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Circulating Endothelial Progenitor Cells Are Reduced in Peripheral Vascular Complications of Type 2 Diabetes Mellitus

Gian Paolo Fadini, MD,* Marta Miorin, BS,† Monica Facco, BS,† Sondra Bonamico,† Ilenia Baesso, BS,† Franco Grego, MD,‡ Mirko Menegolo, MD,‡ Saula Vigili de Kreutzenberg, MD,* Antonio Tiengo, MD,* Carlo Agostini, MD,† Angelo Avogaro, MD, PHD*

Padova, Italy

OBJECTIVES We sought to establish whether a reduction in endothelial progenitor cells (EPCs) has a putative role in peripheral vascular disease (PVD) of type 2 diabetic patients.

BACKGROUND Peripheral vascular disease is a common and severe complication of diabetes mellitus. Impaired collateralization of diabetic vasculopathy has been extensively shown, but causes leading to its pathogenesis are not fully understood. Recently, EPCs have been found to contribute to vascular repair and angiogenesis. Diabetes has been associated with low levels of circulating EPCs, but no data are available in the literature on the relationship between EPCs and PVD in diabetes.

METHODS Flow cytometric analysis was used to quantify circulating progenitor cells (CPCs, CD34+) and EPCs (CD34+KDR+) in 51 patients and 17 control subjects.

RESULTS The CPCs and EPCs from diabetic patients were reduced by 33% and 40%, respectively, compared with healthy subjects ($p < 0.001$). An inverse correlation was found between the number of EPCs and the values of fasting glucose ($r = -0.49$, $p = 0.006$). Peripheral vascular disease was associated with a 47% reduction in EPCs ($p < 0.0001$) and EPC levels directly correlated with the ankle-brachial index ($r = 0.70$, $p = 0.01$). The subgroup of diabetic patients with PVD also had reduced CPCs by 32% ($p = 0.037$), whereas patients with ischemic foot lesions had the lowest levels of both EPCs and CPCs ($p = 0.02$).

CONCLUSIONS Our data demonstrate decreased EPC levels in diabetic patients and, for the first time, show that PVD is associated with an extensively low number of EPCs. Depletion of circulating EPCs in diabetic patients may be involved in the pathogenesis of peripheral vascular complications. (J Am Coll Cardiol 2005;45:1449–57) © 2005 by the American College of Cardiology Foundation

Peripheral vascular disease (PVD) is a common and severe complication of diabetes mellitus (DM) and is characterized by a high prevalence, early development, and rapid progression. In diabetes, there is also an impaired collateralization of vascular ischemic beds (1,2), but mechanisms that hinder ischemia-induced neovascularization in diabetes remain elusive (3).

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Emerging evidence indicates that bone marrow-derived endothelial progenitor cells (EPCs) take part in postnatal neovascularization (4). The EPCs co-express surface markers of both hematopoietic stem cells (CD34 and CD133) and endothelial cells (VEGF-R2, also known as KDR) (5,6). The EPCs express endothelial phenotype in culture, promote in vivo re-endothelization (7), and are able to be incorporated into new vessels in animal models of hindlimb ischemia (8,9).

The EPCs are reduced in the presence of risk factors for coronary artery disease (CAD), endothelial dysfunction, hypercholesterolemia, smoking, and chronic renal failure (10–14). Lambiase et al. (15) have shown that poor coronary collateral development is associated with reduced numbers of circulating EPCs. Thus, depletion of circulating EPCs may contribute to both endothelial dysfunction, as an early event in the atherogenetic process, and poor collateralization, as a late event leading to clinical manifestations of atherosclerosis and cardiovascular disease progression.

Recently, circulating EPC reduction and dysfunction have been reported in both type 1 and type 2 diabetic patients (16,17): these alterations are likely to be involved in the pathogenesis of vascular complications of DM, but there are no data on this topic currently available in the literature.

Therefore, the aim of this study was to evaluate circulating EPC numbers in type 2 diabetic patients with and without PVD to establish whether an EPC reduction in diabetic patients has a putative role in the pathogenesis of peripheral vascular complications.

METHODS

Patients. Fifty-one patients were recruited for this study and classified into three groups: diabetics with (n = 24) and without (n = 16) PVD and nondiabetics with PVD (n =

From the *Department of Clinical and Experimental Medicine, Division of Metabolic Diseases; †Department of Clinical and Experimental Medicine, Clinical Immunology and Hematology; and ‡Department of Medical and Surgical Sciences, Division of Vascular Surgery, University of Padova, School of Medicine, Padova, Italy.

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Abbreviations and Acronyms

ABI = ankle-brachial index
AER = albuminuria excretion rate
CAD = coronary artery disease
CPC = circulating progenitor cell
DM = diabetes mellitus
EPC = endothelial progenitor cell
PVD = peripheral vascular disease

11). Diabetic patients were selected from the Division of Metabolic Diseases, whereas nondiabetic patients were selected from the Division of Vascular Surgery of the University of Padova. Seventeen healthy control subjects were recruited from the local community. Ethics committee approval and written, informed consent from all subjects were obtained.

