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Arterial Erectile Dysfunction: Reliability of Penile Doppler Evaluation Integrated With Serum Concentrations of Late Endothelial Progenitor Cells and Endothelial Microparticles

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ABSTRACT: We recently showed the diagnostic value of a new immunophenotype of blood endothelial progenitor cells (EPC) ($CD45^-/CD34^+/CD144^+$) and endothelial microparticles (EMP) ($CD45^-/CD144^+$/annexin V^+) in patients with arterial erectile dysfunction (AED), particularly in patients with associated late-onset hypogonadism and/or metabolic syndrome. In addition, we evaluated the effects of androgen replacement therapy, aerobic physical activity, and tadalafil administration on these markers. The aim of this study was to evaluate the serum concentrations of EPCs and EMPs in a large cohort of patients with AED according to severity of cavernous arterial insufficiency evaluated by penile Doppler. A total of 120 patients (aged 58.0 ± 6.0 years) with AED were enrolled in this study. Patients were classified into 3 groups based on value of peak systolic velocity (PSV). Group A: 37 patients with PSV <25 cm/s (severe arterial insufficiency); group B: 40 patients with PSV between 25 and 29 cm/s (moderate arterial insufficiency); group C: 43 patients with PSV between 30 and 34 cm/s (mild arterial insufficiency). Twenty patients (aged 60.0 ± 3.0 years) with psychogenic erectile dysfunction (PED) represented the control group. EPC and EMP blood concentrations were evaluated by flow cytometry. Patients with AED had significantly higher blood pressure, triglyceride levels, homeostasis model assessment index of insulin resistance, cavernous artery acceleration time, and intima-media thickness than those with PED, whereas International Index of Erectile Function score, high-density lipoprotein cholesterol level, and cavernous artery PSV were lower than those in PED. Both EPC and EMP levels were significantly higher in patients with AED compared with patients with PED. Among 3 groups of patients with AED, there were no significant differences in metabolic parameters examined, but group A showed significantly higher values of cavernous artery acceleration time and intima-media thickness than group B and group C. Finally, group A showed serum concentrations of EPCs and EMPs significantly higher compared with other groups with AED. Patients with AED showed worse metabolic parameters, cavernous artery parameters, and EPC and EMP levels compared with patients with PED. Among patients with AED, those with PSV <25 cm/s showed worse findings of endothelial dysfunction. This suggests that AED is an expression of endothelial damage and that this original immunophenotype of EPCs and EMPs may be considered a predictor of endothelial dysfunction in patients with AED. Finally, this study confirmed the reliability of penile Doppler evaluation integrated with these serum markers of endothelial dysfunction.

Key words: Endothelial dysfunction, endothelial apoptosis, diagnostic marker.

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We recently demonstrated the diagnostic value of a new immunophenotype of blood endothelial progenitor cells (EPC) and endothelial microparticles (EMP) in patients with arterial erectile dysfunction (AED), in particular in patients with associated late-onset hypogonadism (LOH) and/or metabolic syndrome. In addition, we evaluated the effects in vivo of androgen replacement therapy, aerobic physical activity, and tadalafil administration on these markers (La Vignera et al, 2011a–f). The immunophenotype of blood EPCs examined is represented by cells $CD45^-/CD34^+/CD144^+$, and the antigenic cluster of the EMPs is the $CD45^-/CD144^+$/annexin V^+ or $CD45^-/CD34^+/CD144^+$ (La Vignera et al, 2011a–f). In the first study, we showed that the serum concentrations of EPCs and EMPs (annexin V^+) were significantly higher in patients with AED and LOH compared with patients with AED alone (La Vignera et al, 2011e). In the second study, we observed that patients with AED combined with LOH not treated with androgen replacement therapy showed serum concentrations of EPCs and EMPs ($CD45^-/CD34^+/CD144^+$) significantly higher compared with treated patients after 6 months (La Vignera et al, 2011b). In the third study, we demonstrated that after a standard protocol of aerobic physical activity (150 minutes of moderate-intensity aerobic activity per week), the

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serum concentrations of EPCs and EMPs ($CD45^-$/$CD34^+$/$CD144^+$) in selected patients with AED were significantly lower compared with controls (La Vignera et al, 2011a). In the fourth study, we examined patients with AED combined with metabolic syndrome, showing that these patients have higher serum concentrations of EPCs and EMPs (annexin V^+) compared with healthy men. After tadalafil administration, the concentration of EPCs but not EMPs (annexin V^+) increased, suggesting that this compound may play a role on the endothelial repair response (La Vignera et al, 2011c). In the last 2 studies, we used the EMPs (annexin V^+) as a marker of endothelial apoptosis after tadalafil discontinuation in patients with AED, showing that the positive effect of a chronic therapy on endothelium was maintained for 6 months (La Vignera et al, 2011f) and that the administration of an endothelial antioxidant compound prolonged these effects (La Vignera et al, 2011d).

In clinical practice, the dynamic penile Doppler and in particular, evaluation of the peak systolic velocity (PSV) are important tools for diagnosis and classification of patients with AED (Foresta et al, 2008). On the basis of these premises, the aim of the study was to evaluate the serum concentrations of EPCs and EMPs in a large cohort of patients with AED according to severity of cavernous arterial insufficiency evaluated by penile Doppler.

