



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Bone marrow-derived progenitor cells in the early phase of ischemic stroke: relation with stroke severity and discharge outcome

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Bone marrow-derived progenitor cells in the early phase of ischemic stroke: relation with stroke severity and discharge outcome / Cesari F; Nencini F; Nesi M; Caporale R; Giusti B; Abbate R; Gori AM; Inzitari D. - In: JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM. - ISSN 0271-678X. - STAMPA. - 29:(2009), pp. 1983-1990.

Availability:

The webpage <https://hdl.handle.net/2158/368986> of the repository was last updated on 2018-02-28T18:59:11Z

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

Stroke

American Stroke
AssociationSM

JOURNAL OF THE AMERICAN HEART ASSOCIATION

A Division of American
Heart Association



Bone Marrow-Derived Progenitor Cells in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

Francesca Pescini, Francesca Cesari, Betti Giusti, Cristina Sarti, Enza Zicari, Silvia Bianchi, Maria T. Dotti, Antonio Federico, Maurizio Balestrino, Adriano Enrico, Carlo Gandolfo, Anna M. Gori, Rosanna Abbate, Leonardo Pantoni and Domenico Inzitari

Stroke 2010;41;218-223; originally published online Dec 24, 2009;

DOI: 10.1161/STROKEAHA.109.563726

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214
Copyright © 2010 American Heart Association. All rights reserved. Print ISSN: 0039-2499. Online
ISSN: 1524-4628

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

<http://stroke.ahajournals.org/cgi/content/full/41/2/218>

Subscriptions: Information about subscribing to Stroke is online at
<http://stroke.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters
Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax:
410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/reprints>

Bone Marrow-Derived Progenitor Cells in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

Francesca Pescini, MD; Francesca Cesari, MSc; Betti Giusti, MSc, PhD; Cristina Sarti, MD, PhD; Enza Zicari, MD; Silvia Bianchi, PhD; Maria T. Dotti, MD; Antonio Federico, MD; Maurizio Balestrino, MD, PhD; Adriano Enrico, MD; Carlo Gandolfo, MD, PhD; Anna M. Gori, MSc; Rosanna Abbate, MD; Leonardo Pantoni, MD, PhD; Domenico Inzitari, MD

Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited disease due to cerebral microangiopathy presenting with variable pictures, including stroke, progressive cognitive impairment, and disability. Mechanisms leading from vessel structural changes to parenchymal damage and eventually to clinical expression are not fully understood. Among pathogenic processes, endothelial dysfunction has been hypothesized. Endothelial progenitor cells and circulating progenitor cells (CPCs) derived from bone marrow participate in endothelium structure and function maintenance and contribute to ischemic area revascularization. No data are available about these cells in CADASIL. Our objective in this study was to evaluate endothelial progenitor cells and CPCs role in CADASIL.

Methods—Twenty-nine patients with CADASIL and 29 sex- and age-matched control subjects were enrolled. Cells were measured in peripheral blood using flow cytometry. Endothelial progenitor cells were defined as positive for CD34/KDR, CD133/KDR, and CD34/CD133/KDR; and CPCs as positive for CD34, CD133, and CD34/CD133.

Results—Endothelial progenitor cells were significantly lower in patients with CADASIL than in control subjects (CD34/KDR: 0.05 versus 0.1 cells/ μ L, $P=0.005$; CD133/KDR: 0.07 versus 0.1 cells/ μ L, $P=0.006$; CD34/CD133/KDR: 0.05 versus 0.1 cells/ μ L, $P=0.001$). The difference remained significant after adjusting for age, sex, and statin use. CPCs were not significantly lower in CADASIL, but patients with stroke or dementia had significantly reduced CPC levels than patients without (CD34: 1.68 versus 2.95 cells/ μ L, $P=0.007$; CD133: 1.40 versus 2.82 cells/ μ L, $P=0.004$; CD34/CD133: 1.44 versus 2.75 cells/ μ L, $P=0.004$). CPC levels significantly correlated with cognitive and motor performance measures.

Conclusions—We have documented an association between endothelial progenitor cells and CPCs and CADASIL, extending previous data about the presence of endothelial dysfunction in this disease and its potential role in modulating phenotype. (*Stroke*. 2010;41:218-223.)