Lower extremity vascular disease was diagnosed by a history of claudication or rest pain, bilateral pulse examination (dorsal pedal, posterior tibial, popliteal, and femoral arteries), ankle-brachial index (ABI), ultrasonography performed bilaterally at levels of femoral and popliteal arteries (n = 25), and eventually angiography (n = 7). The ABI was measured as follows: patients were asked to rest supine for 30 min, then bilateral brachial and ankle (dorsalis pedis and posterior tibial arteries) systolic blood pressures were measured through use of a hand-held 5-MHz Doppler scanning probe. The ABI was calculated for each leg by dividing the average of the two ankle arterial pressures by the average of the left and right brachial artery pressures. If brachial artery pressures differed by ≥10 mm Hg, the highest brachial artery pressure was used. Patients with an ABI of <0.90 were considered suggestive of PVD, whereas an ABI >1.40 was considered indicative of the presence of noncompressible arteries.

Carotid atherosclerosis was assessed by bilateral carotid artery ultrasonography, and, when available, angiography (n = 6).

Patients were considered to have CAD in the presence of electrocardiographic (ECG) findings unambiguously suggestive of a past myocardial infarction (n = 3), a positive ECG at exercise test (n = 2), an echocardiographic stress test positive for inducible ischemia (n = 3), or evidence of significant coronary artery stenosis on a coronary angiogram (n = 4).

In control subjects, the absence of type 2 DM and

Table 1. Patient Characteristics

Characteristic	Diabetics With PVD (n = 24)	Diabetics Without PVD (n = 16)	Controls With PVD (n = 11)	Controls Without PVD (n = 17)	p Value
Age (yrs)	68.7	69.5	68.0	50.1*	<0.001
Gender (% male)	70.1	56.2	90.9	41.2	0.04
BMI (kg/m^2)	27.5	29.3	26.6	26.6	NS
Waist (cm)	103.5	107.1	96.6*	93.6*	0.01
Laboratory					
Fasting glucose (mg/dl)	222.1	176.2	92.6*	93.7*	<0.001
HbA_{1c} (%)	8.9	10.9	—	—	0.03
Total cholesterol (mg/dl)	177.5	193.2	196.0	166.5	NS
LDL cholesterol (mg/dl)	111.7	115.1	134.8	90.0	NS
HDL cholesterol (mg/dl)	45.7	45.1	52.0	55.0	NS
Triglycerides (mg/dl)	133.3	182.9	102.1	76.3	NS
Anamnesis					
Hypertension (%)	21 (88)	10 (63)	7 (64)	6 (35)*	0.01
CAD (%)	9 (37)	—	3 (27)	—	NS
PVD (%)	24 (100)	—	11 (100)	—	NS
Carotid (%)	15 (63)	—	7 (63)	—	NS
Lower extremity (%)	18 (75)	—	6 (55)	—	NS
Foot lesions (%)	8 (33)	—	1 (10)	—	NS
Retinopathy (%)	7 (29)	3 (19)	—	—	NS
Smoke (%)	3 (13)	3 (19)	4 (36)	3 (18)	NS
Familiarity (%)	9 (39)	4 (25)	1 (9)	8 (47)	NS
Metabolic syndrome (%)	12 (50)	5 (31)	4 (36)	2 (12)	NS
Therapy					
Insulin (%)	14 (58)	6 (38)	—	—	NS
Oral antidiabetics (%)	11 (46)	7 (44)	—	—	NS
Statin (%)	10 (42)	2 (13)*	3 (27)	0*	0.006
Beta-blockers (%)	3 (13)	1 (6)	1 (9)	0	NS
Calcium antagonists (%)	8 (33)	3 (19)	2 (18)	0	NS
ACE inhibitors (%)	18 (75)	13 (81)	4 (36)*	0*	<0.001
Diuretics (%)	16 (67)	4 (25)*	3 (27)*	0*	<0.001
Antiaggregants (%)	15 (63)	6 (38)	3 (27)*	0*	<0.001
Anticoagulants (%)	2 (8)	1 (6)	0	0	NS

*$p < 0.05$. Data are presented as the mean value or number (%) of subjects.

ACE = angiotensin-converting enzyme; BMI = body mass index; CAD = coronary artery disease; HbA_{1c} = glycosylated hemoglobin; HDL and LDL = high- and low-density lipoprotein; PVD = peripheral vascular disease.

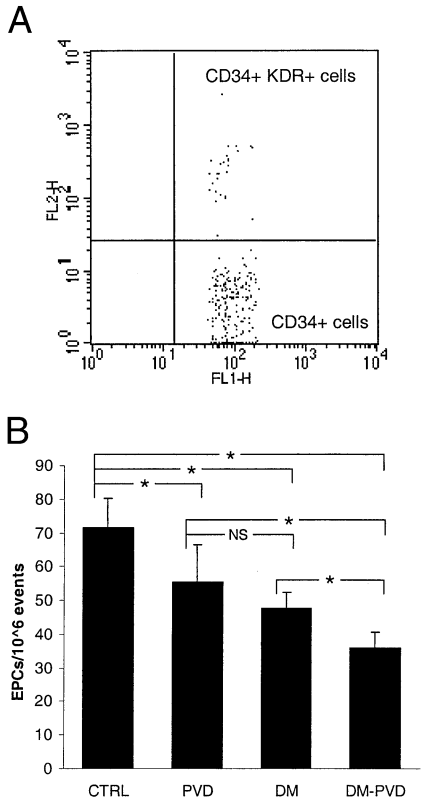

Figure 1. Circulating endothelial progenitor cells (EPCs) and circulating progenitor cells (CPCs) were identified by flow cytometry with low cytoplasmic granularity and with the expression of cell surface antigens, such as CD34 and VEGF-R2 (KDR). **(A)** Representative flow cytometry analysis of a blood sample from a patient with high EPC count. **(B)** The number of EPCs from peripheral blood in the four groups of subjects. Mean values ± SE. CTRL = control subjects; NS = not statistacally significant. *$p < 0.05$.

impaired glucose tolerance was documented by means of fasting glucose and 2-h glucose determination or oral glucose tolerance test. The absence of cardiovascular diseases was evaluated by a clinical history and examination, carotid ultrasonography, and, when available, echocardiography and coronary angiography.

