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New Immunophenotype of Blood Endothelial Progenitor Cells and Endothelial Microparticles in Patients With Arterial Erectile Dysfunction and Late-Onset Hypogonadism

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ABSTRACT: Blood endothelial progenitor cells (EPC) and microparticles (EMP) have been proposed as markers of endothelial dysfunction. The aim of this study was to evaluate a new immunophenotype of EPCs and EMPs in patients with arterial erectile dysfunction (ED) and late-onset hypogonadism (LOH). Fifty patients (58.2 ± 0.7 years) with ED and LOH were enrolled in this study. Their EPC and EMP concentrations were compared with those of 20 patients with arterial ED alone (61.2 ± 1.2 years) and of 20 healthy men (controls; 61.4 ± 1.2 years). EPC ($CD45_{neg}$/$CD34_{pos}$/$CD144_{pos}$) and EMP ($CD45_{neg}$/$CD144_{pos}$/annexin V_{pos}) blood concentrations were evaluated by flow cytometry. Patients with ED and LOH or ED alone had significantly higher blood pressure, triglycerides, homeostasis model assessment index of insulin resistance, cavernous artery acceleration time, and intima-media thickness than controls, whereas International Index of Erectile Function score, high-density lipoprotein cholesterol, and cavernous artery peak systolic velocity and resistance index were lower than those of controls. Both EPCs and EMPs were significantly higher in patients with ED and LOH compared with patients with ED alone or controls. Patients with ED alone had EPCs and EMPs significantly higher than controls. In conclusion, patients with ED and LOH showed worse metabolic parameters and cavernous artery parameters, measured by dynamic penile echo color Doppler, and higher EPCs and EMPs compared with patients with ED alone. This suggests that LOH is an additional vascular risk factor and that EPCs and EMPs may be considered predictors of endothelial dysfunction in patients with ED and LOH.

Key words: Endothelial dysfunction, androgen, andropause, hormone, penis.

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Male hypogonadism can be considered a surrogate marker of incident cardiovascular disease (CVD). A recent study showed a marked improvement in the homeostasis model assessment index of insulin resistance (HOMA), carotid intima-media thickness (IMT), and high-sensitivity C-reactive protein in hypogonadal patients treated with testosterone undecanoate for 12 months compared with patients receiving placebo. After 24 months, testosterone undecanoate reduced fasting glucose and waist circumference and improved surrogate markers of atherosclerosis without changes in body mass index (BMI) (Aversa et al, 2010).

Erectile dysfunction (ED) of arterial origin, CVDs, and male hypogonadism share an endothelial dysfunction that has a well-established role in the pathogenesis of both atherosclerosis and plaque instability (Widlansky et al, 2003; Sugiyama et al, 2004). Hence, a significant proportion of men with ED exhibit early signs of coronary artery disease (CAD), and this group may develop more severe CAD than men without ED. The time interval between the onset of ED symptoms and the occurrence of CAD symptoms or of cardiovascular events is estimated to be 2–3 years and 3–5 years, respectively; this time interval allows elimination of the risk factors eventually present (Feldman et al, 2000; Russel et al, 2004; Baumhakel et al, 2006; Gazzaruso et al, 2008; Jackson et al, 2010).

Hypogonadism may play a significant role in the pathophysiology of ED. A threshold level of testosterone may be necessary for normal erectile function. Testosterone replacement therapy must be given to hypogonadal patients, and it is beneficial in patients with ED and hypogonadism. A number of laboratory and human studies have shown the combination of testosterone and other ED treatments, such as phosphodiesterase type 5 (PDE5) inhibitors, to be beneficial

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in patients with ED and hypogonadism, who do not respond satisfactorily to the administration of PDE5 inhibitor alone (Mikhail, 2006; Shabsigh et al, 2006; Diemer, 2010).

Endothelial dysfunction may be evaluated in many ways, and recently this has been done by estimating the number of circulating endothelial progenitor cells (EPC) and endothelial microparticles (EMP) (Costa and Virag, 2009). EPCs are progenitor cells similar to the embryonic angioblast; they derive from the mesoderm and have a common precursor with the hematopoietic stem cells, the hemangioblast. EPCs may also originate from transdifferentiated monocyte/macrophages (Rehman et al, 2003). The first EPC phenotype was defined by the presence of the following antigens in blood cells: CD34, CD133, and VEGFR2 (or KDR) (Asahara et al, 1997). However, recent observations suggest that this EPC phenotype is rich in hematopoietic progenitor cells and expresses the panleukocyte antigen CD45. Moreover, these cells have been reported to be unable to form endothelial colonies in vitro. Two types of EPCs have been described to date in vitro: early EPCs and late EPCs. Although early EPCs and late EPCs share common features, such as expression of CD31 and CD34, they have distinct characteristics with respect to morphology, proliferative potential, and functional characteristics such as tube formation (Lin et al, 2000; Rehman et al, 2003; Ingram et al, 2005; Yoon et al, 2005; Yoder et al, 2007). Because early EPCs do not adopt a typical endothelial phenotype in vitro and enhance neovascularization in an indirect paracrine fashion in vivo, early EPCs were redefined as angiogenic cells instead of EPCs by some (Rehman et al, 2003; Yoon et al, 2005; Yoder et al, 2007). Late EPCs, on the other hand, bear typical endothelial characteristics in vitro and were shown to contribute more directly to neovascularization by providing new endothelial cells and vessels in vivo, and therefore probably act more as true EPCs in the literal sense (Lin et al, 2000; Rehman et al, 2003; Ingram et al, 2005; Yoon et al, 2005; Yoder et al, 2007).

