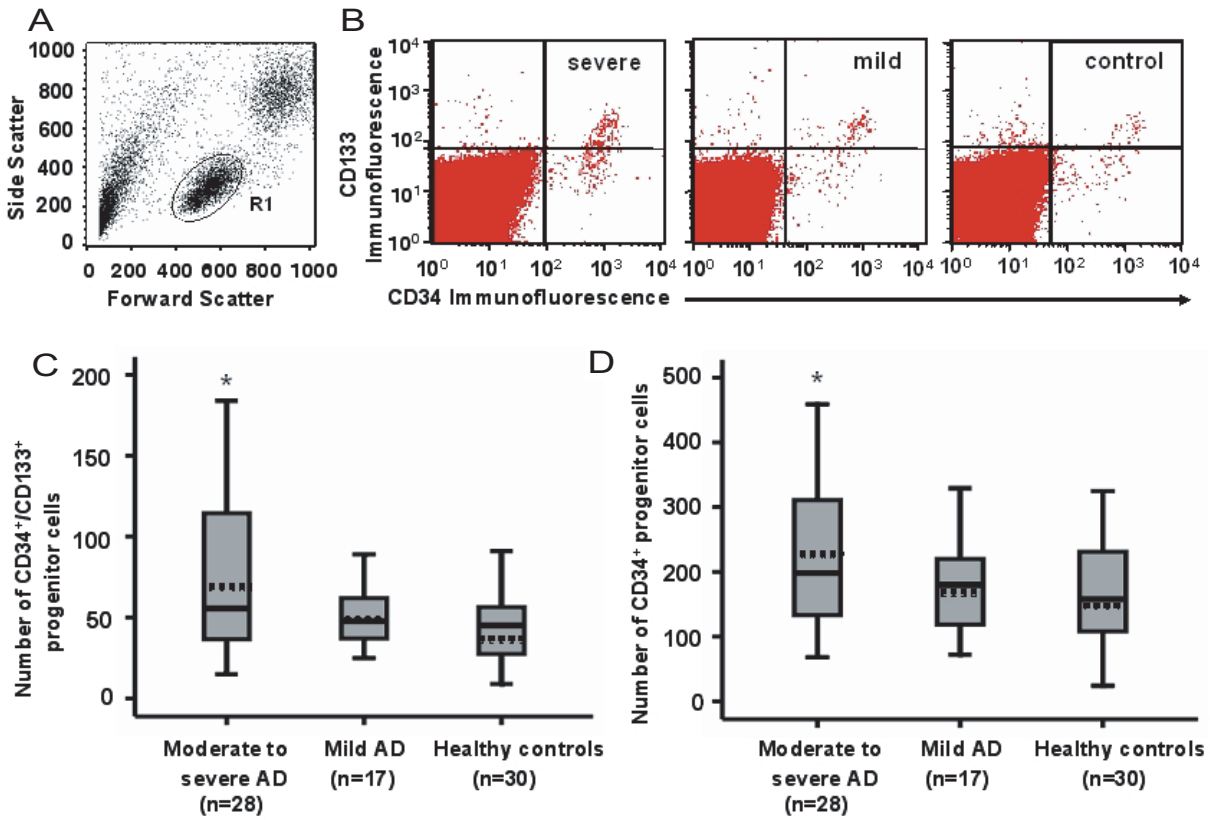


Fig. 1. Number of circulating $CD34^+/CD133^+$ and $CD34^+$ progenitor cells in Alzheimer's disease (AD) patients. (A) A representative cell sorting histogram of peripheral blood mononuclear cells is shown. The progenitor cell populations were identified within the lymphocyte population (R1). (B) Representative immunofluorescence histograms are presented showing the cells positive for CD34 and CD133 biomarker in a patient with severe dementia (left panel), with mild dementia (middle panel), and in a healthy control (right panel). (C, D) The mean (dot line), median (simple line), and the 25th and 75th percentile (upper and lower boundaries) of the number of $CD34^+/CD133^+$ and $CD34^+$ cells in AD patients with moderate to severe dementia, mild dementia, and in age-matched healthy controls are depicted. The data showed a normal distribution; hence, statistical significance was calculated performing the ANOVA-test with a Scheffe post hoc analysis. $^*P < 0.005$.