Key Words: CADASIL ■ endothelial dysfunction ■ phenotype ■ progenitor cells ■ small vessel

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare inherited disease linked with a nonarteriosclerotic nonamyloid microangiopathy. It is caused by missense point mutations of the *NOTCH3* gene (chromosome 19p13.1).^{1,2} The disease is clinically characterized by migraine, early-onset transient ischemic attack/stroke, cognitive disturbances, mood disorders, and more rarely seizures.³ It has a progressive course leading to disability and dementia in three fourths of patients.⁴ The neuroimaging hallmarks are severe and

diffuse leukoencephalopathy, typically involving the anterior temporal pole and the external capsule, and multiple subcortical lacunar infarcts. The disease phenotype may be variable ranging from minimal symptoms at late-age to full-blown dementia and disability in middle-aged patients.^{3,5,6} Variability is only partially explained by the effect possibly exerted by genetic or acquired cofactors.^{7,8}

It has been hypothesized that the structural microvessel changes lead to impaired vasoreactivity with consequent reduced cerebral perfusion and tissue damage. Cerebral

Received July 26, 2009; final revision received September 24, 2009; accepted October 27, 2009.

From the Departments of Neurological and Psychiatric Sciences (C.S., L.P., D.I., F.P.) and Medical and Surgical Critical Care (F.C., B.G., A.M.G., R.A.), Thrombosis Center, University of Florence, Florence, Italy; the Neurometabolic Unit (E.Z., S.B., M.T.D., A.F.), Institute of Neurological Sciences, University of Siena, Siena, Italy; the Department of Neuroscience (M.B., A.E., C.G.), Ophthalmology and Genetics, University of Genova, Genova, Italy; and S. Maria agli Ulivi Center (A.M.G.), Don Carlo Gnocchi IRCCS Foundation, Florence, Italy.

F.P. and F.C. contributed equally to the study.

Correspondence to Francesca Pescini, MD, Department of Neurological and Psychiatric Sciences, University of Florence, Viale Morgagni 85, 50134 Florence, Italy. E-mail francescapescini@virgilio.it

© 2010 American Heart Association, Inc.

Stroke is available at <http://stroke.ahajournals.org>

DOI: 10.1161/STROKEAHA.109.563726

hypoperfusion has been documented in CADASIL using blood flow imaging studies,^{9,10} and, although not univocally, vasoreactivity changes have been reported by transcranial Doppler sonography studies.^{11,12} Altered skin microvessel reactivity¹³ and endothelial-dependent vasodilatation in cerebral and forearm arteries studies have been observed.^{14,15} Over the last years, increasing evidence has accumulated showing that bone marrow-derived cells, named endothelial progenitor cells (EPCs), possess the capacity to home to sites of vascular injury contributing to the maintenance of the homeostasis of vascular endothelium and to in vivo neoangiogenesis.¹⁶ To date, studies that have investigated EPCs in extracerebral vascular diseases have shown that increased levels of EPCs are associated with a reduced risk of vascular events and with better outcomes, including death from cardiovascular causes.¹⁷ Only few studies have addressed EPCs in cerebrovascular diseases reporting a lower EPC number in patients with both stable and acute stroke compared with control subjects¹⁸ and a better outcome in patients with increased EPC number after stroke.^{19,20}

Experimental studies suggest a role of the more undifferentiated bone marrow-derived circulating progenitors cells (CPCs) in neovascularization of cerebral ischemic areas.²¹ Furthermore, in one human study, CPC levels were correlated inversely with the number of cerebral infarctions and directly with preserved cerebral perfusion, suggesting that they could play a role in the maintenance of cerebral circulation after stroke.²²

In the framework of a prospective study (Microvascular LEukoencephalopathy prospective multicenter Study [MILES], conducted in Italy) aimed at evaluating clinical, biochemical, and genetic factors and mechanisms possibly modulating the phenotypic expression of genetic and sporadic leukoencephalopathies, we have evaluated the possible role of both EPCs and CPCs in CADASIL.

Materials and Methods

This was a case-control study: consecutive patients with CADASIL were matched 1:1 for age (± 3 years) and sex with healthy control subjects.

Patients with CADASIL were selected from case series already existing in 3 of the 4 centers (Florence, Siena, and Genoa) participating in MILES. Inclusion criteria were to be ≥ 18 years old, free from stroke and myocardial infarction in the previous 6 months and from severe comorbidities with impact on clinical status and on short-term prognosis; the Mini Mental State Examination²³ score had to be ≥ 18 . Genetic diagnosis had been established by the detection of a *NOTCH3* mutation as previously reported.⁵ The patients were divided into 2 groups according to clinical picture severity; the severe group was composed of patients with stroke or dementia and the mild group of patients without stroke and dementia.

For each patient with CADASIL, a control subject matched according to the previously mentioned criteria was recruited among the hospital employers and their relatives in one center. Control subjects were excluded if they had a history of cerebro- and cardiovascular diseases, peripheral arteriopathy, cancer, or dementia (a standard questionnaire was used for the screening).