Materials and Methods

Patient Selection

A total of 120 patients with AED were enrolled during a screening of 4 months conducted at our center (S.L., E.V., and A.C. enrolled the patients). The patients had a mean age ± SEM of 58.0 ± 6.0 years (range, 52–64 years). The diagnosis of AED was made when all of the following criteria were fulfilled: 1) International Index of Erectile Function (IIEF5) score <21 (Rosen et al, 1999), 2) cavernosal artery PSV <35 cm/s 10 and 20 minutes after the intracavernosal injection of alprostadil (20 μg) by echo color Doppler (Benson et al, 1993), and 3) acceleration time (AT) >110 ms (Speel et al, 2003). Patients with unsymmetrical vascular parameters (> or <2 cm/s) between the 2 cavernous arteries (PSV – end-diastolic velocity) were excluded from the study. During enrollment to the study, no patient with AED showed previous or current drug treatments for conditions other than the ED; in addition, no patient showed previous or current specific treatments for ED. Finally, no patient reported previous or concomitant organic diseases. Smokers were excluded from enrollment.

Twenty age-matched (60.0 ± 3.0 years; range, 57–63 years) men with psychogenic ED (PED) alone were selected. The diagnosis of PED was made when all of the following criteria were fulfilled: 1) normal vascular parameters (dynamic penile echo color Doppler); 2) normal serum concentrations of total testosterone, luteinizing hormone, estradiol, prolactin, and thyroid-stimulating hormone; 3) absence of the following cardiovascular risk factors: cigarette smoking; hypertension; diabetes or impaired glucose tolerance; dyslipidemia; obesity; previous cardiovascular, cerebrovascular, and peripheral vascular events; use of drugs for the treatment of cardiovascular risk factors; 4) negative neurologic physical examination; and 5) score >16 in the scale n.3 of the Structured Interview on Erectile Dysfunction questionnaire (Petrone et al, 2003).

Blood was withdrawn from all patients (10 mL) for EPC and EMP measurements by flow cytometry. Blood samples were taken from each patient in heparinized tubes and evaluated by flow cytometry within 6 hours. Blood collection was performed for all patients at 8:00 AM after 12 hours of fasting. The protocol was approved by the internal institutional review board, and an informed written consent was obtained from each patient.

Dynamic Penile Echo Color Doppler

Dynamic penile echo color Doppler was performed following intracavernosal injection of alprostadil (Pfizer, New York, New York). Doppler evaluations were performed with an Aplio XV ultrasound machine (Toshiba, Rome, Italy) equipped with a 6- to 13-MHz multifrequency linear probe. The cavernous intimal thickness was measured in the proximal tract of the cavernous artery, using the best rectilinear portion at low magnification. Afterward, the selected portion was studied at high magnification (×24), regulating the partial and total B-mode gain to reduce the noise at the minimum level. Cavernous intimal thickness was measured in a semiquantitative manner, using a dedicated preexisting software available in the Aplio XV and always steering the angle parallel to the lumen (Caretta et al, 2009).

According to value of PSV obtained, the patients were classified into 3 groups. Group A: 37 patients with PSV <25 cm/s (severe arterial insufficiency); group B: 40 patients with PSV between 25 and 29 cm/s (moderate arterial insufficiency); and group C: 43 patients with PSV between 30 and 34 cm/s (mild arterial insufficiency).

Blood EPC and EMP Determination

EPC and EMP concentrations were determined as previously reported (La Vignera et al, 2011e). Briefly, the evaluation was performed in blood following incubation in erythrocyte-lysing solution (Versalyse; Beckman Coulter, Milan, Italy) for 1 minute. The suspension was then washed twice with phosphate-buffered saline and centrifuged, and the pellet was incubated in phosphate-buffered saline containing the appropriate monoclonal antibodies (Table 1) at room temperature for 20 minutes.

EPC Measurement—Direct staining of r-phycoerythrin covalently bound to Texas red (energy-coupled dye [ECD])–conjugated anti–human CD45 (10 μL) (Beckman Coulter), fluorescein isothiocyanate (FITC)–conjugated anti–human CD34 (20 μL) (Beckman Coulter), and r-phycoerythrin–conjugated anti–human CD144 (20 μL) (Beckman Coulter)

Table 1. *Significance of the monoclonal antibodies used to identify EPCs and EMPs*

CD45	This antigen is expressed on the surface of all human leukocytes; EPCs and EMPs do not express this antigen
CD34	This antigen is expressed in all lines of hematopoietic progenitor cells as the most primitive totipotent stem cells; it is maximally expressed in primitive endothelial stem cells and is gradually lost when the progenitors differentiate into mature endothelial cells
CD144	CD144 or vascular endothelial cadherin is specifically localized to the interendothelial cell junction; it seems to be important in maintaining endothelial permeability because monolayers of transfected cells show a calcium-dependent reduction in permeability
Annexin V	Phosphatidylserine (PS), a negative phospholipid localized on the inner side of the plasma membrane, is exposed at the cell surface during apoptosis; annexin V, a calcium-binding protein and phospholipid, binds preferentially to PS with high affinity