In all patients body mass index, waist circumference, risk factors for cardiovascular diseases, and pharmacologic history were assessed. Diabetic patients underwent a metabolic evaluation, including fasting glucose, immunoreactive insulin and C-peptide, glycated hemoglobin, and lipid profile. Renal function and albuminuria excretion rate (AER) were also evaluated. The presence of diabetic retinopathy was assessed by ophthalmologic examination. A diagnosis of the metabolic syndrome was considered when patients met the criteria of either the World Health Organization or the Adult Treatment Panel III from the National Cholesterol Education Program (18,19).

Quantification of peripheral blood EPCs and CPCs by flow cytometry. After an overnight fast, blood samples were obtained through a 20-gauge butterfly needle inserted into a forearm vein. All samples were processed after 1 to 2 h. Peripheral blood progenitor cells were analyzed for the expression of cell-surface antigens with direct two-color analysis using fluorescein isothiocyanate (FITC)-conjugated and phycoerythrin (PE)-conjugated monoclonal antibodies (mAbs) by flow cytometric analysis (FACScan, Becton Dickinson, Sunnyvale, California), as previously reported (10,13,15). Briefly, 150 μl of peripheral blood was incubated with 10 μl of FITC-conjugated anti-human CD34 mAb (Becton Dickinson) and 10 μl of PE-conjugated anti-human KDR mAb (R&D Systems Inc., Minneapolis, Minnesota), followed by incubation at 4°C for 30 min. Control isotype immunoglobulin (Ig)G1 and IgG2a antibody were obtained from Becton Dickinson. The frequency of peripheral blood cells positive for these reagents was determined by a two-dimensional side-scatter fluorescence dot-plot analysis of the samples, after appropriate gating, stained with the different reagents, as previously reported. Briefly, initially we gated CD34+ peripheral blood cells and then examined the resulting population for dual expression of KDR. Circulating progenitor cells (CPCs) were defined as CD34+ cells, whereas EPCs were defined as CD34+ and KDR+ cells. For FACS analysis, 5×10^5 cells were acquired and scored using a FACScan analyzer (Becton Dickinson). Data were processed using the Macintosh CELLQuest software program (Becton Dickinson). The instrument set-up was optimized daily by analyzing the expression of peripheral blood lymphocytes labeled with anti-CD4 FITC/CD8 PE/CD3 PECy5/CD45 APC four-color combination.

Statistical analysis. Data are expressed as the mean value ± SD, except as otherwise specified. Results from flow cytometry are expressed as the number of cells per one million events. Comparisons between two or more groups were performed by the unpaired Student *t* test and analysis of variance, respectively. The chi-square test was used for dichotomous variables. Statistical associations between clinical conditions or risk factors and cell counts were examined by multivariate analysis using multiple linear regression. Correlations of blood glucose and ABI with progenitor cells were assessed by Pearson's coefficient (r), whereas the correlation between risk factors and progenitor cells was assessed by Spearman's coefficient (rho).

Statistical significance was accepted if the null hypothesis could be rejected at $p \leq 0.05$.

RESULTS

Patient characteristics. Subject characteristics are presented in Table 1. The group of diabetic patients was representative of a mixed type 2 diabetic population with and without macrovascular complications. Patients had a wide range in their duration of diabetes (1 to 30 years). Twenty-two percent of patients (n = 15) were receiving statin therapy for more than six months. Pathologic AER (>30 mg per 24 h) was present in seven diabetic patients with PVD (29%) and four diabetics without PVD (25%). Chronic renal failure was present in one diabetic patient

