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Arterial Erectile Dysfunction and Peripheral Arterial Disease: Reliability of a New Phenotype of Endothelial Progenitor Cells and Endothelial Microparticles

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ABSTRACT: The aim of this study was to evaluate whether the blood concentrations of a new immunophenotype of circulating late endothelial progenitor cells (EPC) and endothelial microparticles (EMP) varies in patients with arterial erectile dysfunction (aED) and abnormalities in other arterial districts. To accomplish this, cavernous artery peak systolic velocity (PSV), acceleration time (AT), and intima-media thickness (IMT) were determined after intracavernous administration of alprostadil by echo-color Doppler in 80 consecutive patients (age range, 50–75 years). Fifteen patients had aED alone (group A) and served as controls; 22 had aED plus atheroma plaques and/or increased IMT of the common carotid artery (group B); 20 had aED plus lower limb artery abnormalities (group C); and 23 had aED plus carotid and lower limb artery abnormalities (group D). EPC and EMP blood concentrations were evaluated by flow cytometry. Blood mononuclear cells with the immunophenotype $CD45_{neg}/CD34_{pos}/CD144_{pos}$ were defined as EPCs, whereas $CD45_{neg}/CD144_{pos}$/annexin V_{pos} cells were defined as EMPs. Group B and C patients had a similar PSV, AT, and IMT at the level of the cavernous arteries. Their PSV values were significantly lower and mean values of AT and IMT significantly higher compared with group A patients. Patients of group D had a significantly lower PSV and significantly higher AT and IMT compared with all other groups. As far as serum concentrations of EPCs and EMPs, group D patients had significantly higher EPC and EMP mean values compared with all other groups. Group B and C patients had similar EPC and EMP values. This study showed that a more generalized peripheral atherosclerotic process is associated with a more severe penile artery insufficiency and endothelial dysfunction. Moreover, this study confirms the diagnostic reliability of the immunophenotype of EPCs and EMPs chosen in the clinical practice.

Key words: Late endothelial progenitor cells, carotid abnormalities, lower limb artery abnormalities.

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It is known that erectile dysfunction (ED) is a potential marker of vascular damage in patients with cardiovascular risk factors. Therefore, the development of diagnostic tools that may help the clinician to anticipate the diagnosis of endothelial dysfunction in these patients is of paramount importance. Indeed, the evaluation of endothelial progenitor cells (EPC) and endothelial microparticles (EMP) represents a reliable method to screen the endothelial alterations in patients with ED (Esposito et al, 2007; Foresta et al, 2010). In this regard, we have recently showed that the integration between penile echo-color Doppler and serum concentrations of an original phenotype of EPCs ($CD45_{neg}/CD34_{pos}/CD144_{pos}$) and EMPs ($CD45_{neg}/CD144_{pos}$/annexin V_{pos}) is reliable for the diagnostic evaluation of patients with vascular arterial ED (aED; La Vignera et al, 2012d). In particular, we found that patients with more severe cavernous artery insufficiency had higher serum concentrations of EPCs and EMPs compared with patients with mild and moderate level of arterial dysfunction. Moreover, we previously reported the diagnostic value of this EPC and EMP phenotype in other categories of patients with aED, such as those with associated late onset hypogonadism or metabolic syndrome. In addition, we evaluated the effects of androgen replacement therapy, aerobic physical activity, and tadalafil administration on these markers (La Vignera, 2011; La Vignera et al, 2011a,b,c,d; 2012a,c,d). Finally, we have previously shown that a more generalized peripheral atherosclerotic process is associated with a more severe penile artery insufficiency (Vicari et al, 2006); therefore, patients with aED should undergo an extensive echo-duplex examination (Vicari et al, 2006).