0.032). The number of circulating $CD34^+/CD133^+$ progenitor cells inversely correlated with MMSE scores in patients with AD ($n = 45$, $r = -0.312$; $P = 0.037$; Fig. 2A) and with CDR scale in our total population ($n = 75$, $r = -0.333$; $P = 0.003$; Fig. 2B).

### *Influence of age on the number of $CD34^+/CD133^+$ progenitor cells in AD patients*

The number of $CD34^+/CD133^+$ progenitor cells in our total population seemed to be significantly influenced by age, as shown in our univariate analysis of variance (Table 2). Indeed, a significant inverse correlation was observed between the number of circulating $CD34^+/CD133^+$ progenitor cells and age in our total population ($n = 75$, $r = -0.246$, $P = 0.033$; Fig. 3A). In order to further define the association of age with the number of progenitor cells, we separately investigated the potential correlation of age with $CD34^+/CD133^+$ cells in only the AD group. Similar correlations were observed between age and the number of $CD34^+/CD133^+$ progenitor cells ($r = -0.37$, $P = 0.015$) or $CD34^+$ cells ($r = -0.319$, $P = 0.037$).

### *Number of $CD34^+/CD133^+$ progenitor cells and plasma SDF-1 levels in AD patients*

Although no significant differences of SDF-1 plasma levels were observed among our groups, an inverse correlation was identified between the number of circulating $CD34^+/CD133^+$ progenitor cells and SDF-1 plasma levels ($n = 75$, $r = -0.25$, $P = 0.032$; Fig. 3B). In a similar manner the number of $CD34^+$ progenitor cell inversely correlated with plasma levels of SDF-1 ($r = -0.279$, $P = 0.017$). A subgroup analysis of only the AD patients showed similar results for plasma

Table 2
Univariate analysis of variance for the number of $CD34^+$ / $CD133^+$ progenitor cells and possible confounders for cognitive status

| Characteristics | Factor | P value |
|---|---|---|
| | Female | 0.055 |
| | Age (years) | 0.037 |
| CVRFs | Hypertension | 0.480 |
| | Hypercholesterinemia | 0.366 |
| | Diabetes mellitus | 0.932 |
| | Smoking | 0.463 |
| | Family history of CAD | 0.498 |
| Medication | Aspirin | 0.666 |
| | Statin | 0.769 |
| | Antihypertensives | 0.869 |
| | ChEI | 0.618 |
| Groups (moderate to severe vs. mild AD vs. healthy controls) | | 0.006 |

ChEI, cholinesterase inhibitor; CVRFs, cardiovascular risk factors; CAD, coronary artery disease.