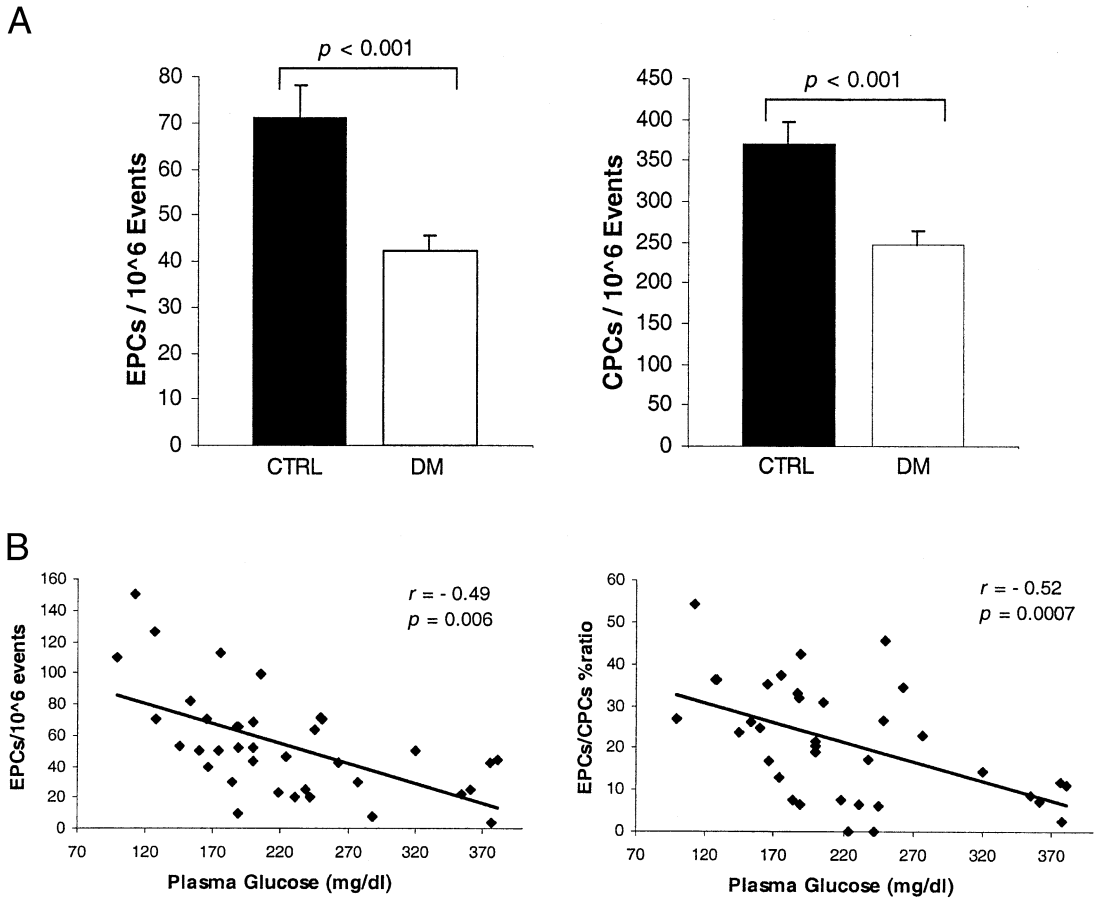

Figure 2. (A) Diabetic patients have lower levels of circulating endothelial progenitor cells (EPCs) when compared to controls. Circulating progenitor cells (CPCs) from diabetics display a similar reduction, indicating that the effect of diabetes per se is not specifically targeted to endothelial progenitors. **(B)** Circulating EPCs and EPC/CPC ratio were negatively correlated with blood glucose at time of blood collection. Higher glucose concentrations were associated with low absolute numbers of EPCs and low endothelial fraction of all progenitors. Mean values ± SE. CTRL = control subjects; DM = diabetes mellitus.

with PVD (<1%) and in three diabetics without PVD (19%); none of these patients was on hemodialysis.

Circulating EPCs and CPCs are reduced in type 2 diabetic patients. Flow cytometry was used to determine the number of circulating peripheral blood CD34+ cells (CPCs) and CD34+KDR+ cells (EPCs). Because only 0.02% to 0.07% of white blood cells where CD34+, EPC and CPC counts were expressed for one million cytometric events. The EPC/CPC percent ratio was taken to represent the extent of endothelial differentiation of generic circulating progenitors (Fig. 1A). Figure 1B illustrates EPC counts in the four groups of subjects taken into consideration. The analysis of variance revealed a significant difference among the groups ($p = 0.008$). Differences were then analyzed with the least significant difference post-hoc test.

Circulating EPC and CPC levels were significantly lower in diabetic patients compared with healthy control subjects (42 ± 21 vs. 71 ± 38, $p < 0.001$; and 247 ± 95 vs. 370 ± 139, $p < 0.001$, respectively) (Fig. 2A).

In diabetic patients, linear regression analysis revealed that both the number of EPCs and the EPC/CPC percent ratio negatively correlated with the value of fasting glucose on day of blood collection ($r = -0.49$, $p = 0.006$ and $r = -0.52$, $p = 0.0007$, respectively) (Fig. 2B). No significant correlations were found with glycated hemoglobin levels.

In the multivariate analyses, of all risk factors for atherosclerotic disease, DM was the most significantly associated with a reduced EPC count (Table 2, Fig. 3A). In patients with a diagnosis of the metabolic syndrome, an EPC reduction was even more evident (35 ± 18 vs. 71 ± 37, $p < 0.001$) (Fig. 3B). Moreover, the number of risk factors significantly correlated with both EPCs (rho = −0.48, $p = 0.003$) and CPCs (rho = −0.39, $p = 0.02$) (Fig. 3C).