The relevance of these studies relates to 1) identification of a different phenotype of EPCs as a useful

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diagnostic marker in the clinical practice; 2) description of a dynamic model where the reparative response of EPCs is associated with simultaneous evidence of endothelial injury in the same patient (increased EMPs positive for annexin V); and 3) evaluation of different clinical models of patients with ED. However, there is no evidence regarding the serum concentrations of this phenotype of EPCs and EMPs in patients with aED and signs of atherosclerosis in other arterial districts. On this basis, the aim of the present study was to evaluate the serum concentrations of EPCs and EMPs in a cohort of patients with aED and/or carotid and/or lower arterial limb atherosclerosis. A second purpose was to test the reliability of these markers in another diagnostic category of patients frequently found in the clinical practice.

Materials and Methods

Patient Selection

We enrolled 80 consecutive patients (mean age 64.0 years, range 50–75 years) with ED (median duration 6.0 years, range 1.0–9.0 years) due to cavernous artery insufficiency diagnosed by of dynamic echo-color Doppler following intracavernous administration of alprostadil. The diagnosis of aED was made when all the following criteria were fulfilled: 1) cavernous artery peak systolic velocity (PSV) <35 cm/s, 10 and 20 minutes after the intracavernous injection of alprostadil (20 μg) by echo-color Doppler (Benson et al, 1993); and 2) acceleration time (AT) >110 milliseconds (Speel et al, 2003). Each patient underwent duplex ultrasonography (combined real-time and Doppler ultrasonography) of the carotid and lower limb arteries to evaluate the concomitant presence of atherosclerosis in these arterial districts.

The protocol was approved by the institutional review board and an informed written consent was obtained from each patient.

Clinical History and Physical Examination

All the patients were asked information regarding their habits and medical history. Each of them fulfilled the 5-item version of the International Index of Erectile Function questionnaire (IIEF-5; Cappelleri et al, 1999). The clinical history included the identification of the main vascular risk factors, such as cigarette smoking, hypertension, hyperlipidemia, and diabetes mellitus. In particular, patients were considered smokers if they smoked more than 20 cigarettes a day for at least 1 year. Hypertension was defined as systolic blood pressure ≥140 mm/Hg and/or diastolic blood pressure ≥90 mm/Hg (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003). Hyperlipidemia was defined as a total serum cholesterol concentration exceeding 200 mg/dL and/or total cholesterol/high-density lipoprotein ratio greater than 5 and/or serum triglyceride concentration exceeding 140 mg/dL (European Association for Cardiovascular Prevention & Rehabilitation et al, 2011). Diabetes mellitus was defined by clinical history and/or the results of the 75 g oral glucose tolerance test (fasting glycemia equal to or greater than 126 mg/dL and/or glycemia 120 minutes after oral glucose loading equal to or greater than 200 mg/dL) (Alberti and Zimmet, 1998).

A clinical examination was conducted to evaluate the presence of penile malformations, reduced testicular volume (<12 mL according to Prader orchidometer), penile sensitivity, cremasteric reflex, and femoral artery pulsation. The physical examination also included auscultation of any carotid arterial murmurs and palpation of the arteries in the penis and lower extremities.

Patients were excluded from the study if they had severe hypertension (grade 3; European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003) and/or they were taking a complicated multidrug antihypertensive regimen, and if they had severe hyperlipidemia (total serum cholesterol concentration exceeding 280 mg/dL and/or serum triglyceride concentration exceeding 350 mg/dL) and/or glycemia >200 mg/dL. In addition, patients with serum concentrations of total testosterone <350 ng/dL were excluded from this study because we found that low serum testosterone concentration is associated with high levels of this phenotype of EPCs and EMPs (La Vignera et al, 2011d). Finally, patients with a resistance index (penile Doppler parameter suggestive of venous leakage) <0.75 were excluded.

Dynamic Penile Echo-Color Doppler

Dynamic penile echo-color Doppler was performed following intracavernosal injection of alprostadil (Caverject; Pfizer, New York, New York). Doppler evaluations were performed by Aplio XV ultrasound machine equipped with a 6–13-MHz multifrequency linear probe (Toshiba, Rome, Italy). The cavernous intima 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 ×), regulating the partial and total B-mode gain to reduce the noise at the minimum level. Cavernous intima thickness was measured in a semi-quantitative manner, using dedicated pre-existing software available in the Aplio XV, always steering the angle parallel to the lumen (Caretta et al, 2009).