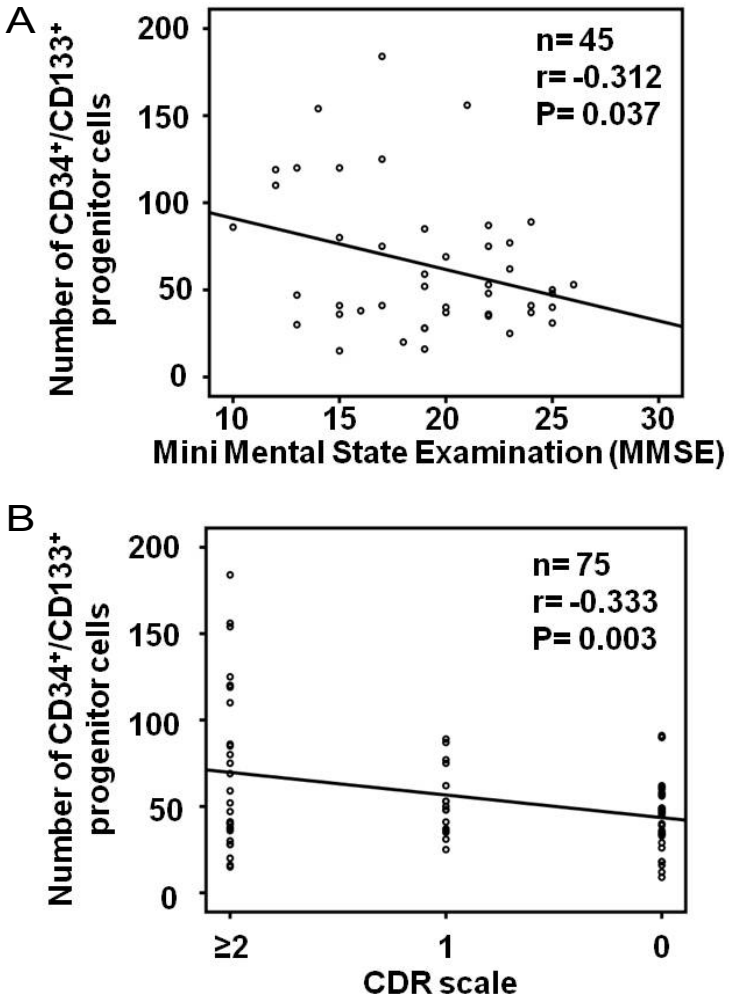


Fig. 2. The number of circulating $CD34^+/CD133^+$ progenitor cells inversely correlates with the cognitive status in Alzheimer's disease (AD) patients, measured by MMSE score ($n = 45$, $r = -0.312$; $P = 0.037$; A) and in our total population, measured by CDR scale ($n = 75$; $r = -0.333$; $P = 0.003$; B).