Abbreviations: EMP, endothelial microparticle ($CD45^-/CD144^+$/annexin V^+); EPC, endothelial progenitor cell ($CD45^-/CD34^+/CD144^+$).

were performed for EPC flow cytometry detection. Each sample was analyzed by flow cytometry (EPICS XL; Coulter Electronics, Milan, Italy) using the following gating strategy (Figure 1, upper panels: patients with PED; lower panels: patient with AED): histograms 1 report the forward vs side scatter dot plot, 3 different cell populations were identified: gate F, lymphocytes; gate I, monocytes; gate J: polymorphonuclear cells; histograms 2 report $CD45^+$ (gate E) and $CD45^-$ (gate G) cells; and histograms 3 report $CD45^-$ cells with dual expression of CD34 and CD144, which were defined EPCs reported as percentage of total events.

EMP Detection—The measurement of blood EMPs requires careful attention to collection and processing of blood samples. While the cellular elements of blood are separated from the plasma containing EMPs, careful attention must be paid to centrifugation speed. In our experience, a 2-step centrifugation using 1500 $\times$ *g* for 10 minutes and then 13,000 $\times$ *g* for 10 minutes results in platelet-free plasma (when assessed by flow cytometry and light microscopy). The second centrifugation step is particularly efficient at rendering plasma relatively "platelet free." Flow cytometric analysis of blood EMPs appears to be the most favored method to characterize blood EMPs. Typically, EMPs are identified as particles with a forward angle light scatter smaller than an internal standard consisting of 1- to 1.5-μm sized latex particles. ECD anti–human CD45 (10 μL) (Beckman Coulter), r-phycoerythrin–conjugated anti–human CD144 (20 μL) (Beckman Coulter), and FITC-conjugated annexin V (5 μL) (Beckman Coulter) were used for EMP flow detection by flow cytometry. To exclude microparticles originating from leukocytes, we considered only events

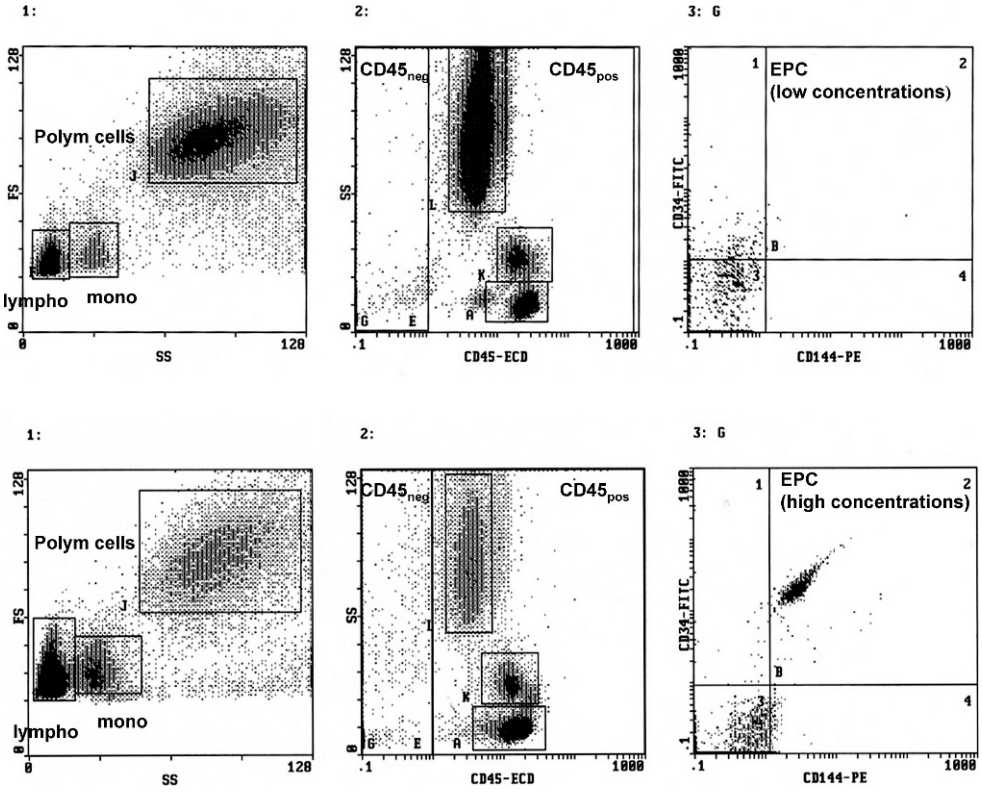

Figure 1. Representative flow cytometric scattergrams showing the gating strategy used in **(Upper Panels)** patients with psychogenic erectile dysfunction and **(Lower Panels)** a patient with late arterial erectile dysfunction. Both histograms 1 report the forward vs side scatter dot plot: 3 different cell populations can be identified (gate F: lymphocytes; gate I: monocytes; gate J: polymorphonuclear cells). Both histograms 2 report (gate E) $CD45^+$ and (gate G) $CD45^-$ cells. Both histograms 3 report only the $CD45^-$ cells (gate G) subdivided according to the expression of CD34 and CD144 antigen staining. Endothelial progenitor cells (EPC) were defined as $CD45^-/CD34^+/CD144^+$, whereas endothelial microparticles were $CD45^-/CD34^-/CD144^+$.