Carotid and Lower Arterial Limb Echo-Color Doppler Evaluation

Carotid and lower limb arterial assessments were performed according to specific general ultrasound principles (Gareth and Phillips, 2000), involving both a grading of any stenosis and an attempt to characterize the plaque or intima-media thickness (IMT).

A carotid or lower limb stenosis >50% of the diameter reduction was judged hemodynamically significant (Bluth et al, 1988; Moneta et al, 1992). Plaques were classified as homogeneous if they were relatively uniform in texture, consisting usually of dense fibrous tissue and/or containing areas of variable echo pattern. They were defined as heterogeneous if there was at least 1 well-defined focal sonolucent area and its surface was irregular (Gareth and Phillips, 2000). Carotid artery IMT was measured by B-mode ultrasonography using a

Table 1. *Age and sexual characteristics of patients with arterial erectile dysfunction (ED) alone (group A), ED plus carotid abnormalities (group B), ED plus lower limb artery abnormalities (group C), or ED plus carotid and lower limb artery abnormalities (group D)*

	Age, y (Range)	ED Duration, y (Range)	IIEF-5 Score (Range)	Grading of ED (IIEF-5 Score Distribution Frequency), %		
				Severe (0–10)	Moderate (11–16)	Mild (17–21)
Group A (n = 15)	62.0 ± 4.0 (51–73)	3.2 (1.4–5)	14.0 ± 1.0 (6–21)	40.0 (n = 6)	26.7 (n = 4)	33.3 (n = 5)
Group B (n = 22)	61.0 ± 6.0 (50–75)	3.5 (2.0–5)	12.0 ± 1.1 (6–20)	41.0 (n = 9)	27.2 (n = 6)	31.8 (n = 7)
Group C (n = 20)	61.5 ± 5.0 (51–72)	3.8 (3–6)	12.0 ± 2.0 (5–20)	40.0 (n = 8)	30.0 (n = 6)	30.0 (n = 6)
Group D (n = 23)	62.3 ± 3.0 (50–75)	4.8 (3–8)[a]	8.0 ± 1.0[a] (5–19)	39.1 (n = 9)	30.4 (n = 7)	30.4 (n = 7)

Abbreviation: IIEF-5, 5-item version of the International Index of Erectile Function questionnaire.

[a] $P < .01$ vs penile arterial insufficiency alone (group A) (analysis of variance followed by Duncan's test).

7.5-MHz high-resolution transducer with the patient in a supine position. IMT, that is, the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line, was measured at 3 different points on each side of the carotid artery; the maximal value was used as a selection criterion.

Fifteen patients had aED alone (group A) and made up the control group; 22 had aED plus atheroma plaques and/or marked IMT (stenosis >50% of the diameter reduction) of the common carotid artery (group B); 20 had aED plus lower limb artery abnormalities (stenosis >50% of the diameter reduction) (group C); 23 had aED plus carotid and lower limb artery abnormalities (group D).

Blood EPC and EMP Determination

EPCs ($CD45_{neg}/CD34_{pos}/CD144_{pos}$) and EMPs ($CD45_{neg}/CD144_{pos}$/annexin V_{pos}) were determined as previously reported (La Vignera et al, 2012d). Briefly, their evaluation was performed in blood following incubation in erythrocyte lysing solution (Versalyse; IL, Milan, Italy). 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 (CD45, CD34, CD144, annexin V) at room temperature for 20 minutes. 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.

Statistical Analysis

Results are shown as mean ± SEM throughout the study. The data were analyzed by 1-way analysis of variance followed by Duncan's multiple range test or χ^2 analysis, as appropriate. The SPSS 9.0 software for Windows was used for statistical evaluation (Chicago, Illinois). A statistically significant difference was accepted when the P value was lower than .05.