A closer look at the EPC biology indicates a progressive loss of CD133 and CD34 antigens and the expression of CD31, VE-cadherin (CD144), and Vwf, following their mobilization into the general circulation (Papayannopoulou, 2004; Schatteman et al, 2007). Thus the absence of CD45 and the concomitant presence of CD34 and CD144 antigens assure that these cells are true endothelial stem/progenitor cells (Güven et al, 2006; Case et al, 2007; Schatteman et al, 2007; Timmermans et al, 2007). In addition, KDR is down-regulated during the maturation of immature CD45 hematopoietic precursors (Hirai et al, 2005). On the other hand, endothelial cells differentiate from mesodermic progenitors and interconnect to form a primary vascular plexus. This process is called vasculogenesis and occurs only in visceral derivatives and somites (Pardanaud et al, 1996). The primary plexus is extended by angiogenesis, which involves sprouting of new vessels and various steps of remodeling, allowing the formation of a complex vascular network with vessels of different sizes. VE-cadherin is exclusively and constitutively expressed at interendothelial junctions (Lampugnani et al, 1992). VE-cadherin was detected in all developing vessels as well as in the adult vasculature (Breier et al, 1996). Functionally, VE-cadherin is able to promote the assembly of the junctional complex and to develop homotypic adhesive reactivity (Lampugnani et al, 1995; Navarro et al, 1995). On this basis, we chose to evaluate the following EPC phenotype: $CD45_{neg}/CD34_{pos}/CD144_{pos}$.

In addition to EPCs, EMPs may be found in the general circulation. Cellular microparticles (MP) are fragments of the plasma membrane that are shed by virtually all cells undergoing stress conditions, including cell activation and apoptosis. Since the description of ''platelet dust'' (Wolf, 1967), numerous studies have reported the presence of subcellular vesicles in centrifuged plasma. Although long considered to be cellular debris, blood MPs are more recently considered reflective of cellular stimulation, activation, and degeneration/apoptosis. By general consensus, MPs are small in size ($\leq$1.5 μm), expose the anionic phospholipid phosphatidylserine on the outer leaflet of their membrane, and bear surface membrane antigens reflecting their cell of origin. MPs that arise from the cellular components of blood and the endothelial lining of blood vessels are referred to as blood MPs. EMPs are released as a consequence of endothelial dysfunction, atherogenesis, and endothelial apoptotic processes (Shet, 2008).

Many studies have evaluated the correlation between EPCs or EMPs with the severity of CVD (Schwartzenberg et al, 2007; Nozaki et al, 2010). Only a few have simultaneously assessed these biomarkers and correlated them with the arterial condition (Sabatier et al, 2009; Curtis et al, 2010). There are no studies that have evaluated directly EMPs in hypogonadal patients.

Therefore, the present study was undertaken to evaluate the number of a new immunophenotype of EPCs ($CD45_{neg}/CD34_{pos}/CD144_{pos}$) and of EMPs ($CD45_{neg}/CD144_{pos}$/annexin V_{pos}) (Table 1) in patients with ED of arterial origin and late-onset hypogonadism (LOH). To accomplish this, 50 patients with arterial dysfunction–based ED and LOH (Wang et al, 2009) were selected. The numbers of circulating EPCs and EMPs were determined and compared with those of patients with arterial ED alone and normal, age-matched men (controls).

Table 1. *Significance of the monoclonal antibodies used to identify endothelial progenitor cells (EPCs) and endothelial microparticles (EMPs)*[a]

Antibody	Significance
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 it is gradually lost when the progenitors differ in mature endothelial cells.
CD144	CD144 or vascular endothelial cadherin is specifically localized in the interendothelial cell junction. It seems important in maintaining endothelial permeability, because monolayers of transfected cells show a calcium-dependent reduction in permeability.

[a] EPCs: $CD45_{neg}/CD34_{pos}/CD144_{pos}$; EMPs: $CD45_{neg}/CD144_{pos}$/annexin V_{pos}.

Materials and Methods

Patient Selection

Fifty patients with arterial ED and LOH were enrolled. They had a mean age of 59.3 ± 0.5 years (range, 50–64 years). The diagnosis of arterial ED was made when all the following criteria were fulfilled: 1) International Index of Erectile Function (IIEF-5) score <21 (Rosen et al, 1999); 2) cavernosal artery peak systolic velocity (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 milliseconds (Speel et al, 2003). The diagnosis of LOH was made according to the criteria established by the major scientific societies (Wang et al, 2009). A serum sample for total testosterone (TT) determination was obtained between 0700 and 1100 hours. Only patients with TT concentrations <230 ng/dL were enrolled in this group. Smokers and diabetic patients were excluded.