EPCs and CPCs in patients with PVD. Of all clinical conditions, PVD, but neither CAD nor microvascular complications (Table 2, Fig. 4), was associated with a strong reduction in EPCs (37 ± 22 vs. 70 ± 37, $p < 0.0001$) and in the endothelial committed fraction of CPCs (14 ± 9% vs. 23 ± 13%, $p = 0.003$), as compared with controls and all non-PVD patients. On the contrary, the total number of CPCs did not statistically differ between vascular and nonvascular patients, but when considering all patients with PVD, the subgroup of diabetics had significantly lower total

Table 2. Multiple Linear Regression Analyses Between Progenitor Cells (Dependent Variables) and Risk Factors or Clinical Conditions (Independent Variables)

	EPC Number		CPC Number	
	Beta Coefficient*	p Value†	Beta Coefficient*	p Value†
Risk factors	(r^2 = 0.27)		(r^2 = 0.25)	
Smoke	0.233	0.071	−0.053	0.330
Familiarity	0.084	0.232	−0.076	0.259
Hypercholesterolemia	−0.093	0.188	0.112	0.190
Gender (M)	−0.188	0.121	−0.023	0.425
Age >50 years	−0.166	0.152	0.016	0.452
Diabetes	−0.388	0.0007	−0.543	<0.0001
Obesity	0.111	0.182	−0.024	0.420
Hypertension	0.142	0.188	0.139	0.164
ANOVA	—	0.015	—	0.032
Clinical conditions	(r^2 = 0.17)		(r^2 = 0.05)	
PVD	−0.441	0.0006	−0.144	0.151
CAD	0.093	0.231	−0.070	0.303
Retinopathy	−0.022	0.425	−0.017	0.445
AER >30 mg/24h	0.049	0.346	−0.093	0.243
Chronic renal failure	−0.090	0.241	−0.057	0.331
ANOVA	—	0.049	—	0.317

*Standardized regression coefficient. †One-sided p value.
AER = albuminuria excretion rate; ANOVA = analysis of variance; CPC = circulating progenitor cell; EPC = endothelial progenitor cell; M = male; other abbreviations as in Table 1.

CPC counts (239 ± 96 vs. 349 ± 22, p = 0.037) (Fig. 5A). No differences in EPC and CPC levels were found between carotid and lower extremity vascular disease (p = 0.27). In a subgroup of 20 patients with PVD, both EPCs and CPCs showed a direct and significant correlation with the values of ABI (Fig. 5B). Finally, diabetic patients with ischemic foot lesions due to end-stage PVD had even lower EPC and CPC numbers as compared with controls and diabetic patients with PVD but without foot lesions (27 ± 25 vs. 54 ± 31, p = 0.02 and 182 ± 68 vs. 275 ± 89, p = 0.008, respectively) (Fig. 5C).

The PVD patients on long-term statin therapy had higher EPC levels (66 ± 36 vs. 34 ± 21, p = 0.002) and higher EPC/CPC percent ratios (26 ± 11% vs. 12 ± 7%, p < 0.001) than did the patients with PVD who were not treated with statins (Fig. 5D).

DISCUSSION

In this study, we show that: 1) type 2 diabetic patients have a 40% mean reduction in peripheral blood EPC numbers; 2) PVD is characterized by very low EPC levels; and 3) diabetic patients with peripheral vascular complications have a more profound reduction of all circulating progenitors.

The ability of organisms to spontaneously develop collateral vessels represents an important response to vascular occlusive diseases that partly determines the severity of residual tissue ischemia. Recent studies demonstrate that postnatal neovascularization does not rely exclusively on sprouting from existing vessels, but also involves a subset of bone marrow-derived progenitor cells (4). The mobilization of EPCs from bone marrow to peripheral blood has been reported in patients after acute myocardial infarction and acute coronary syndromes (20–22), but the literature lacks data on EPC variations in the presence of PVD.

Diabetes mellitus is characterized by endothelial dysfunction (23–25) and a three- to four-fold increase in cardiovascular risk, and diabetic vasculopathy is an important source of morbidity and mortality. Both type 1 and type 2 DM have been associated with low levels and poor function of circulating EPCs (16,17); however, no data are available on the relationships between EPC pathophysiology and vascular complications of DM.

In most published studies, circulating EPCs are determined by a culture method (10,11,14,16,17): EPCs are defined as fibronectin-adherent peripheral blood-derived cells uptaking acetylated low-density lipoprotein and binding Ulex-selectin in culture. However, these methods are time-dependent, and results vary largely on culture conditions. In fact, endothelial cells cultured from peripheral blood do not correspond to the actual population of circulating EPCs but include mature circulating endothelial cells and monocyte/macrophage-derived cells assuming an endothelial phenotype in culture (26). Moreover, the amounts of cultured EPCs do not provide information on the absolute number of circulating EPCs, because they depend not only on the initial number of progenitors, but also on adhesion, proliferation, and survival of plated EPCs, resulting from complex cellular interactions in the culture environment (27).

Recently, Peichev et al. (28) showed that CD34+CD133+KDR+ cells give rise to endothelial cells in vitro and that three-color fluorescence analysis of this cell subset may be used to identify and quantify circulating EPCs.

In this study, CD34+ cells are defined as CPCs, whereas CD34+KDR+ cells are defined as EPCs.