Both patients and control subjects were assessed for the presence of vascular risk factors and drug use. In the CADASIL group, a standardized protocol was also applied to assess: (1) detailed medical history mainly focused on the typical disease disturbances; (2) clinical and functional status by means of neurological examination and documentation of the Disability Assessment for Dementia scale²⁴; (3) cognitive performance, applying the neuropsychological

battery used in the European multicenter "Leukoaraiosis and Disability in the Elderly Study" (LADIS)²⁵; (4) mood disturbances using the Geriatric Depression Scale²⁶; and (5) motor performance using a slightly modified Short Physical Performance Battery.²⁷ Duplex and transcranial Doppler sonography was also performed in all patients with CADASIL to exclude extracranial and intracranial artery stenosis.

The study complies with the Declaration of Helsinki and was approved by the local ethic committee of each center. All subjects gave informed consent.

Definition of Associated Diseases and Risk Factors

Hypertension was defined according to the National High Blood Pressure Education Program Coordinating Committee²⁸ or to the use of antihypertensive medications as verified by the physician. Diabetes mellitus was defined in agreement with the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus²⁹ or by the use of specific medications. Dyslipidemia was defined according to the National Cholesterol Education Program (Adult Treatment Panel III).³⁰ Smoking was considered as present in case of current or previous history. Dementia was defined according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.³¹

Blood Collection

Blood samples were obtained from an antecubital vein in the morning after an overnight fasting and were collected into evacuated plastic tubes (BD Vacutainer Systems, Plymouth, UK) containing ethylenediaminetetraacetate 0.17 mol/L for EPC and CPC evaluation.

Because inflammatory events are known to influence EPC number,³² blood was withdrawn after excluding the occurrence of infectious events (defined according to previously published criteria³³) in the previous 15 days.

Flow Cytometric Analysis of EPCs and CPCs

EPC and CPC number was assessed contemporarily using flow cytometry as previously described with minor modifications.^{32,34} Briefly, 200 μ L of peripheral venous blood was incubated for 20 minutes in the dark with the appropriated monoclonal antibodies and 300 000 cells within the leukocyte gate were acquired using a FACSCanto analyzer (Becton Dickinson, San Jose, Calif). Data were processed using BD FACS Diva software. Cells positive for CD34/KDR, CD133/KDR, and CD34/CD133/KDR were considered EPCs. By using a modification of the International Society of Hematotherapy and Graft Engineering guidelines,³⁵ CPCs were defined as cells forming a cluster with low side scatter and low-to-intermediate CD45 staining and positive for CD34, CD133, and CD34/CD133.

Statistical Analysis

To assess the statistical significance of differences in clinical data and progenitor cell numbers between patients with CADASIL and control subjects, the χ^2 test for categorical variables and Mann-Whitney test for numeric variables were used. Logistic regression analysis, including age, drug use, and sex as variables possibly influencing the cells number, was performed to test the independence of associations. In this analysis, the logarithm of the cell number was used for a better evaluation of the OR.

To study possible associations between cell numbers and the clinical manifestations, patients with CADASIL were divided into 2 groups (severe and mild) as previously defined. χ^2 test and Mann-Whitney tests were applied for comparisons between these groups. Correlation analysis with nonparametric test (Spearman) was performed to establish the possible relationship between CPC and EPC numbers and patient performances on cognitive, functional, and motor tests.

All analyses were performed using the SPSS (Statistical Package for Social Sciences, Chicago, Ill) software for Windows (Version 15.0).

Table 1. Demographic Data, Vascular Risk Factors and Therapies of Patients With CADASIL (Mild and Severe Groups and All Patients) and Control Subjects