Table 2. *Clinical, laboratory, and penile dynamic echo color Doppler parameters (mean ± SEM) in patients with AED classified for different severities of penile arterial insufficiency and PED*

Parameters	AED (n = 120)	PED (n = 20)	AED (A) (n = 37)	AED (B) (n = 40)	AED (C) (n = 43)
Age, y	58.0 ± 6.0	60.0 ± 3.0	58.0 ± 3.0	60.0 ± 4.0	57.0 ± 4.0
Body mass index, kg/m^2	28.0 ± 0.2	24.4 ± 0.6[a]	27.8 ± 0.2	28.1 ± 0.2	27.8 ± 0.4
IIEF5	8.0 ± 2.0	16.0 ± 2.0[a]	5.0 ± 3.0[b]	9.0 ± 1.0	8.0 ± 2.0
Systolic blood pressure, mm Hg	144.5 ± 2.5	129.5 ± 1.2[a]	145.0 ± 2.0	142.5 ± 2.5	142.0 ± 2.0
Diastolic blood pressure, mm Hg	95.0 ± 3.0	78.0 ± 0.7[a]	98.0 ± 2.0	93.0 ± 2.0	94.0 ± 3.0
Total cholesterol, mg/dL	215.0 ± 8.4	160.0 ± 10.3[a]	220.0 ± 5.0	214.0 ± 4.0	212.0 ± 6.0
HDL cholesterol, mg/dL	40.2 ± 1.2	53.3 ± 3.1[a]	39.2 ± 1.0	40.0 ± 1.0	41.2 ± 1.2
Triglycerides, mg/dL	224.9 ± 2.9	156.1 ± 3.3[a]	226.5 ± 3.0	222.5 ± 2.0	223.0 ± 3.0
HOMA index	2.3 ± 0.02	1.47 ± 0.05[a]	2.4 ± 0.02	2.2 ± 0.02	2.1 ± 0.06
Total testosterone, ng/dL	442.1 ± 13.4	446.7 ± 14.5	437.1 ± 7.4	444.1 ± 10.0	440.1 ± 11.2
Peak systolic velocity, cm/s	26.3 ± 7.0	52.5 ± 2.1[a]	20.1 ± 1.6[b]	27.0 ± 2.0[c]	32.0 ± 2.0
End-diastolic velocity, cm/s	1.2 ± 0.1	1.3 ± 0.4	1.4 ± 0.5	1.2 ± 0.2	1.6 ± 0.4
Acceleration time, ms	133.8 ± 30.0	84.2 ± 2.6[a]	163.8 ± 80[b]	145.0 ± 6.0[c]	112.8 ± 5.0
Resistance index	0.95 ± 0.2	0.98 ± 0.2	0.93 ± 0.3	0.95 ± 0.3	0.95 ± 0.2
Intima-media thickness, mm	0.48 ± 0.4	0.14 ± 0.01[a]	0.52 ± 0.2[b]	0.48 ± 0.2[c]	0.45 ± 0.3

Abbreviations: AED, arterial erectile dysfunction; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IIEF5, International Index of Erectile Function; PED, psychogenic erectile dysfunction.

[a] $P < .05$ vs all categories of AED.

[b] $P < .05$ vs groups B and C.

[c] $P < .05$ vs group C.

within the CD45$^-$ gate. CD144$^+$ events expressing phosphatidylserine in the outer membrane leaflet following annexin V staining were defined as EMPs. They were reported as percentage of total events. Appropriate isotype controls were used for each staining procedure as negative controls to set the appropriate regions. Flow cytometric analysis was conducted for 600 s or 100,000 events, whichever occurred first (Jy et al, 2004; Khan et al, 2005; Shet, 2008; Mariucci et al, 2010; Masouleh et al, 2010; van Ierssel et al, 2010). The same operator, blinded with respect to the sample origin (controls or patients), performed all tests throughout the study.

Statistical Analysis

Results are reported as means ± SEM throughout the study. The data were analyzed by a Student's *t* test for comparison between AED and PED and 1-way analysis of variance (ANOVA) followed by the Duncan multiple range test for evaluation of 3 groups of patients with AED. Correlation analysis was conducted by a Pearson correlation test. The software SPSS 9.0 for Windows was used for statistical evaluation (SPSS Inc, Chicago, Illinois). A statistically significant difference was accepted when $P < .05$.

Results

Age did not differ significantly between patients with AED compared with those with PED; among the 3 groups of patients with AED with different severities of arterial insufficiency. Instead, patients with AED showed worse metabolic parameters compared with patients with PED, whereas there were no significant differences for these parameters among the 3 groups of patients with AED. Serum total testosterone levels did not differ significantly between AED and PED or among different groups of AED (Table 2).