Results

The mean age of the control group was not significantly different from mean ages of patients of groups B, C, and D (Table 1). The patients of group D had a significantly longer ED duration and greater ED severity, evaluated by the IIEF-5 questionnaire, whereas IIEF-5 score frequency distributions were similar in the 4 groups of patients with aED (Table 1).

The frequency of the major cardiovascular risk factors was not significantly different among groups (Figure 1). Group D showed a higher frequency of triple combination of cardiovascular risk factors (Table 2).

Patients with arterial abnormalities at the carotid (group B) or lower limb (group C) levels had similar PSV, AT, and IMT, but respectively a significant lower PSV and significant higher values of AT and IMT compared with the control group. Interestingly, patients with signs of peripheral atherosclerosis in both districts had PSV not only lower than that of the controls (group A), but also significantly lower than that of patients of groups B and C, as well as higher values of AT and IMT compared with all other groups. This suggests that a more severe peripheral atherosclerosis is associated with a more profound impairment of penile artery blood flow (Table 3).

Finally, group D patients had significantly higher mean values of EPCs and EMPs compared with all other

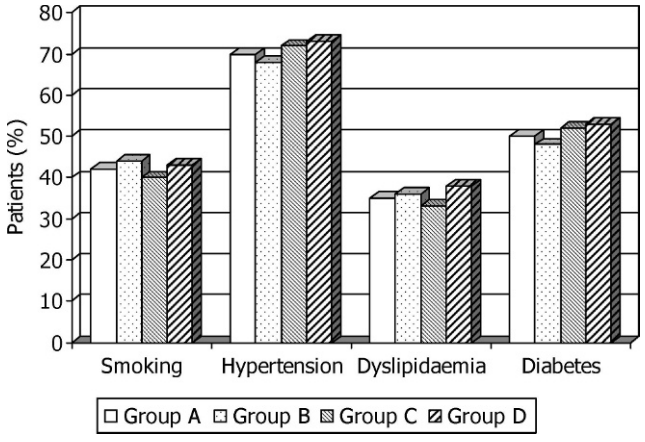

Figure 1. Frequency of arterial risk factors in patients with arterial erectile dysfunction (ED) alone (group A), ED plus carotid abnormalities (group B), ED plus lower limb artery abnormalities (group C), or ED plus carotid and lower limb artery abnormalities (group D).

Table 2. *Percentage and number of patients exhibiting 1 or more arterial risk factors in presence of arterial erectile dysfunction (ED) alone (group A), ED plus carotid abnormalities (group B), ED plus lower limb artery abnormalities (group C), or ED plus carotid and lower limb artery abnormalities (group D)*

	Arterial Risk Factors		
	1, % (No. of Patients)	2, % (No. of Patients)	3, % (No. of Patients)
Group A (n = 15)	33.3 (5)	33.3 (5)	33.3 (5)
Group B (n = 22)	31.8 (7)	31.8 (7)	36.4 (8)
Group C (n = 20)	30.0 (6)	35.0 (7)	35.0 (7)
Group D (n = 23)	13.0 (3)[a]	39.1 (9)	47.9 (11)[a]

[a] $P < .01$ vs all other matched groups (χ^2).

groups (Figure 2). Patients of groups B and C showed similar values of EPCs and EMPs. Finally, these groups showed higher values of EPCs and EMPs compared with controls, but this difference did not reach statistical significance.

Discussion

It is widely demonstrated that ED and generalized atherosclerosis often share the same cardiovascular risk factors, such as smoking, diabetes, hypertension, dyslipidemia, and obesity; moreover, the atherosclerosis process worsens the severity of aED (Virag et al, 1985; Jackson, 1999; Kaiser et al, 2004). In this regard, the results of the present study showed that a low percentage of patients with aED had an isolated penile arterial dysfunction, whereas the vast majority of the patients had a concomitant atherosclerosis in other arterial districts, confirming our previous observations (Vicari et al, 2006). Although patients with aED and multidistrict atherosclerosis had a similar mean age and frequency of cardiovascular risk factors compared with patients with aED alone or in combination with carotid or lower limb artery abnormalities, they had a significantly longer ED duration and a lower IIEF-5 score. Moreover, group D patients also comprised a higher percentage of patients with 3 variously combined arterial risk factors, and they showed lower values of PSV and respectively higher values of AT and IMT. Finally, with regard to patients with peripheral atherosclerosis, the results of this study suggest that the circulating levels of these markers of endothelial dysfunction (EPCs and EMPs) were significantly higher in patients with more extensive forms of atherosclerosis, confirming its diagnostic reliability in the evaluation of patients with cardiovascular risk factors. In fact, in previous studies investigating this phenotype of EPCs and EMPs, we showed that their blood concentrations