Twenty age-matched (61.3 ± 1.1 years; range, 48–66 years) men with arterial ED alone were selected as diseased controls to establish the numbers of EPCs and EMPs in absence of hypogonadism. A group (n = 20) of age-matched (62.6 ± 1.1 years; range, 50–68 years) healthy men (no ED, no LOH) were selected as controls. These men had an IIEF-5 score >21, PSV >50 cm/s, AT <90 milliseconds, and TT >350 ng/dL.

All patients and controls underwent blood withdrawal (10 mL) for EPC and EMP measurements by flow cytometry. Blood samples were taken from each subject in heparinized tubes and evaluated by flow cytometry within 6 hours. Blood collection was performed for all patients at 0800 hours 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 (Caveject; Pfizer, New York, New York). Doppler evaluations were performed by Aplio XV (Toshiba, Rome, Italy) ultrasound machine equipped with a 6–13-MHz multifrequency linear probe. The cavernous intimal thickness was measured in the proximal tract of the cavernous artery, choosing the best rectilinear portion at low magnification. Afterward, the selected portion was studied at high magnification (×24 zoom), regulating the partial and total B-mode gain to reduce the noise to the minimum level. Cavernous intimal thickness was measured in a semiquantitative manner, using dedicated pre-existing software available in the Aplio XV, always steering the angle parallel to the lumen (Caretta et al, 2009).

Blood EPC and EMP Determination

EPC and EMP evaluation was performed in blood following incubation in erythrocyte lysing solution (Versalyse; IL, Milan, Italy) for 1 minute. The suspension was then washed twice with phosphate buffer solution (PBS) and centrifuged, and the pellet was rapidly incubated in PBS containing the appropriate monoclonal antibodies at room temperature for 20 minutes.

EPC Measurement—Phycoerythrin covalently bound to Texas red (ECD)–conjugated anti-human CD45 (IL), fluorescein isothiocyanate (FITC)–conjugated anti-human CD34 (IL), and r-phycoerythrin (PE)–conjugated anti-human CD144 (IL) were used for EPC flow cytometry detection.

Each sample was analyzed by flow cytometry (EPICS XL; Coulter Electronics, IL) using the following gating strategy (Figure 1; upper panels, control; lower panels, patient with ED and LOH):

- Histograms 1 report the forward vs side scatter dot plot. Three different cell populations were identified: gate F, lymphocytes; gate I, monocytes; and gate J, polymorphonuclear cells.
- Histograms 2 report $CD45_{pos}$ (gate E) and $CD45_{neg}$ (gate G) cells.
- Histograms 3 report $CD45_{neg}$ cells with the dual expression of CD34 and CD144, which were defined as EPCs and were reported as percentage of total events.

EMP Detection—The measurement of blood EMPs requires careful attention to collection and processing of blood samples. While separating the cellular elements of blood from the plasma containing EMPs, careful attention must be paid to centrifugation speed. In our experience, a 2-step centrifugation using 1500 × *g* for 10 minutes and then 13 000 × *g* for 10 minutes resulted 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 MPs appears to be the most favored method to characterize blood MPs. Typically, EMPs are identified as particles with a forward angle light scatter smaller than an internal standard consisting of 1–1.5-μm-sized latex particles.