Patients With AED vs PED

Patients with AED had an IIEF5 score significantly lower than patients with PED (Student's *t* test; $P < .05$). Patients with AED had a significantly lower PSV compared with patients with PED (Student's *t* test; $P < .05$) (Table 2). Similar to PSV, patients with AED had significantly higher AT and cavernosal intima-media thickness (IMT) than patients with PED (Student's *t* test; $P < .05$). Finally, the end-diastolic velocity and resistance index did not differ significantly between AED and PED (Table 2).

Patients with AED had significantly higher levels of EPCs compared with patients with PED (Student's *t* test; $P < .05$) (Figure 2A). The levels of EMPs were significantly higher in patients with AED compared with patients with PED (Student's *t* test; $P < .05$) (Figure 2B).

Patients With Mild, Moderate, or Severe Penile Arterial Insufficiency

Group A had an IIEF5 score significantly lower compared with that of group B and group C (ANOVA; $P < .05$). Similar to PSV, group A had significantly higher AT and cavernosal IMT compared with group B and group C (ANOVA; $P < .05$). Finally, the end-diastolic velocity and resistance index did not differ significantly among the 3 groups (Table 2).

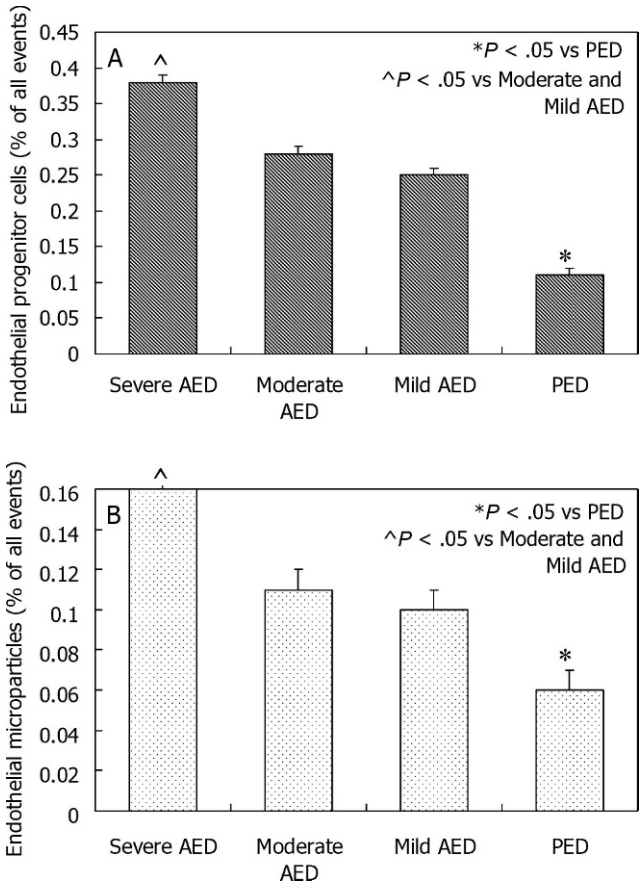

Figure 2. Percentage of **(Panel A)** circulating endothelial progenitor cells (immunophenotype $CD45^-/CD34^+/CD144^+$) and **(Panel B)** endothelial microparticles (immunophenotype $CD45^-/CD34^+$/annexin V^+) in patients with arterial erectile dysfunction (AED) classified for different severities of penile arterial insufficiency and patients with psychogenic erectile dysfunction (PED).

Group A had significantly higher levels of EPCs compared with other groups (ANOVA; $P < .05$) (Figure 2A). The levels of EMPs were significantly higher in patients of group A compared with patients of other groups (ANOVA; $P < .05$) (Figure 2B).

Finally, levels of EPCs and EMPs showed significant correlation with PSV, AT, and IMT (Table 3).

Discussion

The results of this study showed that patients with AED had greater penile vascular damage and higher serum concentrations of EPCs and EMPs than patients with PED; in addition, there was a significant correlation between these markers and arterial parameters of penile Doppler analysis. Moreover, among patients with AED, those with lower PSV and higher arterial AT showed worse IIEF5 scores and endothelial dysfunction. Finally, these results suggest the reliability of penile Doppler evaluation integrated with serum concentrations of late EPCs and EMPs in these patients.

It is now well documented that the measurement of levels of both EPCs and EMPs may be proposed as biomarkers of cardiovascular disease and atherosclerotic complications and progression (Chironi et al, 2009; Dotsenko, 2010). Recently we evaluated these immunophenotypes of EPCs ($CD45^-/CD34^+/CD144^+$) and EMPs ($CD45^-/CD144^+$/annexin V^+) in patients with AED (La Vignera et al, 2011c–f). These results suggest that these immunophenotypes of EPCs and EMPs may be considered predictors of endothelial dysfunction in patients with ED.

EPCs are subpopulations of leukocytes that may differentiate into mature endothelial cells both in vitro and in vivo (Real et al, 2008). The relevant contribution of these cells in the processes of re-endothelization at sites of endothelial injury and neovascularization has also been confirmed (Asahara et al, 1997; Werner et al, 2003; Kong et al, 2004). Circulating EPC repair capability of the damaged endothelium suggests that these cells play a key role in maintaining endothelial homeostasis. As a result, the number of EPCs may reflect the ''vascular health'' of an individual, and it is has been shown to be an independent predictor of cardiovascular disease (Güven et al, 2006).