Using a sensitive and specific technique, we found a

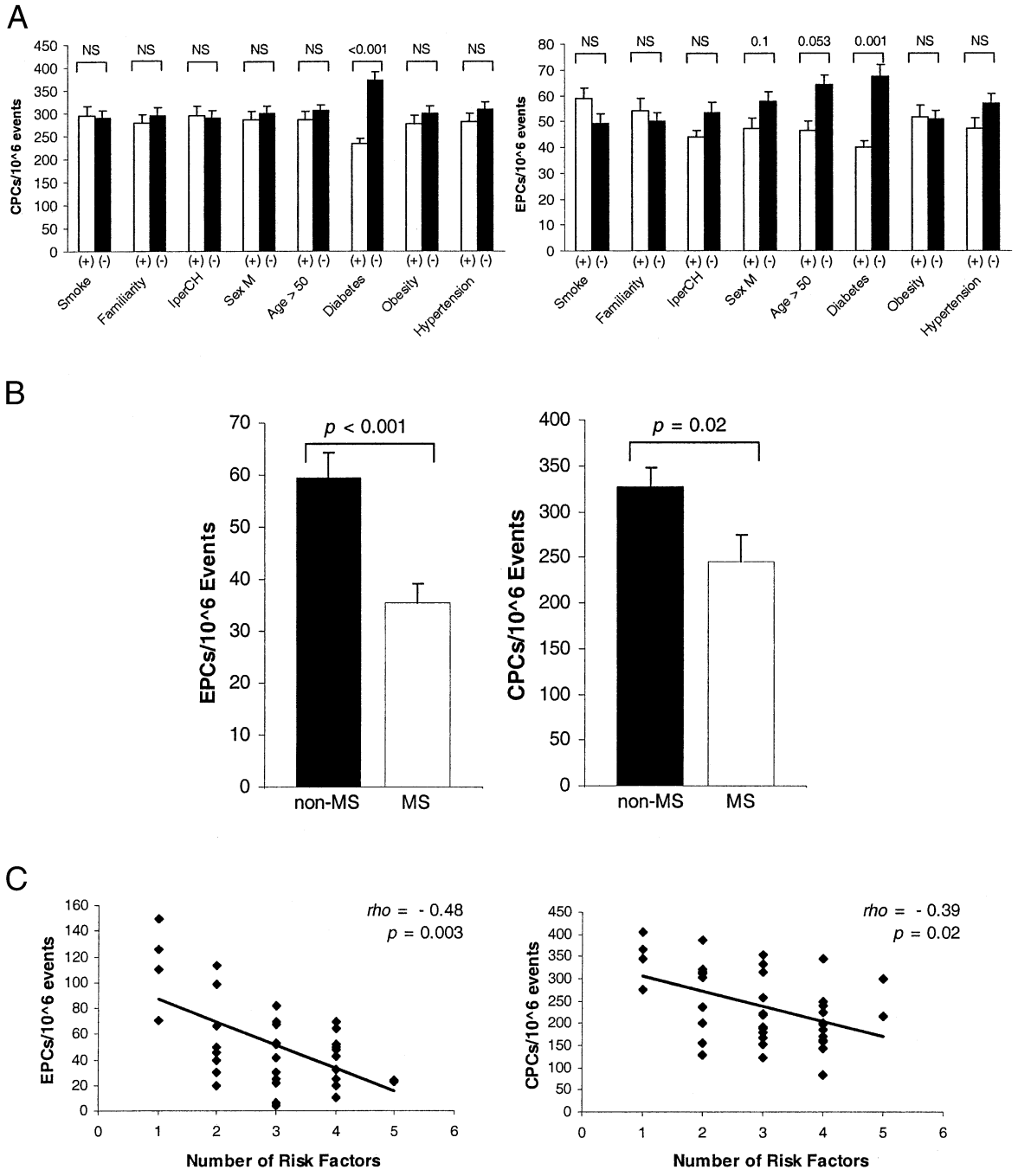

Figure 3. **(A)** Effect of individual risk factors on the numbers of endothelial progenitor cells (EPCs) and circulating progenitor cells (CPCs). **(B)** In patients fulfilling diagnostic criteria for metabolic syndrome, EPC levels were even lower, while CPCs were reduced to a lesser extent. **(C)** Linear regression analysis showed a significant negative correlation between the number of risk factors in diabetic patients and their circulating EPC levels. A milder negative correlation with CPC counts was also present. Mean values ± SE. IperCh = hypercholesterolemia; MS = metabolic syndrome; NS = not statistically significant.

reduction in circulating EPCs in diabetic patients. We also found a significant correlation between plasma glucose at the time of blood collection and absolute EPC numbers, as well as the EPC/CPC percent ratio (endothelial fraction of all CPCs). This finding indicates that the current rather than the previous metabolic control could influence the EPC count and offers this possible explanation: a lower EPC/CPC ratio may reflect a shortened peripheral survival of EPCs rather than a weak bone marrow mobilization, which should also involve CPCs. According to this hypothesis, preliminary data from our patients confirm that rapid metabolic recompensation is followed by an increase in the EPC number and EPC/CPC ratio (data not shown). It has been reported that high glucose is able to induce apoptosis of cultured endothelial cells (29). Given their endothelial phenotype, EPCs exposed to high glucose may undergo