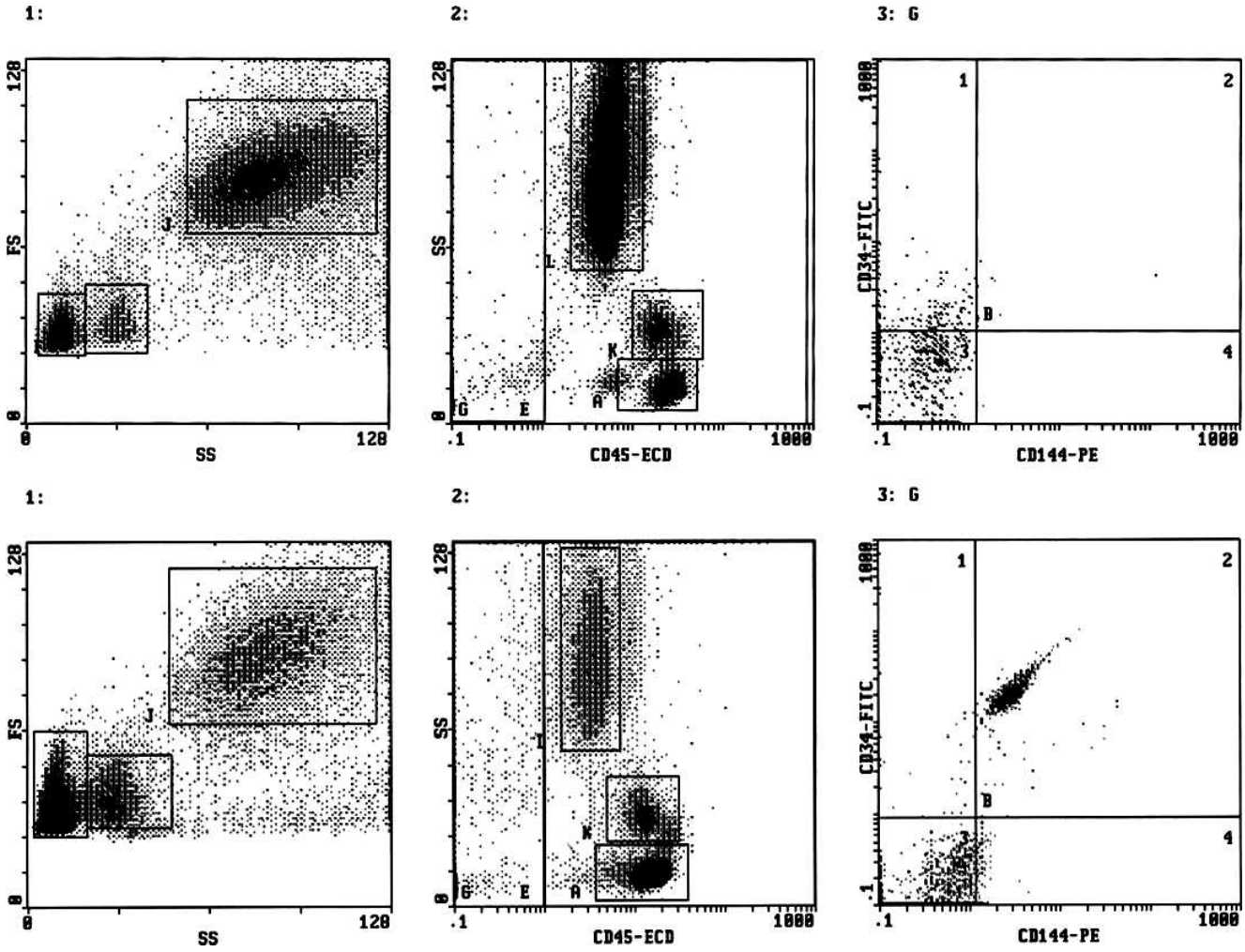

Figure 1. Representative flow cytometric scattergrams showing the gating strategy used in a normal healthy man (upper panels) and in a patient with late-onset hypogonadism and erectile dysfunction (lower panels). Both scattergrams 1 report the forward vs side scatter dot plot: 3 different cell populations can be identified (gate F, lymphocytes; gate I, monocytes; gate J, polymorphonuclear cells). Both scattergrams 2 report the $CD45_{pos}$ (gate E) and $CD45_{neg}$ (gate G) cells; gate A, lymphocytes; gate K, monocytes; gate L, polymorphonuclear cells. Both scattergrams 3 report only the $CD45_{neg}$ cells (gate G) subdivided according to the expression of CD34 and CD144 antigen staining. Endothelial progenitor cells were defined as $CD45_{neg}/CD34_{pos}/CD144_{pos}$, whereas endothelial microparticles were defined as $CD45_{neg}/CD34_{neg}/CD144_{pos}$.

ECD anti-human CD45 (IL), PE conjugated anti-human CD144 (IL), and FITC-conjugated annexin V (IL) were used for EMP flow detection by flow cytometry. To exclude MPs originating from leukocytes, we considered only events within the $CD45_{neg}$ gate. $CD144_{pos}$ 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 seconds 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 the tests throughout the study.

Statistical Analysis

Results are shown as mean ± SEM. Statistical analysis was performed by 1-way analysis of variance (ANOVA) followed by the Duncan multiple range test. Statistical analysis was conducted using SPSS 10.0 for Windows (IBM Corporation, Somers, New York). A P value lower than .05 was accepted as statistically significant.

Results

Age, BMI, and total cholesterol did not differ significantly between patients with ED and LOH, patients with ED alone, and controls (Table 2). Both groups of patients with ED had significantly lower high-density

Table 2. *Clinical, laboratory, and penile dynamic echo color Doppler parameters (mean ± SEM) in patients with arterial erectile dysfunction (ED) and late-onset hypogonadism (LOH), ED alone, and controls*

Parameter	ED and LOH (n = 50)	ED Alone (n = 20)	Controls (n = 20)
Age, y	58.2 ± 0.7	61.2 ± 1.2	61.4 ± 1.2
Body mass index, kg/m^2	27.0 ± 0.2	27.2 ± 0.4	26.7 ± 0.3
International Index of Erectile Function–5	9.2 ± 0.2[a,b]	13.1 ± 0.4[a]	22.7 ± 0.3
Systolic blood pressure, mmHg	141.7 ± 1.1[a]	140.3 ± 1.4[a]	129.5 ± 1.2
Diastolic blood pressure, mmHg	91.8 ± 0.7[a]	91.6 ± 0.9[a]	78.0 ± 0.7
Total cholesterol, mg/dL	210.6 ± 6.4	204.4 ± 5.9	203.0 ± 8.3
HDL cholesterol, mg/dL	42.4 ± 0.8[a]	43.8 ± 1.8[a]	53.3 ± 3.1
Triglycerides, mg/dL	196.9 ± 6.9[a]	189.5 ± 11.8[a]	156.1 ± 3.3
HOMA index	2.1 ± 0.02[a,b]	2.0 ± 0.04[a]	1.47 ± 0.05
Total testosterone, ng/dL	180.1 ± 3.4[a,b]	422.8 ± 5.3[a]	476.7 ± 14.5
Peak systolic velocity, cm/s	24.1 ± 0.6[a,b]	32.1 ± 0.4[a]	52.5 ± 2.1
End diastolic velocity, cm/s	1.7 ± 0.1[a]	1.2 ± 0.3	1.0 ± 0.4
Acceleration time, ms	133.2 ± 2.6[a,b]	113.3 ± 5.0[a]	88.2 ± 2.6
Resistance index	0.86 ± 0.01[a,b]	0.90 ± 0.03[a]	0.96 ± 0.02
Intima-media thickness, mm	0.43 ± 0.01[a,b]	0.37 ± 0.01[a]	0.14 ± 0.01