	CADASIL Patients, Mild Group (N=13)	CADASIL Patients, Severe Group (N=16)	CADASIL Patients, Mild Versus Severe, <i>P</i>	CADASIL Patients, All (N=29)	Control Subjects (N=29)	CADASIL Patients Versus Control Subjects, <i>P</i>
Mean age, years (\pm SD)	47.7 (\pm 14.6)	60.0 (\pm 12.6)	0.03†	54.5 (\pm 14.6)	54.1 (\pm 14.6)	0.87†
Males, no. (%)	5 (38%)	7 (44%)	0.77*	12 (41%)	12 (41%)	1.00*
Education, years (mean \pm SD)	10.4 (\pm 3.7)	8.3 (\pm 3.4)	0.10†	9.2 (\pm 3.6)	—	—
Vascular risk factors						
Hypertension, no. (%)	3 (23%)	8 (50%)	0.14*	11 (38%)	10 (35%)	1.00*
Hypercholesterolemia, no. (%)	5 (38%)	6 (40%)	0.93*	11/28 (39%)	10 (34%)	0.79*
Hypertriglyceridemia, no. (%)	3 (23%)	2 (13%)	0.50*	5/28 (18%)	3 (10%)	0.47*
Diabetes mellitus, no. (%)	0	1 (6%)	0.36*	1 (3%)	0	1.00*
Smoking, no. (%)	4 (31%)	3 (19%)	0.45*	7 (24%)	7 (24%)	1.00*
Drug use						
Antiaggregants	4 (31%)	14 (87%)	0.002*	18 (62%)	1 (3%)	0.001*
Statins	3 (23%)	4 (25%)	0.90*	7 (24%)	3 (10%)	0.30*
Angiotensin converting enzyme inhibitors	1 (8%)	5 (31%)	0.12*	6 (21%)	4 (14%)	0.73*

* χ^2 .

†Mann–Whitney test.

Results

Twenty-nine patients with CADASIL belonging to 24 unrelated families and 29 control subjects were enrolled. Each group was composed of 12 males and 17 females; the mean age was 54.5 ± 14.6 years (range, 29 to 81 years) and 54.1 ± 14.6 years (range, 29 to 84 years), respectively, for patients and control subjects. Demographic data, risk factors, and drug use of patients (all and the 2 severity groups) and

control subjects are reported in Table 1. Comparing patients with control subjects, there was a similar distribution of vascular risk factors, whereas patients were more frequently on antiaggregants. Patients who were more severely affected were older and used more frequently antiaggregants than patients with a milder picture. Table 2 reports the distribution of CADASIL typical features and the mean values obtained in patients on neurocognitive tests and on functional, motor,

Table 2. Clinical Features and Mean Scores of the Cognitive, Motor, Functional, and Mood Tests/Scales Performed in All Patients With CADASIL and in the 2 Severity Subgroups (Severe Versus Mild)

	All CADASIL Patients (N=29)	Severe (N=16)	Mild (N=13)	Severe Versus Mild, <i>P</i>
Stroke, no. (%)	14 (48%)	14 (87%)	0	<0.001†
TIA, no. (%)	8 (28%)	8 (50%)	0	0.004†
Dementia, no. (%)	3 (10%)	3 (19%)	0	0.099†
Mild cognitive impairment, no. (%)	13/26 (50%)	6/14 (43%)	7/12 (58%)	0.431†
Psychiatric disturbances, no. (%)	21 (72%)	13 (82%)	8 (61%)	0.238†
Migraine, no. (%)	15 (52%)	9 (56%)	6 (50%)	0.457†
Seizures, no. (%)	2/27 (7%)	2/14 (14%)	0	0.173†
Gait disturbances, no. (%)	11/28 (39%)	9/15 (60%)	2 (15%)	0.023†
Dysphagia, no. (%)	8/28 (29%)	7/15 (47%)	1 (8%)	0.023†
MMSE, mean (\pm SD)	26.5 (\pm 4.4)	25.3 (\pm 4.7)	27.9 (\pm 3.2)	0.038*
Trial Making Test-A, mean time (\pm SD)	77.4 (\pm 64.0)	101.6 (\pm 74.0)	47.2 (\pm 30.5)	0.006*
Trial Making Test-B, mean time (\pm SD)	168.0 (\pm 96.1)	211.0 (\pm 91.9)	114.2 (\pm 73.6)	0.009*
Stroop test (interference trial) mean time (\pm SD)	46.7 (\pm 31.2)	61.5 (\pm 35.4)	32.8 (\pm 16.5)	0.007*
Verbal fluency, mean words, no. (\pm SD)	17.7 (\pm 9.4)	13.6 (\pm 8.0)	21.5 (\pm 9.0)	0.020*
Delayed word recall, mean (\pm SD)	4.4 (\pm 3.0)	5.2 (\pm 3.3)	3.8 (\pm 2.4)	0.202*
Short Physical Performance Battery, mean (\pm SD)	9.2 (\pm 3.1)	7.8 (\pm 3.6)	10.6 (\pm 1.7)	0.022*
Disability Assessment for Dementia, mean (\pm SD)	89.8% (\pm 19.1)	83.0% (\pm 24.0)	98.0% (\pm 4.0)	0.128*
Geriatric Depression Scale	4.4 (\pm 3.2)	4.4 (\pm 3.2)	4.5 (\pm 3.2)	0.912*

*Mann–Whitney test.

† χ^2 .