Other researchers (Urbich and Dimmeler, 2004; Foresta et al, 2005) have evaluated different phenotypes and expression of premature differentiation lines; the phenotype evaluated in this study expresses a subsequent

Table 3. *Correlation between circulating EPCs or EMPs and parameters of dynamic penile echo color Doppler in 120 patients with AED*

Parameter	EPCs	EMPs
IIEF5 score	$r = -0.10$, P = NS	$r = -0.06$, P = NS
Peak systolic velocity, cm/s	$r = -0.75$, $P < .05$	$r = -0.80$, $P < .05$
End-diastolic velocity, cm/s	$r = -0.03$, P = NS	$r = -0.10$, P = NS
Acceleration time, ms	$r = -0.69$, $P < .001$	$r = -0.66$, $P < .001$
Resistance index	$r = -0.03$, P = NS	$r = 0.10$, P = NS
Intima-media thickness, mm	$r = -0.79$, $P < .001$	$r = -0.80$, $P < .05$

Abbreviations: AED, indicates arterial erectile dysfunction; EMP, endothelial microparticle ($CD45^-/CD144^+$/annexin V^+); EPC, endothelial progenitor cell ($CD45^-/CD34^+/CD144^+$); IIEF5, International Index of Erectile Function; NS, not significant.

dysfunctional phase, with a different kinetic. The increase in the number of these more endothelial-regenerating EPCs may be able to rebuild the endothelial monolayer of the corpus cavernosum with amelioration of erectile function and protection from disease progression in these patients. The elevated number of EPCs found in patients with AED confirms previous studies that found a higher number of EPCs in patients with cardiovascular risk factors (Adams et al, 2004; Massa et al, 2005; Sandri et al, 2005; Güven et al, 2006).

In patients with coronary artery disease, several mechanistic possibilities have been advanced to explain the increase in the levels of EPCs. This activation may result from a variety of proinflammatory cytokines released in patients with coronary ischemia (Cho et al, 2003; Werner et al, 2003). Moreover, exercise-induced ischemia is associated with an increased number of circulating EPCs (Adams et al, 2004; Massa et al, 2005; Sandri et al, 2005). Patients with unstable angina have an increased number of EPCs compared with patients with stable angina, and the stabilization of angina in these patients resulted in a 2-fold decrease in the number of circulating EPCs (George et al, 2004). In addition, patients with AED had increased expression of endothelial apoptosis and initial platelet adhesion. Pathophysiologic externalization of specific substances belonging to the vessel wall (after endothelial injury), usually not in contact with the blood (subintimal area), represent the signal that when captured by surface receptor platelets results in their adhesion; this mechanism could help explain a compensatory increase in serum concentrations of EPCs in these patients (La Vignera, 2011).

EMPs are regarded as markers of cardiovascular dysfunction with a prothrombotic role associated with risk factors (Puddu et al, 2010). The higher number of EMPs found in patients with AED suggests more severe vascular damage in these patients compared with patients with PED. Elevated EMP levels ($CD31^+/CD42^-$ and $CD31^+/CD42^+$) have been reported in patients with ED and diabetes mellitus, and they were found to be independently involved in the pathogenesis of ED (Foresta et al, 2005). In this study, EMPs inversely correlated with the IIEF5 score in patients both with and without diabetes, and multivariate analysis corrected for age, anthropometric indices, glucose, plasma lipids, flow-mediated dilation, and platelet microparticles identified EMP as the only independent predictor of the IIEF5 score (Foresta et al, 2005). In addition, patients with ED with or without diabetes mellitus have significantly higher EMPs ($CD62^+$) than men without diabetes without ED. The EMPs ($CD62^+$)/EMPs ($CD31^+$) ratio, an index of endothelial activation (high ratio) or apoptosis (low ratio), was lowest in patients with diabetes and ED. These findings suggest that EMP level increases in patients with diabetes and ED are consistent with increased apoptotic activity (Esposito et al, 2008). The results of the present study conducted in patients with AED imply a similar conclusion. These patients share a common picture of endothelial damage and markers of this dysfunction with patients with atherosclerosis.

Increased IMT is an important expression of morphostructural alteration in patients with atherosclerosis, and the higher endothelial regenerative response is probably of a compensatory nature (Dotsenko, 2010). Bone marrow–derived EPCs circulating in the peripheral blood migrate toward their target tissue where they differentiate and contribute to the formation of new vessels (Asahara et al, 1997). During this process, they are exposed to shear stress generated by interstitial fluid flow and blood flow, and it has been reported that when cultured EPCs undergo shear stress in a flow-loading device, their differentiation into mature endothelial cells accelerates significantly (Yamamoto et al, 2003). In recent evidence, other researchers showed that the levels of a different phenotype of EPCs were increased in newly diagnosed untreated patients with hypertension with arterial stiffening but normal carotid IMT; the EPC numbers correlated with gp91phox (a source of oxidative stress), a reactive oxygen species and fibrinogen, suggesting that the EPC number may be increased in early phases of atherosclerosis before the development of wall thickening to maintain an adequate number of EPCs in peripheral blood (Mandraffino et al, 2011). Based on this model, further studies will clarify if correlations exist among oxidative stress, cavernous intimal thickness, and EPC numbers in patients with AED or if this alteration (increased IMT) occurs first for different calibers of the vessel.