Abbreviations: HDL, high-density lipoprotein; HOMA, homeostasis model assessment of insulin resistance.

[a] $P < .05$ vs controls.

[b] $P < .05$ vs ED alone.

lipoprotein cholesterol and higher serum triglycerides and systolic and diastolic blood pressure compared with controls ($P < .05$, ANOVA followed by Duncan test). Patients with ED and LOH had significantly lower IIEF-5 scores compared with patients with ED alone or controls ($P < .05$, ANOVA followed by Duncan test). Patients with ED alone had significantly lower IIEF-5 scores than controls ($P < .05$, ANOVA followed by Duncan test). Similarly, serum TT levels were significantly lower in patients with ED and LOH compared with patients with ED alone or controls ($P < .05$, ANOVA followed by Duncan test). A slight but significant difference was observed between the latter 2 groups ($P < .05$, ANOVA followed by Duncan test). Patients with ED and LOH or ED alone had the HOMA index significantly higher than controls, and the HOMA index was also slightly but significantly higher in patients with ED and LOH compared with patients with ED alone ($P < .05$, ANOVA followed by Duncan test; Table 2).

Both groups of patients with ED had a significantly lower PSV and resistance index compared with controls; patients with ED and LOH had a significantly lower PSV compared with patients with ED alone ($P < .05$, ANOVA followed by Duncan test; Table 2). Similarly to PSV, patients with ED and LOH had significantly higher AT and cavernosal IMT than patients with ED alone and controls; both parameters were significantly higher in patients with ED alone compared with controls ($P < .05$, ANOVA followed by Duncan test). The end diastolic velocity did not differ significantly among groups (Table 2).

Patients with ED and LOH had significantly higher EPCs compared with patients with ED alone or controls. Patients with ED alone had significantly higher EPCs compared with patients with ED alone or controls ($P < .05$, ANOVA followed by Duncan test; Figure 2, panel A). EMPs were significantly higher in patients with ED and LOH compared with patients with ED alone or controls; patients with ED alone had EMPs significantly higher than controls ($P < .05$, ANOVA followed by Duncan test; Figure 2, panel B).

Discussion

This study evaluated the numbers of a newly proposed phenotype (Case et al, 2007; Timmermans et al, 2007) of circulating EPCs as well as EMPs in patients with arterial dysfunction–based ED and LOH because no such data are available in these patients. It is now well documented that the measurement of both EPCs and EMPs may be proposed as a biomarker of CVD and atherosclerotic complication and progression (Chironi et al, 2009; Dotsenko, 2010). We found that patients with arterial ED and LOH had greater penile vascular damage than patients with ED alone and higher circulating EPCs and EMPs. This suggests that LOH exacerbates endothelial dysfunction and that EPCs and EMPs are markers of endothelial function indicative of a more severe vascular damage.

The increased IMT is an important expression of morpho-structural alteration in patients with atherosclerosis, and the higher endothelial regenerative response is probably of a compensatory nature (Yamamoto et al, 2003; Dotsenko, 2010). Bone marrow–derived EPCs circulating in the peripheral blood migrate toward their target tissue, where they differentiate and