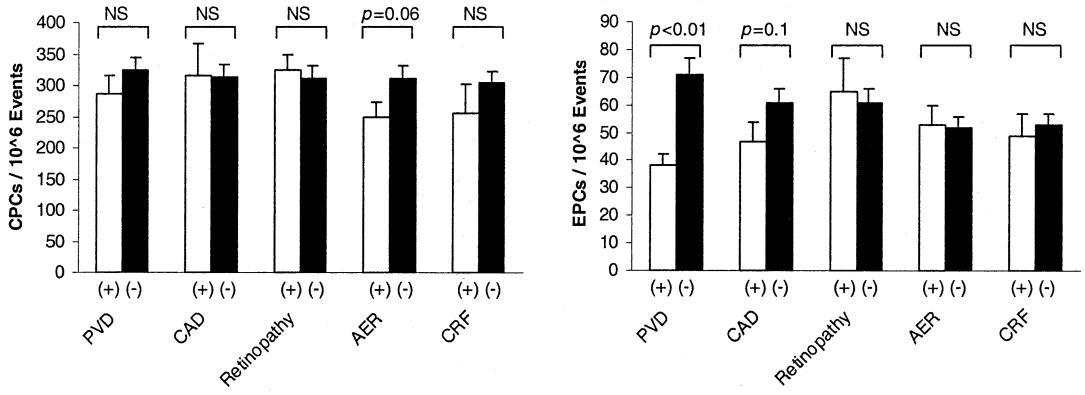

Figure 4. Association between any individual clinical condition present in the study population and variations in endothelial progenitor cell (EPC) and circulating progenitor cell (CPC) levels. Only PVD was associated with significantly reduced EPC numbers, while pathologic AER and microvascular complications had no effect on levels of EPCs and CPCs. Mean values ± SE. AER = albuminuria excretion rate >30 mg per 24 h; CRF = chronic renal failure; NS = not statistically significant.

apoptosis. Future studies are needed to determine the mechanisms involved in EPC reduction in DM, and we are currently trying to establish the effects of acute hyperglycemia on circulating EPCs in vivo.

Our data also suggest that DM is the most relevant risk factor associated with EPC reduction: low circulating EPCs could account for both endothelial dysfunction and poor collateralization typical of diabetics, although we did not perform functional vascular studies on our patients. However, the finding of a lower EPC count in patients with the metabolic syndrome as compared with those without, together with the negative correlation between the number of risk factors and progenitor cell counts (Fig. 3), underscores the importance of risk factor clustering in determining the reduced EPC blood content.

Circulating EPC and CPC reduction has been demonstrated in several clinical conditions characterized by high cardiovascular risk (11–14), although data on stable atherosclerotic disease are few and ambiguous.

In this study, we report for the first time, a profound reduction in circulating EPCs in patients with PVD. In our patients, CAD was characterized by a mild and nonsignificant reduction in EPC levels. This finding apparently contradicts other data in the literature, but it should be noted that Vasa et al. (10) reported a 40% reduction of EPCs in a cohort of patients with CAD, compared with age-matched healthy volunteers; however, their CAD group included not only patients with stable disease, but also patients with acute coronary syndromes or myocardial infarction—clinical events known to be followed by EPC increases (20,21). Heeschen et al. (30) found no differences in CD34+CD133+ cells between patients with chronic myocardial ischemia and healthy controls, but reported poorer angiogenic properties of progenitor cells from patients than from controls. Thus, it appears that the literature provides no definite data on EPC reduction in stable CAD.

The ABI is considered the most objective diagnostic test for lower extremity vascular disease and is also a reliable marker of cardiovascular risk (31): the strong correlation between progenitor cell levels and the ABI suggests that the reduction in vascular progenitor cells is related to the severity of PVD, as well as to global cardiovascular risk in both diabetic and nondiabetic patients with PVD (Fig. 5B).

Among all patients selected for the presence of PVD, those with diabetes had a lower number of EPCs, and those with foot lesions complicating end-stage obstructive vascular disease had the lowest values of both CPCs and EPCs (Fig. 5). Although our study was cross-sectional and does not establish cause-effect relationships, these data suggest that an EPC reduction may have a role in the pathogenesis of PVD. Thus, we would like to propose a possible pathophysiologic model. Diabetes mellitus and clustered risk factors reduce circulating EPC levels: an EPC decrease contributes to endothelial dysfunction, accelerates atherogenetic processes, and leads to vascular diseases. Furthermore, EPC depletion impairs collateralization and favors complications, such as foot lesions. Our observations also offer a possible explanation for the unsatisfactory outcome of patients with critical lower extremity ischemia who have been enrolled in clinical trials of angiogenic therapy with cytokines (32–34). Delivering cytokines (the software) is not sufficient in the absence of the cells primarily involved in new vessel generation (the hardware) (35).

Statin therapy has been associated with an EPC increase. At the time of the study, 42% of diabetic patients with PVD were taking statins: the actual severity of EPC reduction in PVD is unmasked when only patients not treated with statins are considered (Fig. 5D).

Recently, interest in the possible use of EPCs for cellular therapy of critical ischemia syndromes has increased (9,36–38). Nonetheless, it should be noted that EPC dysfunction might limit the use of autologous peripheral blood- or bone marrow-derived progenitors for angiogenic therapy (3,9,17). However, in vitro demonstration of EPC dysfunction is highly unspecific due to the presence of alterations in the whole pool of peripheral blood mononuclear cells (3), and because it does not provide reliable information on EPC biology in vivo. Therefore, a rational basis for EPC