TIA indicates transient ischemic attack; MMSE, Mini Mental State Examination.

Table 3. Median Values of the EPC and CPC Numbers (Cells/Microliter) in the 2 Severity Groups (Mild, 13 and Severe, 16) and All Patients With CADASIL (29) and Control Subjects (29)

	CADASIL Patients, Mild Group Median (Range)	CADASIL Patients, Severe Group Median (Range)	Mild Versus Severe, <i>P</i> *	All CADASIL Patients Median (Range)	Control Subjects Median (Range)	CADASIL Patients Versus Control Subjects, <i>P</i> *
EPCs						
CD34+/kdr+	0.06 (0.001–0.27)	0.04 (0.001–0.15)	0.211	0.05 (0.001–0.27)	0.10 (0.02–0.34)	0.005
CD133+/kdr+	0.09 (0.001–0.31)	0.06 (0.001–0.13)	0.062	0.07 (0.001–0.31)	0.10 (0.02–0.34)	0.006
CD34+/CD133+/Kdr+	0.06 (0.001–0.31)	0.04 (0.001–0.13)	0.124	0.05 (0.001–0.31)	0.10 (0.02–0.34)	0.001
CPCs						
CD34+	2.95 (1.75–4.79)	1.68 (0.72–3.69)	0.007	2.29 (0.72–4.79)	2.61 (0.80–5.19)	0.499
CD133+	2.82 (1.55–4.77)	1.40 (0.66–3.42)	0.004	2.26 (0.66–4.77)	2.60 (0.80–5.19)	0.316
CD34+/133+	2.75 (1.61–4.77)	1.44 (0.66–3.40)	0.004	2.29 (0.66–4.77)	2.55 (0.80–5.19)	0.266

*Mann–Whitney test.

and depression scales. Except for 3 demented patients, global cognitive performances and functional abilities were relatively well preserved. However, as expected, the more severe group had worse performance on neuropsychological and motor tests. In the mild group, 3 patients were asymptomatic and one had migraine only. Considering bone marrow-derived cells, median EPC count was significantly lower in patients with CADASIL with respect to control subjects (Table 3). The difference remained significant after adjusting for age, sex, and statin use in multivariate logistic regression (OR, 9.6; 95% CI, 1.5 to 59.1 for CD34/KDR; OR, 8.9; 95% CI, 1.3 to 59.3 for CD133/KDR; and OR, 13.5; 95% CI, 1.9 to 97.9 for CD34/CD133/KDR). Entering 2 other drugs potentially active on endothelium, namely antiaggregants and angiotensin converting enzyme inhibitors, the strength of the association did not change. Also, CPC levels were lower in patients with CADASIL than control subjects, but the difference did not reach statistical significance (Table 3).

Taking into account disease severity, patients with a more severe clinical picture, compared with those with a milder phenotype, had a statistically significant lower number of CPCs (Table 3). After adjusting for age, this difference remained significant (*P* values and regression coefficient

[Wald statistic], respectively, for CD34, CD133, and CD34/133 were 0.044 and -2.56 [5.75], 0.041 and -2.50 [4.20], and 0.034 and -2.87 [5.65]). The differences in EPC values between patients with a mild or severe picture narrowly failed to reach the statistical significance.

Some correlations between CPC levels and cognitive or motor performances proved significant (Table 4).

Discussion

To the best of our knowledge, this is the first study to evaluate EPCs and CPCs in CADASIL and their possible association with measures of clinical severity. There was an association between low bone marrow-derived circulating cells levels and CADASIL, particularly in patients with the most severe manifestations of the disease. The strength of the association was corroborated by the fact that the number of CPCs was correlated with cognitive and motor performances.

EPCs contribute to the maintenance of the endothelium by replacing injured mature endothelial cells and by serving as a cellular reservoir for the replacement of dysfunctional endothelium. Our finding that patients with CADASIL have a reduced number of circulating EPCs is consistent with previous data, obtained using different markers, pointing to the

Table 4. Correlation Analysis Between CPC and EPC Numbers and the Scores of the Cognitive Tests and Motor, Functional, and Depression Scales*