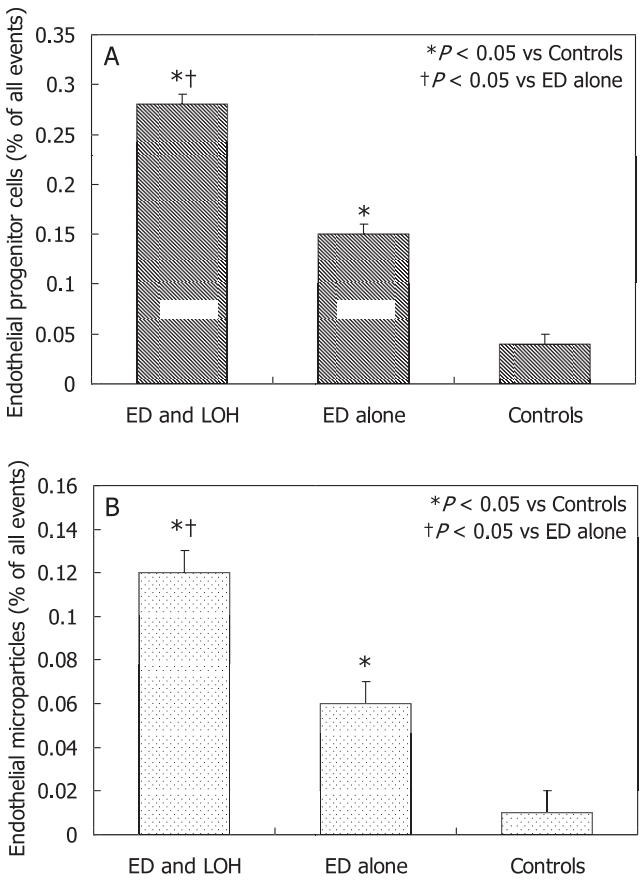

Figure 2. Percentage of circulating endothelial progenitor cells (immunophenotype $CD45_{neg}/CD34_{pos}/CD144_{pos}$) **(A)** and endothelial microparticles (immunophenotype $CD45_{neg}/CD34_{pos}$/annexin V_{pos}) **(B)** in patients with erectile dysfunction (ED) and late-onset hypogonadism (LOH), ED alone, and controls.

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).

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; Urbich and Dimmeler, 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 has been shown to be an independent predictor of CVD (Güven et al, 2006).

A considerable body of evidence suggests that androgen deficiency contributes to the onset and/or progression of CVD. Androgen deficiency, in fact, is associated with increased levels of total cholesterol and low-density lipoprotein, increased production of proinflammatory factors, and increased thickness of the arterial wall (Foresta et al, 2006; Mikhail, 2006; Zitzmann, 2009; Aversa et al, 2010; Diemer, 2010; Francomano et al, 2010; Katabami et al, 2010; Lunenfeld, 2010). Hypotestosteronemia is associated with a low number of circulating EPCs, defined by the phenotype $CD34_{pos}/CD133_{pos}/VEGFR_{pos}$, in young hypogonadal patients (Foresta et al, 2006). On the contrary, EPCs positive for osteocalcin (OCN_{pos}), a subpopulation of EPCs highly correlated with atherosclerosis progression, were significantly increased (Foresta et al, 2009, 2010). The more endothelial-committed EPC ($CD45_{neg}/CD34_{pos}/CD144_{pos}$) phenotype evaluated in this study is probably another subpopulation of EPCs with similar kinetic profile, but different pathophysiologic significance. In fact, EPCs OCN_{pos} is a proatherosclerotic phenotype, while $CD45_{neg}/CD34_{pos}/CD144_{pos}$ EPCs is a compensatory subpopulation.

Other authors (Foresta et al, 2005; Baumhakel et al, 2006; Esposito et al, 2009) have evaluated different phenotypes, expressions of a premature differentiation line; instead, the phenotype evaluated in this study expresses a subsequent dysfunctional phase, with different kinetics. The increase 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 the disease progression in these patients. Indeed, low levels of testosterone decrease the expression of nitric oxide, resulting in endothelial dysfunction, which triggers an endothelial repair response by EPCs (Traish et al, 2009).

The elevated number of EPCs found in patients with arterial ED and LOH 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). However, previous studies have evaluated patients with acute vascular injury; instead, our study examines the effects of long exposure to cardiovascular risk factors (ED and LOH).

In patients with CAD, several mechanistic possibilities have been advanced to explain the increase of EPCs. This activation may result from a variety of proinflammatory cytokines released in patients with coronary ischemia (Cho et al, 2003; Kong et al, 2004). 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 circulating EPCs (George et al, 2004). This study examines the effects of chronic exposure to cardiovascular risk factors; this aspect is original but at the same time requires caution and should not be the final consideration of this issue, but needs further confirmation with other studies.

EMPs are regarded as markers of cardiovascular dysfunction with a prothrombotic role associated with risk factors such as those that LOH promotes (Kintzel et al, 2008; Zitzmann, 2009; Aversa et al, 2010; Katabami et al, 2010; Lunefeld, 2010). The higher number of EMPs found in patients with ED and LOH suggests a more severe vascular damage in these patients compared with patients with ED alone. EMPs ($CD31_{pos}/CD42_{neg}$ and $CD31_{pos}/CD42_{pos}$) have been reported elevated in patients with ED and diabetes mellitus, and they were found independently involved in the pathogenesis of ED (Esposito et al, 2007). In this study, EMPs inversely correlated with the IIEF score in both diabetic and nondiabetic patients, 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 IIEF score (Esposito et al, 2007). In addition, ED patients with or without diabetes mellitus have significantly higher EMPs ($CD62_{pos}$) than nondiabetic men without ED. The EMPs ($CD62_{pos}$)/EMPs ($CD31_{pos}$) ratio, an index of endothelial activation (high ratio) or apoptosis (low ratio), was lowest in ED diabetic patients. These findings suggest that EMP increase in ED diabetic patients is consistent with increased apoptotic activity (Esposito et al, 2008). The results of the present study conducted in patients with ED and LOH imply a similar conclusion. These patients share with atherosclerotic patients a common picture of endothelial damage and markers of this dysfunction. This is sustained by the significant correlation between EPCs or EMPs, both biomarkers of endothelial dysfunction, with indices of ED severity (PSV, AT, and IMT).