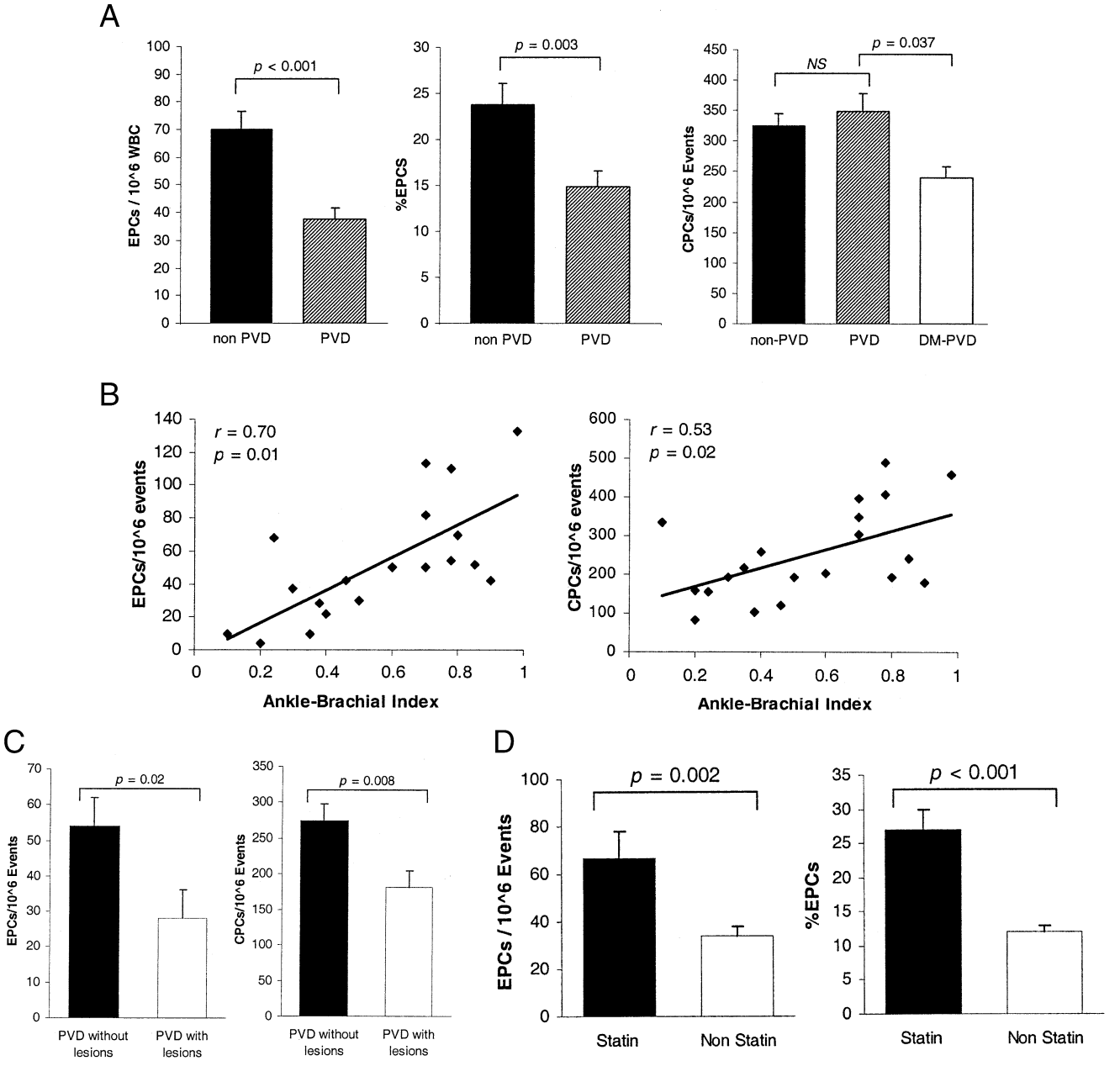

Figure 5. **(A)** Patients with peripheral vascular disease (PVD) had lower levels of endothelial progenitor cells (EPCs) when compared to controls and to non-vascular subjects. In all PVD patients circulating progenitor cells (CPCs) were not significantly lower and thus the EPC/CPC ratio was reduced. Only in diabetic patients with PVD were CPC counts significantly reduced. **(B)** Linear regression analysis showing correlation between EPCs and CPCs with the ankle brachial index (ABI) in patients with lower extremity vascular disease. The ABI values >1.40 were excluded because they suggest the presence of non-compressible arteries due to calcific sclerosis. **(C)** Patients with foot lesions due to end-stage lower extremity vascular disease had significantly lower EPC and CPC levels than PVD patients without foot lesions. **(D)** Circulating EPC numbers and EPC/CPC ratios were higher in PVD patients on statin therapy than in nontreated patients. Mean values ± SE. DM-PVD = diabetic patients with peripheral vascular disease; NS = not statistically significant.

therapy in vascular complications of DM should rely on clearly confirming an EPC reduction in peripheral blood of PVD patients, rather than in vitro functional impairment of their EPCs.

Conclusions. This study reports a reduction in circulating EPCs in type 2 DM and a further, progressive EPC and CPC decrease in diabetic patients with PVD and distal lesions, in relation to ABI values. These data may offer a new pathophysiologic hypothesis for the high incidence of vascular damage in patients with type 2 DM.

Reprint requests and correspondence: Dr. Gian Paolo Fadini, Department of Clinical and Experimental Medicine, University of Padova, School of Medicine, Via Giustiniani 2, 35128 Padova, Italy. E-mail: angelo.avogaro@unipd.it.

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