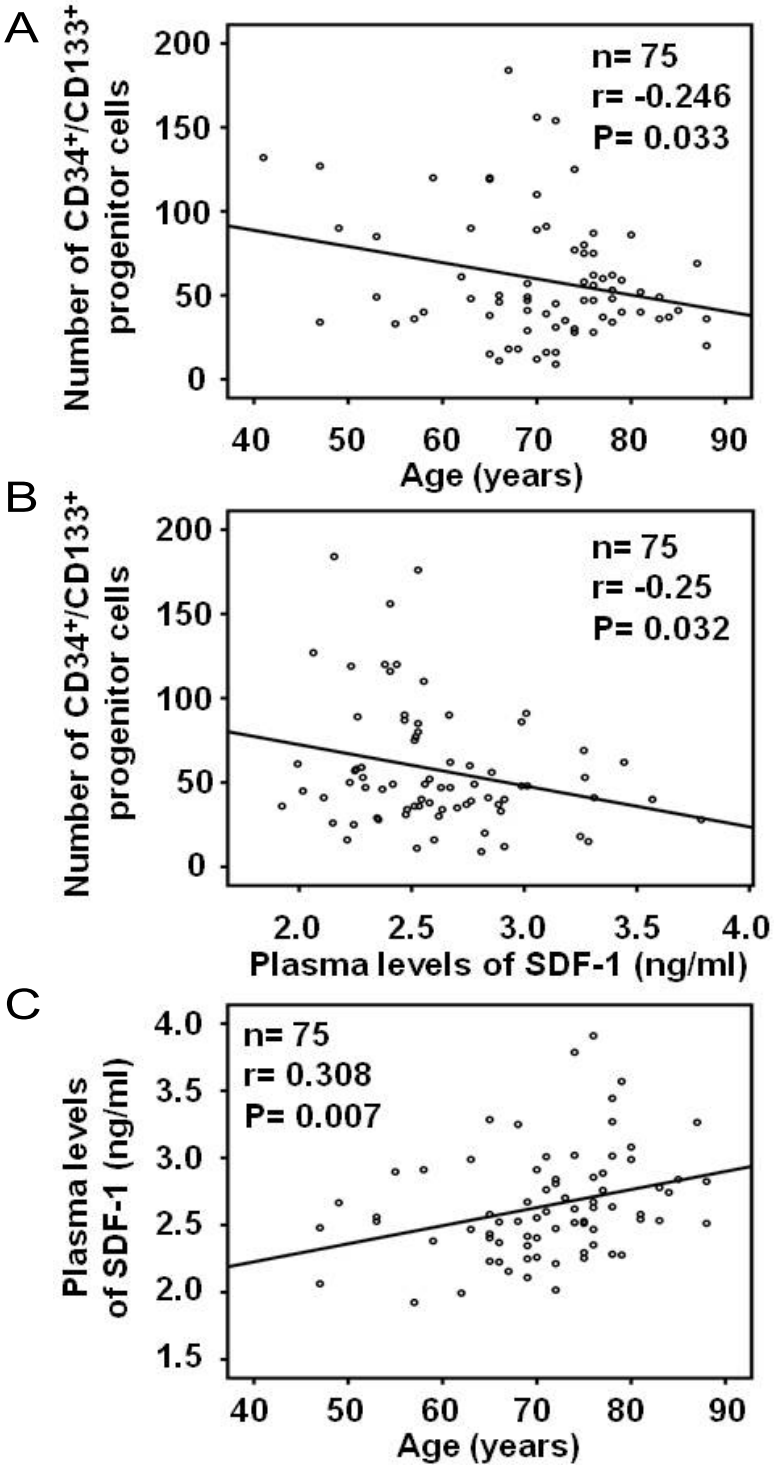


Fig. 3. (A) Inverse correlation between age and the number of $CD34^{+}/CD133^{+}$ progenitor cells in our total population (45 Alzheimer's disease [AD] patients and 30 healthy controls; $r = -0.246$; $P = 0.033$). (B) Plasma levels of the chemokine stromal cell-derived factor-1 (SDF-1) inversely correlates with the number of circulating $CD34^{+}/CD133^{+}$ progenitor cells in our total population. (C) Correlation between plasma levels of SDF-1 and age.

levels of SDF-1 and the number of $CD34^+/CD133^+$ ($r = -0.34$, $P = 0.028$) or $CD34^+$ cells ($r = -0.334$, $P = 0.033$).

*Association of plasma levels of SDF-1 with age in AD*

Since the number of $CD34^+/CD133^+$ progenitor cells inversely correlated with both age and plasma SDF-1, the next question raised was whether age associates with plasma levels of SDF-1. Interestingly, performing a Pearson correlation coefficient test between SDF-1 plasma levels and age, a positive correlation was observed ($n = 75$, $r = 0.308$, $P = 0.007$; Fig. 3C). A similar correlation was found within the AD group ($n = 45$, $r = 0.314$, $P = 0.043$). On the other hand, plasma SDF-1 levels did not correlate with cognitive status in our population.

## DISCUSSION

The major findings of the present study are: 1) The number of $CD34^+/CD133^+$ or $CD34^+$ progenitor cells is increased in AD patients with moderate to severe dementia, but not in AD patients with mild dementia, in comparison to healthy elderly controls independent of cardiovascular risk factors and medication; 2) the number of $CD34^+/CD133^+$ progenitor cells in AD patients is associated with the cognitive status as measured by the MMSE score; 3) age inversely correlates with the number of progenitor cells in AD patients and in our total population. In this context, it is remarkable that we still found significantly increased circulating $CD34^+$ cell levels in AD patients despite of a trend of older age compared to healthy controls; 4) plasma levels of the chemokine SDF-1 inversely correlate with the number of circulating progenitor cells in AD patients and in our total population; 5) plasma SDF-1 levels are positively correlated with age; and 6) concerning the treatment with an ChEI, AD patients treated with an ChEI ($n = 35$) and those without ($n = 10$) showed no significant differences as regards the number of $CD34^+$, and $CD34^+/CD133^+$ progenitor cells, indicating that the treatment of AD patients with an ChEI may not have biased our results.