	CPC			EPC		
	CD34+	CD133+	CD34+/133+	CD34+kdr+	CD133+kdr+	CD34+/CD133+/Kdr+
MMSE	0.376 (0.053)	0.383 (0.049)	0.407 (0.035)	−0.106 (0.598)	0.064 (0.751)	−0.084 (0.675)
TMT-A	−0.401 (0.038)	−0.417 (0.031)	−0.416 (0.031)	0.259 (0.192)	0.073 (0.719)	0.170 (0.397)
TMT-B	−0.492 (0.009)	−0.518 (0.006)	−0.507 (0.007)	0.380 (0.051)	0.325 (0.098)	0.337 (0.086)
Stroop test (interference trial)	−0.499 (0.009)	−0.550 (0.004)	−0.561 (0.003)	0.465 (0.017)	0.224 (0.271)	0.441 (0.024)
Delayed word recall	−0.456 (0.017)	−0.459 (0.016)	−0.428 (0.026)	0.102 (0.614)	0.037 (0.853)	0.151 (0.453)
Verbal fluency	0.514 (0.006)	0.509 (0.007)	0.507 (0.007)	−0.244 (0.220)	−0.090 (0.654)	−0.184 (0.359)
SPPB	0.421 (0.029)	0.402 (0.038)	0.390 (0.044)	−0.087 (0.668)	−0.084 (0.676)	−0.070 (0.728)
DAD scale	0.403 (0.051)	0.368 (0.077)	0.340 (0.105)	−0.104 (0.629)	−0.023 (0.916)	−0.092 (0.668)
GDS	−0.175 (0.384)	−0.147 (0.464)	−0.129 (0.522)	0.037 (0.855)	−0.032 (0.874)	−0.053 (0.791)

*Values represent the Spearman rho; *P* values shown in parentheses.

MMSE indicates Mini Mental State Examination; TMT, Trial Making Test; SPPB, Short Physical Performance Battery; DAD, Disability Assessment for Dementia; GDS, Geriatric Depression Scale.

presence of endothelial dysfunction in CADASIL. Although the disease pathology affects mainly the tunica media, endothelial changes, including cytoplasmic swelling, disruption of tight junctions, and appearance of bundles of microfilaments, have been also reported.³⁶ Altered endothelial-dependent vasodilatation in cerebral and forearm arteries and higher levels of asymmetrical dimethylarginine, a nitric oxide endogenous inhibitor, have been found.^{14,15,37} Even if our findings support the involvement of the endothelium in the pathogenesis of the disease, differently from the experimental setting, in vivo studies do not allow to establish whether this is associated with a failure of angiogenesis. Moreover, it is not possible to determine whether the endothelium is damaged and cells are not being replaced or there might be endothelium degeneration not compensated by increase in EPCs. Further studies are warranted to better elucidate the relationship between EPCs and CADASIL pathogenesis.

Regarding the different behavior of EPCs and CPCs, there are a few possible explanations: one is technical and is related to the fact that EPCs are rare in the circulation; from a biological viewpoint, EPCs and CPCs likely represent different progenitor cell phenotypes with different biological properties. The EPC circulating pool represents a population of more mature cells that are just “committed” to differentiate into endothelial cells able to participate mainly in the processes of the re-endothelialization and revascularization. On the other hand, CPCs are a more heterogeneous and undifferentiated cell population that can evolve in different cell types. They are known for being involved in sustaining the homeostasis of damaged brain. In animal models, it has been shown that within the central nervous system, these cells can give rise to neurons,³⁸ astrocytes,³⁹ and oligodendrocytes⁴⁰ as well as endothelial cells.⁴¹ In vitro studies are able to produce several growth factors.⁴² In a few human studies, their role in angiogenesis^{43,44} and cerebral circulation²² has been shown.

The main limitation of our study is the small number of patients examined. This implies that potential confounders, both those involved in the relation between cell numbers and the disease (for example, age, sex, or drugs⁴⁵) and those influencing the severity of phenotype (for example, hypertension and smoking^{7,8}), cannot be adequately accounted for. Moreover, we cannot exclude completely a chance effect. However, CADASIL remains a rare disease and published series are usually not large. This remains a preliminary observation to be expanded in future studies. Being a cross-sectional study, we are not able to show any data about variations in progenitor cells occurring over time possibly related to aging or to disease progression.

Conclusions

We documented, for the first time, an association between EPCs and CPCs and CADASIL. These data corroborate the hypothesis that endothelial dysfunction in CADASIL plays an active role and may contribute to its phenotypic expression. Further and more robust data are needed to prove this hypothesis. If this will be the case, pharmacological strategies aimed at favoring stem cell mobilization could be considered to limit the clinical and functional consequences of CADASIL.

Acknowledgments

We thank Roberto Caporale, MD, for critical discussion on cytofluorimetric data and Giovanni Pracucci, MD, for statistical analysis.

Sources of Funding

The study was supported by the Italian Ministry of University and Research (Ministero dell'Istruzione Università Ricerca [MIUR]), Programmi di ricerca cofinanziati—2006 (prot. 2006065719).