Table 3. *Medians and 95% confidence intervals of peak systolic velocity (PSV), resistance index (PSV − end diastolic velocity/PSV), acceleration time, and intima-media thickness in patients with arterial erectile dysfunction (ED) alone (group A), ED plus carotid abnormalities (group B), ED plus lower limb artery abnormalities (group C), or ED plus carotid and lower limb artery abnormalities (group D)*

	Lower Limit	Median	Upper Limit
Peak systolic velocity, cm/s			
Group A (n = 15)	21.0	23.0	24.5
Group B (n = 22)	16.2	20.0[a]	21.9
Group C (n = 20)	17.3	19.0[a]	19.9
Group D (n = 23)	12.9	15.0[b]	16.7
Resistance index			
Group A (n = 15)	0.92	0.96	1.00
Group B (n = 22)	0.92	0.95	1.00
Group C (n = 20)	0.94	0.98	1.00
Group D (n = 23)	0.92	0.96	1.00
Acceleration time, mm/s			
Group A (n = 15)	111.0	113.0	120.0
Group B (n = 22)	112.0	118.0[a]	122.0
Group C (n = 20)	112.0	120.0[a]	125.0
Group D (n = 23)	115.0	136.0[b]	140.0
Intima-media thickness, mm			
Group A (n = 15)	0.25 ± 0.6	0.32 ± 0.6	0.38 ± 0.6
Group B (n = 22)	0.32 ± 0.5	0.39 ± 0.6[a]	0.44 ± 0.4
Group C (n = 20)	0.33 ± 0.5	0.41 ± 0.6[a]	0.46 ± 0.8
Group D (n = 23)	0.60 ± 0.4	0.74 ± 0.8[b]	0.83 ± 0.2

[a] $P < .05$ vs group A (χ^2).
[b] $P < .01$ vs all other matched groups (χ^2).