In conclusion, this study showed that patients with arterial ED and LOH have higher blood concentrations of EPCs and EMPs compared with patients with ED alone. Thus, LOH 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 biological balance between the degree of vascular wall damage severity (EMPs) and the extent of the repair mechanism (EPCs).

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 (5302):964–967.

Aversa A, Bruzziches R, Francomano D, Rosano G, Isidori AM, Lenzi A, Spera G. Effects of testosterone undecanoate on cardiovascular risk factors and atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 24-month, randomized, double-blind, placebo-controlled study. *J Sex Med*. 2010;7:3495–3503.

Baumhakel M, Werner N, Bohm M, Nickening G. Circulating endothelial progenitor cells correlate with erectile function in patients with coronary heart disease. *Eur Heart J*. 2006;27:2184–2188.

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.

Breier G, Breviario F, Caveda L, Berthier R, Schnürch H, Gotsch U, Vestweber D, Risau W, Dejana E. Molecular cloning and expression of murine vascular endothelial-cadherin in early stage development of cardiovascular system. *Blood*. 1996;87:630–642.

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.

Case J, Mead LE, Bessler WK, Prater D, White HA, Saadatzadeh MR, Bhavsar JR, Yoder MC, Haneline LS, Ingram DA. Human CD34+ AC133+ VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. *Exp Hematol*. 2007;35:1109–1118.

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.

Costa C, Virag R. The endothelial-erectile dysfunction connection: an essential update. *J Sex Med*. 2009;6:2390–2404.

Curtis AM, Zhang L, Medenilla E, Gui M, Wilkinson PF, Hu E, Giri J, Doraiswamy V, Gunda S, Burgert ME, Moore JS, Edelberg JM, Mohler ER 3rd. Relationship of microparticles to progenitor cells as a measure of vascular health in a diabetic population. *Cytometry B Clin Cytom*. 2010;78:329–337.

Diemer T. Testosterone and erectile dysfunction. *Urol A*. 2010;49: 26–31.

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.

Esposito K, Ciotola M, Giugliano F, Schisano B, Improta L, Improta MR, Beneduce F, Rispoli M, De Sio M, Giugliano D. Endothelial microparticles correlate with erectile dysfunction in diabetic men. *Int J Impot Res*. 2007;19:161–166.

Esposito K, Ciotola M, Maiorino MI, Giugliano F, Autorino R, De Sio M, Jannini E, Lenzi A, Giugliano D. Circulating CD34+ KDR+ endothelial progenitor cells correlate with erectile function and endothelial function in overweight men. *J Sex Med*. 2009;6:107–114.

Feldman HA, Johannes CB, Derby CA, Kleinman KP, Mohr BA, Araujo AB, McKinlay JB. Erectile dysfunction and coronary risk factors: prospective results from the Massachusetts male aging study. *Prev Med*. 2000;30:328–338.

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, Caretta N, Lana A, De Toni L, Biagioli A, Ferlin A, Garolla A. Reduced number of circulating endothelial progenitor cells in hypogonadal men. *J Clin Endocrinol Metab*. 2006;91: 4599–4602.

Foresta C, De Toni L, Biagioli A, Ganz F, Magagna S, Caretta N. Increased levels of osteocalcin-positive endothelial progenitor cells in patients affected by erectile dysfunction and cavernous atherosclerosis. *J Sex Med*. 2009;7:751–757.

Foresta C, De Toni L, Selice R, Garolla A, Di Mambro A. Increased osteocalcin-positive endothelial progenitor cells in hypogonadal male patients. *J Endocrinol Invest*. 2010;33:439–442.

Francomano D, Bruzziches R, Natali M, Aversa A, Spera G. Cardiovascular effect of testosterone replacement therapy in aging male. *Acta Biomed*. 2010;81(suppl 1):101–106.

Gazzaruso C, Solerte SB, Pujia A, Coppola A, Vezzoli M, Salvucci F, Valenti C, Giustina A, Garzaniti A. Erectile dysfunction as a predictor of cardiovascular events and death in diabetic patients with angiographically proven asymptomatic coronary artery disease. A potential protective role for statins and 5-phosphodiesterase inhibitors. *J Am Coll Cardiol*. 2008;51:2040–2044.

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.

Hirai H, Samokhvalov IM, Fujimoto T, Nishikawa S, Imanishi J, Nishikawa S. Involvement of Runx1 in the down-regulation of fetal liver kinase-1 expression during transition of endothelial cells to hematopoietic cells. *Blood*. 2005;106:1948–1955.

Ingram DA, Caplice NM, Yoder MC. Unresolved questions, changing definitions, and novel paradigms for defining endothelial progenitor cells. *Blood*. 2005;106:1525–1531.

Jackson G, Boon N, Eardley I, Kirby M, Dean J, Hackett G, Montorsi P, Montorsi F, Vlachopoulos C, Kloner R, Sharlip I, Miner M. Erectile dysfunction and coronary artery disease prediction: evidence-based guidance and consensus. *Int J Clin Pract*. 2010;64:848–857.

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 Haemostasis*. 2004;2:1842–1851.