Disclosures

None.

References

- Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cécillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserre E. *Notch 3* mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996;383:707–710.
- Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssiere C, Cruaud C, Maciazek J, Weissenbach J, Bousser MG, Bach JF, Tournier-Lasserre E. Strong clustering and stereotyped nature of *Notch3* mutations in CADASIL patients. *Lancet*. 1997;350:1511–1515.
- Chabriat H, Joutel A, Dichgans M, Tournier-Lasserre E, Bousser MG. CADASIL. *Lancet Neurol*. 2009;8:643–653.
- Opherk C, Peters N, Herzog J, Luedtke R, Dichgans M. Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. *Brain*. 2004;127:2533–2539.
- Pescini F, Bianchi S, Salvadori E, Poggesi A, Dotti MT, Federico A, Inzitari D, Pantoni L. A pathogenic mutation on exon 21 of the *NOTCH3* gene causing CADASIL in an octogenarian paucisymptomatic patient. *J Neurol Sci*. 2008;267:170–173.
- Pescini F, Bianchi S, Dotti MT, Federico A, Inzitari D, Pantoni L. First report of a Romanian CADASIL patient following immigration to Italy. *Eur J Neurol*. 2007;14:7–8.
- Singhal S, Bevan S, Barrick T, Rich P, Markus HS. The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. *Brain*. 2004;127:2031–2038.
- Viswanathan A, Guichard JP, Gschwendtner A, Buffon F, Cumurcu R, Boutron C, Vicaute E, Holtmannspötter M, Pachai C, Bousser MG, Dichgans M, Chabriat H. Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. *Brain*. 2006;129:2375–2383.
- Chabriat H, Pappata S, Ostergaard L, Clark CA, Pachot-Clouard M, Vahedi K, Jobert A, Le Bihan D, Bousser MG. Cerebral hemodynamics in CADASIL before and after acetazolamide challenge assessed with MRI bolus tracking. *Stroke*. 2000;31:1904–1912.
- van den Boom R, Lesnik Oberstein SA, Spilt A, Behloul F, Ferrari MD, Haan J, Westendorp RG, van Buchem MA. Cerebral hemodynamics and white matter hyperintensities in CADASIL. *J Cereb Blood Flow Metab*. 2003;23:599–604.
- Pfefferkorn T, von Stuckrad-Barre S, Herzog J, Gasser T, Hamann GF, Dichgans M. Reduced cerebrovascular CO₂ reactivity in CADASIL: A transcranial Doppler sonography study. *Stroke*. 2001;32:17–21.
- Singhal S, Markus HS. Cerebrovascular reactivity and dynamic autoregulation in nondemented patients with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). *J Neurol*. 2005;252:163–167.
- Gobron C, Vahedi K, Vicaute E, Stucker O, Laemmel E, Baudry N, Bousser MG, Chabriat H. Characteristic features of in vivo skin microvascular reactivity in CADASIL. *J Cereb Blood Flow Metab*. 2007;27:250–257.
- Peters N, Freilinger T, Opherk C, Pfefferkorn T, Dichgans M. Enhanced L-arginine-induced vasoreactivity suggests endothelial dysfunction in CADASIL. *J Neurol*. 2008;255:1203–1208.
- Stenborg A, Kalimo H, Viitanen M, Terent A, Lind L. Impaired endothelial function of forearm resistance arteries in CADASIL patients. *Stroke*. 2007;38:2692–2697.
- Isner JM, Asahara T. Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularisation. *J Clin Invest*. 1999;103:1231–1236.

17. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Böhm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med*. 2005;353:999–1007.
18. Ghani U, Shuaib A, Salam A, Nasir A, Shuaib U, Jeerakathil T, Sher F, O'Rourke F, Nasser AM, Schwandt B, Todd K. Endothelial progenitor cells during cerebrovascular disease. *Stroke*. 2005;36:151–153.
19. Sobrino T, Hurtado O, Moro MA, Rodríguez-Yáñez M, Castellanos M, Brea D, Moldes O, Blanco M, Arenillas JF, Leira R, Dávalos A, Lizasoain I, Castillo J. The increase of circulating endothelial progenitor cells after acute ischemic stroke is associated with good outcome. *Stroke*. 2007;38:2759–2764.
20. Yip HK, Chang LT, Chang WN, Lu CH, Liou CW, Lan MY, Liu JS, Youssef AA, Chang HW. Level and value of circulating endothelial progenitor cells in patients after acute ischemic stroke. *Stroke*. 2008;39:69–74.
21. Beck H, Voswinckel R, Wagner S, Ziegelhoeffer T, Heil M, Helisch A, Schaper W, Acker T, Hatzopoulos AK, Plate KH. Participation of bone marrow-derived cells in long-term repair processes after experimental stroke. *J Cereb Blood Flow Metab*. 2003;23:709–717.
22. Taguchi A, Matsuyama T, Moriaki H, Hayashi T, Hayashida K, Nagatsuka K, Todo K, Mori K, Stern DM, Soma T, Naritomi H. Circulating CD34-positive cells provide an index of cerebrovascular function. *Circulation*. 2004;109:2972–2975.
23. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state.' A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189–198.
24. Gelinàs I, Gauthier L, McIntyre M, Gauthier S. Development of a functional measure for persons with Alzheimer's disease: the disability assessment for dementia. *Am J Occup Ther*. 1999;53:471–481.
25. Madureira S, Verdelho A, Ferro J, Basile AM, Chabriet H, Erkinjuntti T, Fazekas F, Hennerici M, O'Brien J, Pantoni L, Salvadori E, Scheltens P, Visser MC, Wahlund LO, Waldemar G, Wallin A, Inzitari D; LADIS Study Group. Development of a neuropsychological battery for the Leukoaraiosis and Disability in the Elderly Study (LADIS): experience and baseline data. *Neuroepidemiology*. 2006;27:101–116.
26. Yesavage JA. Geriatric Depression Scale. *Psychopharmacol Bull*. 1988;24:709–711.
27. Baezner H, Blahak C, Poggesi A, Pantoni L, Inzitari D, Chabriet H, Erkinjuntti T, Fazekas F, Ferro JM, Langhorne P, O'Brien J, Scheltens P, Visser MC, Wahlund LO, Waldemar G, Wallin A, Hennerici MG; LADIS Study Group. Association of gait and balance disorders with age-related white matter changes: the LADIS study. *Neurology*. 2008;70:935–942.
28. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ. The National High Blood Pressure Education Program Coordinating Committee Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*. 2003;42:1206–1252.
29. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2003;26:5–20.
30. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106:3143–3421.
31. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. Washington, DC: American Psychiatry Association; 1994:133.
32. Cesari F, Caporale R, Marcucci R, Cacioli S, Stefano PL, Capalbo A, Macchi C, Vannucci M, Gensini GF, Abbate R, Gori AM. NT-proBNP and the anti-inflammatory cytokines are correlated with endothelial progenitor cells' response to cardiac surgery. *Atherosclerosis*. 2008;199:138–146.
33. Nencini P, Sarti C, Innocenti R, Pracucci G, Inzitari D. Acute inflammatory events and ischemic stroke subtypes. *Cerebrovasc Dis*. 2003;15:215–221.
34. Cesari F, Sofi F, Caporale R, Capalbo A, Marcucci R, Macchi C, Lova RM, Cellai T, Vannucci M, Gensini GF, Abbate R, Gori AM. Relationship between exercise capacity, endothelial progenitor cells and cytokines in patients undergoing cardiac rehabilitation. *Thromb Haemost*. 2009;101:521–526.
35. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J Hematother*. 1996;5:213–226.
36. Ruchoux MM, Muraige CA. Endothelial changes in muscle and skin biopsies in patients with CADASIL. *Neuropathol Appl Neurobiol*. 1998;24:60–65.
37. Rufa A, Bardi P, De Lalla A, Cevenini G, De Stefano N, Zicari E, Auteri A, Federico A, Dotti MT. Plasma levels of asymmetric dimethylarginine in cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy. *Cerebrovasc Dis*. 2008;26:636–640.
38. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science*. 2000;290:1775–1779.
39. Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci U S A*. 1997;94:4080–4085.
40. Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp Neurol*. 2002;174:11–20.
41. Zhang ZG, Zhang L, Jiang Q, Chopp M. Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. *Circ Res*. 2002;90:284–288.
42. Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzakowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ. Numerous growth factors, cytokines, and chemokines are secreted by human CD34+ cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood*. 2001;97:3075–3085.
43. Yoshihara T, Taguchi A, Matsuyama T, Shimizu Y, Kikuchi-Taura A, Soma T, Stern DM, Yoshikawa H, Kasahara Y, Moriaki H, Nagatsuka K, Naritomi H. Increase in circulating CD34-positive cells in patients with angiographic evidence of moyamoya-like vessels. *J Cereb Blood Flow Metab*. 2008;28:1086–1089.
44. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imazumi T; Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet*. 2002;360:427–435.
45. Jung KH, Roh JK. Circulating endothelial progenitor cells in cerebrovascular disease. *J Clin Neurol*. 2008;4:139–147.