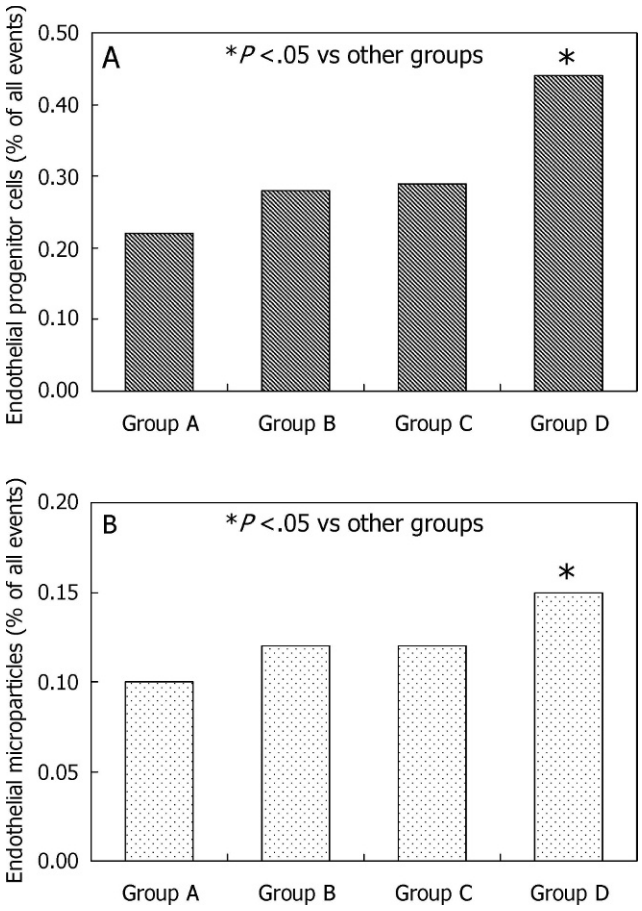

Figure 2. Percentage of circulating endothelial progenitor cells (immunophenotype $CD45_{neg}/CD34_{pos}/CD144_{pos}$) **(A)** and endothelial microparticles (immunophenotype $CD45_{neg}/CD34_{pos}$/annexin V_{pos}) **(B)** among different groups.

increase in patients with aED and particularly in aED patients with late-onset hypogonadism and metabolic syndrome (La Vignera et al, 2011b,d; 2012b).

Endothelial Progenitor Cells

The phenotype of EPCs studied is characterized by the simultaneous expression of CD34 and CD144 on cells negative for CD45. CD34 is an antigen that 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 into mature endothelial cells (Fina et al, 1990). In addition, CD144 or vascular endothelial cadherin is specifically localized in the inter-endothelial cell junction (Breviario et al, 1995), and it seems important in maintaining endothelial permeability, because monolayers of transfected cells show a calcium-dependent reduction in permeability. Finally, CD45 is expressed on the surface of all human leukocytes, and EPCs do not express this antigen (Thomas, 1989). Phenotypic variation, detected by flow cytometry, during EPC culture showed that CD144 is acquired later; indeed, it is almost absent between 5 and 8 days of culture, whereas it is overrepresented between 30 and 32 days of culture, together with CD34 and CD31 (Delva et al, 2008). These findings suggest that the phenotype $CD45_{neg}/CD34_{pos}/CD144_{pos}$ represents a late group of differentiating EPCs. No data have been reported on this EPC phenotype in patients with aED associated with peripheral atherosclerosis, and their blood concentrations are higher than those of other EPC phenotypes evaluated in patients with peripheral arterial disease. This apparent discrepancy may be explained by the different phenotype that expresses a different functional step of the endothelial repair mechanism. In this regard, recently, Güven and colleagues (2006) showed an increased number of EPCs in patients with chronic coronary arterial disease, which is in contrast with previous evidence of a decreased EPC number in these patients (Vasa et al, 2001; Werner et al, 2005). Moreover, limited data are available on the number and the functional role of EPCs in patients with peripheral arterial disease. In particular, Sandri and colleagues showed that exercise training increases circulating EPCs in these patients (Sandri et al, 2005); Fadini and colleagues reported that patients with type 2 diabetes mellitus and concomitant peripheral arterial disease had a lower number of EPCs compared with diabetic patients without peripheral atherosclerosis (Fadini et al, 2006).

Endothelial Microparticles

EMPs are small vesicles that are released from endothelial cells and can be found circulating in the blood. It is important to know that their membrane contains receptors and other cell surface molecules that enable the identification of the endothelial origin of these microparticles and allow them to be distinguished from microparticles deriving from other cells, such as platelets (Davizon and López, 2009). In our laboratory, the phenotype characterization of EMP provides the simultaneous expression of vascular endothelial (VE) cadherin, a Ca^{++}-dependent cell adhesion molecule, which is expressed in atherosclerotic lesions by endothelial cells and is associated with neovascularization (Corada et al, 2001), and annexin V, which is used to detect cells that have externalized phosphatidylserine on the cell surface, a feature found in early apoptosis (Vermes et al, 1995).