Several reports showed an increase of hematopoietic stem and progenitor cell count early after an acute brain damage such as traumatic brain injury and cerebral ischemia, suggesting a contribution of bone marrow cells in brain regeneration [24–26]. In accordance with these data of patients with acute brain damage, we also found an increase of progenitor cells in AD patients with moderate to severe dementia. However, this increase was not apparent in AD patients with mild dementia, indicating that a relevant upregulation of these cells only takes place in AD patients with a more advanced stage of the disease, when considerable neuronal loss has already occurred. The group of CDR $\geqslant 2$ seems rather heterogeneous in the means of the number of circulating progenitor cells. Further studies are needed to investigate exactly the prognostic value of this difference of progenitor cells among patients with moderate to severe AD. The potential clinical relevance of these findings has recently been demonstrated by Taguchi and colleagues [27], showing that circulating $CD34^+$ HSCs have prognostic value for neurologic function in patients with past cerebral infarction. Hematopoietic stem and progenitor cells could take part in tissue healing processes of the brain through several mechanisms. 1) An important mechanism seems to be the expression of neurotrophic factors [28]. 2) Progenitor cells are capable to enhance endogenous neurogenesis of neural stem cells in the CNS via angiogenesis [29–31]. 3) In addition, *in vitro* studies have demonstrated the ability of HSCs to transdifferentiate into neuron-like cells [12, 32]. Thus, the number of circulating progenitor cells is thought to be a marker of cerebrovascular function and repair capacity of the brain [15].

However, heterogeneous data have been reported regarding the number of circulating $CD34^+$ cells in AD patients. A recent study has demonstrated decreased circulating $CD34^+$ HSCs in early AD patients [33]. As limiting factors of this study, there is given no information about age, gender distribution, MMSE scores, and treatment of the included patients and the time point of blood collection, which may have influenced the results. In contrast, Taguchi et al. [27] showed a significant decrease of circulating $CD34^+$ HSCs only in patients with vascular-type cognitive impairment, but not in AD patients with different severity of dementia. However, there is no information about MMSE scores and the time point of blood collection, an imbalance regarding the use of NSAIDs between the patients with vascular dementia (58%) and AD (4%), and a small sample size, which may have biased the results from this study. Furthermore, the previous studies measured only $CD34^+/CD45^{low}$ cells, which characterize a heterogenous population of blood cells [34]. In the present study, we investigated cells positive for both CD34 and CD133 biomarkers which are well characterized as early proangiogenic precursor cells [11,23,35–40]. These limitations and methodological differences may

explain the different findings as regards the number of circulating $CD34^+$ cells in AD patients compared with healthy controls and do not allow a direct comparison with the findings from our present study.

In the present study, we describe to the best of our knowledge for the first time an association between plasma SDF-1 and the number of $CD34^+/CD133^+$ progenitor cells in AD patients, showing a potential mechanism involved in the regulation of progenitor cell trafficking in AD patients or generally in elderly patients. In accordance to our data, it has been recently reported in the Bruneck Study that plasma SDF-1 levels inversely correlate with the number of cultured endothelial progenitor cells (EPC), possessing also a long-term predictive value for circulating EPC number [41]. In the present study we report a similar association between plasma SDF-1 levels with the number of circulating $CD34^+/CD133^+$ progenitor cells, indicating that SDF-1, probably influenced by aging mechanisms as indicated by the correlation with age, may regulate the number of progenitor cells in elderly people including AD patients. Decreased plasma SDF-1 levels are also reported in other diseases including unstable coronary artery disease [42] and non-Hodgkin's lymphoma [43], where low plasma levels of SDF-1 characterize patients with good mobilization outcome (increased $CD34^+$ cells) after administration of granulocyte colony-stimulating factor. Mechanism leading to reduced plasma levels of SDF-1 include binding to peripheral vascular lesions facilitating the domiciliation of progenitor cells to target organs, binding to its receptors CXCR4 and CXCR7 on the surface of progenitor and mature blood cells, and degradation after exposure to increased number of progenitor and mononuclear cells through carboxypeptidase M [44].

In conclusion, our findings show an upregulation of circulating progenitor cells in AD patients, increasing with the severity of dementia and influenced by age and SDF-1 plasma levels. This increase of circulating $CD34^+/CD133^+$ progenitor cells could take part in tissue healing processes of the brain in AD.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=130).

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