Katabami T, Kato H, Asahina T, Hinohara S, Shin T, Kawata T, Ohta A, Iwamoto T, Tanaka Y. Serum free testosterone and metabolic syndrome in Japanese men. *Endocr J*. 2010;57:533–539.

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

Kintzel PE, Chase SL, Schultz LM, O'Rourke TJ. Increased risk of metabolic syndrome, diabetes mellitus, and cardiovascular disease in men receiving androgen deprivation therapy for prostate cancer. *Pharmacotherapy*. 2008;28:1511–1522.

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.

Lampugnani M-G, Corada M, Caveda L, Breviario F, Ayalon O, Geiger B, Dejana E. The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, b-catenin, and a-catenin with vascular endothelial cadherin (VE-cadherin). *J Cell Biol*. 1995;129:203–217.

Lampugnani M-G, Resnati M, Raiteri M Pigott R, Pisacane A, Houen G, Ruco LP, Dejana E. A novel endothelial-specific membrane protein is a marker of cell-cell contacts. *J Cell Biol*. 1992;118: 1511–1522.

Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origin of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest*. 2000;105:71–77.

Lunenfeld B. The relationship between sex hormones and the metabolic syndrome. *Acta Biomed*. 2010;81(suppl 1):79–84.

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(1 pt 1):e40–e48.

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.

Mikhail N. Does testosterone have a role in erectile function? *Am J Med*. 2006;119:373–382.

Navarro P, Caveda L, Breviario F, Mântodeanu I, Lampugnani M-G, Dejana E. Catenin-dependent and -independent functions of vascular endothelial cadherin. *J Biol Chem*. 1995;270:30965–30972.

Nozaki T, Sugiyama S, Sugamura K, Ohba K, Matsuzawa Y, Konishi M, Matsubara J, Akiyama E, Sumida H, Matsui K, Jinnouchi H, Ogawa H. Prognostic value of endothelial microparticles in patients with heart failure. *Eur J Heart Failure*. 2010;12:1223–1228.

Papayannopoulou T. Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization. *Blood*. 2004;103:1580–1585.

Pardanaud L, Luton D, Prigent M, Bourcheix L-M, Catala M, Dieterlen-Lièvre F. Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. *Development*. 1996;122:1363–1371.

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.

Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation*. 2003;107(8):1164–1169.

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.

Russel ST, Khandheria BK, Nehra A. Erectile dysfunction and cardiovascular disease. *Mayo Clin Proc*. 2004;79:782–794.

Sabatier F, Camoin-Jau L, Anfosso F, Sampol J, Dignat-George F. Circulating endothelial cells, microparticles and progenitors: key

players towards the definition of vascular competence. *J Cell Mol Med*. 2009;13:454–471.

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.

Schatteman GC, Dunnwald M, Jiao C. Biology of bone marrow-derived endothelial cell precursors. *Am J Physiol Heart Circ Physiol*. 2007;292:1–18.

Schwartzenberg S, Deutsch V, Maysel-Auslender S, Kissil S, Keren G, George J. Circulating apoptotic progenitor cells: a novel biomarker in patients with acute coronary syndromes. *Arterioscler Thromb Vasc Biol*. 2007;27:e27–e31.

Shabsigh R, Rajfer J, Aversa A, Traish AM, Yassin A, Kalinchenko SY, Buvat J. The evolving role of testosterone in the treatment of erectile dysfunction. *Int J Clin Pract*. 2006;60:1087–1092.

Shet AS. Characterizing blood microparticles: technical aspects and challenges. *Vasc Health Risk Manage*. 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.

Sugiyama S, Kugiyama K, Aikawa M, Nakamura S, Ogawa H, Libby P. Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression: involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. *Arterioscler Thromb Vasc Biol*. 2004;24:1309–1314.

Timmermans F, Van Hauwermeiren F, De Smedt M, Raedt R, Plasschaert F, De Buyzere ML, Gillebert TC, Plum J, Vandekerckhove B. Endothelial outgrowth cells are not derived from CD133+ cells or CD45+ hematopoietic precursors. *Arterioscler Thromb Vasc Biol*. 2007;27:1572–1579.

Traish AM, Saad F, Feeley RJ, Guay A. The dark side of testosterone deficiency: III. Cardiovascular disease. *J Androl*. 2009;30:477–494.

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.

Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, Kaufman JM, Legros JJ, Lunenfeld B, Morales A, Morley JE, Schulman C, Thompson IM, Weidner W, Wu FC. Investigation, treatment and monitoring of late-onset hypogonadism in males. *Int J Androl*. 2009;32:1–10.

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:e17–e24.

Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol*. 2003;42:1149–1160.

Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol*. 1967;13:269–288.

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.

Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prehal JT, Ingram DA. Re-defining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood*. 2007;109:1801–1809.

Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY, Kim TY, Cho HJ, Kang HJ, Chae IH, Yang HK, Oh BK, Park YB, Kim HS. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation*. 2005;112:618–627.

Zitzmann M. Testosterone deficiency, insulin resistance and the metabolic syndrome. *Nat Rev Endocrinol*. 2009;5:673–681.