Limitations of the Study

The clinical assessment of vascular severity was made by penile dynamic Doppler, which is not considered a necessary first screening for all patients with ED. The advantage of ultrasound is the ability to screen patients to identify a normal arterial response of cavernous arteries. Men with sexual dysfunction and comorbidities are more likely to have multiorgan vascular dysfunction and may necessitate further testing because erectile failure may be the first presenting symptom requiring investigation and treatment even in the absence of cardiovascular risk factors (Aversa and Sarteschi, 2007). However there is a significant difference between pharmacologically induced erection using alprostadil and natural erectile function. A dose of alprostadil 20 μg will cause direct smooth muscle relaxation and may result in erection, but this bypasses the normal neuromuscular initiation and maintenance of erection.

In conclusion, this study showed that patients with AED have higher blood concentrations of EPCs and EMPs compared with patients with PED. Thus, AED may be considered an additional vascular risk factor, and these biomarkers may be regarded as indices of endothelial dysfunction. The simultaneous evaluation of EPCs and EMPs may better monitor the biologic balance between the degree of the vascular wall damage severity (EMPs) and the extent of the repair mechanism (EPCs). In addition, this study confirmed the key diagnostic role of penile dynamic Doppler in the evaluation of these patients, suggesting the reliability of ultrasound evaluation integrated with these serum markers.

References

Adams V, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, Tarnok A, Gielen S, Emmrich F, Schuler G, Hambrecht R. Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. *Arterioscler Thromb Vasc Biol*. 2004;24:684–690.

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997; 275:964–697.

Aversa A, Sarteschi LM. The role of penile color-duplex ultrasound for the evaluation of erectile dysfunction. *J Sex Med*. 2007; 4:1437–1447.

Benson CB, Aruny JE, Vickers MA Jr. Correlation of duplex sonography with arteriography in patients with erectile dysfunction. *Am J Roentgenol*. 1993;160:71–73.

Caretta N, Palego P, Schipilliti M, Ferlin A, Di Mambro A, Foresta C. Cavernous artery intima-media thickness: a new parameter in the diagnosis of vascular erectile dysfunction. *J Sex Med*. 2009; 6:1117–1126.

Chironi GN, Boulanger CM, Simon A, Dignat-George F, Freyssinet JM, Tedgui A. Endothelial microparticles in diseases. *Cell Tissue Res*. 2009;335:143–151.

Cho HJ, Kim HS, Lee MM, Kim DH, Yang HJ, Hur J, Hwang KK, Oh S, Choi YJ, Chae IH, Oh BH, Choi YS, Walsh K, Park YB. Mobilized endothelial progenitor cells by granulocyte-macrophage colony-stimulating factor accelerate reendothelization and reduce vascular inflammation after intravascular radiation. *Circulation*. 2003;108:2918–2925.

Dotsenko O. Stem/progenitor cells, atherosclerosis and cardiovascular regeneration. *Open Cardiovasc Med J*. 2010;4:97–104.

Esposito K, Ciotola M, Giugliano F, Sardelli L, Giugliano F, Maiorino MI, Beneduce F, De Sio M, Giugliano D. Phenotypic assessment of endothelial microparticles in diabetic and nondiabetic men with erectile dysfunction. *J Sex Med*. 2008;5:1436–1442.

Foresta C, Caretta N, Lana A, Cabrelle A, Palù G, Ferlin A. Circulating endothelial progenitor cells in subjects with erectile dysfunction. *Int J Impot Res*. 2005;17:288–290.

Foresta C, Palego P, Schipilliti M, Selice R, Ferlin A, Caretta N. Asymmetric development of peripheral atherosclerosis in patients with erectile dysfunction: an ultrasonographic study. *Atherosclerosis*. 2008;197:889–895.

George J, Goldstein E, Abashidze S, Deutsch V, Shmilovich H, Finkelstein A, Herz I, Miller H, Keren G. Circulating endothelial progenitor cells in patients with unstable angina: association with systemic inflammation. *Eur Heart J*. 2004;25:1003–1008.

Güven H, Shepherd RM, Bach RG, Capoccia BJ, Link DC. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J Am Coll Cardiol*. 2006;48:1579–1587.

Jy W, Horstman LL, Jimenez JJ, Ahn YS, Biró E, Nieuwland R, Sturk A, Dignat-George F, Sabatier F, Camoin-Jau L, Sampol J, Hugel B, Zobairi F, Freyssinet JM, Nomura S, Shet AS, Key NS, Hebbel RP. Measuring circulating cell-derived microparticles. *J Thromb Haemost*. 2004;2:1842–1851.

Khan SS, Solomon MA, McCoy JP Jr. Detection of circulating endothelial cells and endothelial progenitor cells by flow cytometry. *Cytometry B Clin Cytom*. 2005;64:1–8.

Kong D, Melo LG, Gnecchi M, Zhang L, Mostoslavsky G, Liew CC, Pratt RE, Dzau VJ. Cytokine-induced mobilization of circulating endothelial progenitor cells enhances repair of injured arteries. *Circulation*. 2004;110:2039–2046.

La Vignera S. Increased expression of endothelial-platelet dysfunctional pathway in patients with arterial erectile dysfunction. *Int Angiol*. 2011;30:408–414.

La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero A. Aerobic physical activity improves endothelial function in the middle-aged patients with erectile dysfunction. *Aging Male*. 2011a;14(4):265–272.

La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero A. Original immunophenotype of blood endothelial progenitor cells and microparticles in patients with isolated arterial erectile dysfunction and late onset hypogonadism: effects of androgen replacement therapy. *Aging Male*. 2011b;14(3):183–189.

La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Circulating endothelial progenitor cells and endothelial microparticles in patients with arterial erectile dysfunction and metabolic syndrome. *J Androl*. 2011c;33(2):202–209.

La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Endothelial antioxidant compound prolonged the endothelial antiapoptotic effects registered after tadalafil treatment in patients with arterial erectile dysfunction. *J Androl*. 2011d;33(2):170–175.

La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. New immunophenotype of blood endothelial progenitor cells and endothelial microparticles in patients with arterial erectile dysfunction and late onset hypogonadism. *J Androl*. 2011e;32(5):509–517.

La Vignera S, Condorelli RA, Vicari E, D'Agata R, Calogero AE. Endothelial apoptosis decrease following tadalafil administration in patients with arterial ED does not last after its discontinuation. *Int J Impot Res*. 2011f;23(5):200–205.

Mandraffino G, Sardo MA, Riggio S, Loddo S, Imbalzano E, Alibrandi A, Saitta C, Cinquegrani M, Mormina EM, Saitta A. Circulating progenitor cells are increased in newly diagnosed untreated hypertensive patients with arterial stiffening but normal carotid intima-media thickness. *Hypertens Res*. 2011;34:876–883.

Mariucci S, Rovati B, Bencardino K, Manzoni M, Danova M. Flow cytometric detection of circulating endothelial cells and endothelial progenitor cells in healthy subjects. *Int J Lab Hematol*. 2010; 32:40–48.

Masouleh BK, Baraniskin A, Schmiegel W, Schroers R. Quantification of circulating endothelial progenitor cells in human peripheral blood: establishing a reliable flow cytometry protocol. *J Immunol Methods*. 2010;357:38–42.

Massa M, Rosti V, Ferrario M, Campanelli R, Ramajoli I, Rosso R, De Ferrari GM, Ferlini M, Goffredo L, Bertoletti A, Klersy C, Pecci A, Moratti R, Tavazzi L. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood*. 2005;105:199–206.

Petrone L, Mannucci E, Corona G, Bartolini M, Forti G, Giommi R, Maggi M. Structured interview on erectile dysfunction (SIEDY): a new, multidimensional instrument for quantification of pathogenetic issues on erectile dysfunction. *Int J Impot Res*. 2003;15:210–220.

Puddu P, Puddu GM, Cravero E, Muscari S, Muscari A. The involvement of circulating microparticles in inflammation, coagulation and cardiovascular diseases. *Can J Cardiol*. 2010;26:140–145.

Real C, Caiado F, Dias S. Endothelial progenitors in vascular repair and angiogenesis: how many are needed and what to do? *Cardiovasc Hematol Disord Drug Targets*. 2008;8:185–193.

Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Pena BM. Development and evaluation of an abridged, 5 item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. *Int J Impot Res*. 1999;11:319–326.

Sandri M, Adams V, Gielen S, Linke A, Lenk K, Kränkel N, Lenz D, Erbs S, Scheinert D, Mohr FW, Schuler G, Hambrecht R. Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies. *Circulation*. 2005;111:3391–3399.

Shet AS. Characterizing blood microparticles: technical aspects and challenges. *Vasc Health Risk Manag*. 2008;4:769–774.

Speel TG, van Langen H, Wijkstra H, Meuleman EJ. Penile duplex pharmaco-ultrasonography revisited: revalidation of the parameters of the cavernous arterial response. *J Urol*. 2003;169:216–220.

Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res*. 2004;95:343–353.

van Ierssel SH, Van Craenenbroeck EM, Conraads VM, Van Tendeloo VF, Vrints CJ, Jorens PG, Hoymans VY. Flow cytometric detection of endothelial microparticles (EMP): effects of centrifugation and storage alter with the phenotype studied. *Thromb Res*. 2010;125:332–339.

Werner N, Junk S, Laufs U, Link A, Walenta K, Bohm M, Nickenig G. Intravenous transfusion of endothelial progenitor cells reduces neointima formation after vascular injury. *Circ Res*. 2003;93:17–24.

Yamamoto K, Takahashi T, Asahara T, Ohura N, Sokabe T, Kamiya A, Ando J. Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. *J Appl Physiol*. 2003;95:2081–2088.