The results of the present study show that EMP blood concentrations are significantly higher in patients with aED and carotid and lower limb artery atherosclerosis compared with patients affected by isolated aED. This confirms our previous results regarding the increase of this marker when the severity of the vascular damage is

greater (La Vignera et al, 2012d) and the results of other studies that have shown the involvement of EMPs as biological messengers in the pathophysiology of different cardiovascular disorders, including atherogenesis and thrombosis (Shantsila et al, 2010). Moreover, increased levels of EMPs ($CD105_{pos}$ and $CD11a_{pos}$) have previously been reported to be associated with carotid inward remodeling even before atherosclerosis is detectable (Chironi et al, 2010). Finally, other authors have investigated the diagnostic role of EMPs positive for annexin V and/or VE-cadherin. In particular, Sinning and colleagues (2011) showed that the level of circulating $CD31_{pos}$/annexin V_{pos} EMPs is an independent predictor of cardiovascular events in patients with stable coronary artery disease and may be useful for risk stratification. Bernard and colleagues (2009) reported for the first time an association between plasma EMP-$CD144_{pos}$ and coronary noncalcified plaques assessed by multidetector computed tomography in a population of type 2 diabetic patients, suggesting that EMPs might be used as a surrogate marker of unstable plaques and might help to improve cardiovascular prediction in diabetic patients with intermediate risk. In addition, other authors have shown elevated levels of VE-$cadherin_{pos}$ EMPs in patients with type 2 diabetes mellitus and coronary artery disease (Koga et al, 2005).

Diagnostic Advantages of This Phenotype of EPCs and EMPs

Five main approaches have been used to detect EPCs in the blood. They consist of morphological identification, isolation with magnetic beads, in vitro culture, fluorescent microscopy, and flow cytometry; the latter is the technique most frequently used for the enumeration of EPCs, though it presents some technical difficulties. The detection of EPCs with flow cytometry analysis is characterized by rare events and, depending on the sample (blood preparation and patients from whom blood is obtained), the number of EPCs detected can vary from 1 in 10 000 to 1 in 1 million events. At this level of detection, obtaining a low background is crucial for an accurate estimation of such rare events. In fact, the presence of debris, dead cells, fluorescence, and microscopic ''dark'' bubbles are all factors that can introduce false-positive events. These obstacles can be overcome by using serum blocking to prevent nonspecific binding of antibodies to the sample, dye test to exclude dead cells, and gating techniques to exclude unwanted cell lines, as well as a very accurate cytometer cleaning procedure before use. Another important aspect relates to the correct phenotype of EPCs and EMPs to be identified. In fact, despite the fact that EPCs are often characterized by the surface marker CD34 together with vascular endothelial growth factor receptor 2, and because of the lack of annexin V binding specificity and sensitivity as well as the large panel of available markers for EMP characterization, the optimal procedure to be used for the detection and quantification of these cells remains uncertain. This is mainly for the following 4 reasons: 1) monoclonal antibodies used for identification of these cell types do not recognize epitopes present only in endothelial cells; 2) a clear separation between mature and immature endothelial cells cannot be easily achieved; 3) other cell lines apparently have the ability to transdifferentiate into endothelial cells; and 4) these cells are present in rather small amounts in the peripheral blood (Asahara et al, 1997; Shet, 2007).

In our experience, the reliability of this phenotype of EPCs and EMPs in different clinical models (isolated aED and aED associated with hypogonadism, metabolic syndrome, or peripheral atherosclerosis), the easiness of the methodology, and the possibility of exploring the pathophysiology dynamically make them excellent diagnostic markers of endothelial dysfunction in patients with ED. The available lines of evidence indicate that shedding of EMPs from the parental cells is not just a passive process accompanying cellular dysfunction and apoptosis, but a tightly regulated mechanism implicated in the interactions among various cell types (Shantsila et al, 2010). We do not think that this test may replace penile echo-color Doppler, but it may certainly improve its diagnostic accuracy, suggesting the presence of endothelial dysfunction in patients with quantitative alterations of vascular parameters, which can be notoriously altered by the experience of the operator and the patient's anxiety (La Vignera et al, 2012d). Finally, another important aspect is that this test may help the clinician to understand whether the treatment strategy is curative or only a palliative on endothelial dysfunction of these patients (La Vignera, 2011; La Vignera et al, 2011a,b,c,d; 2012a,b,c,d).

Conclusions

In conclusion, the present study confirms the diagnostic accuracy of the phenotype of ECPs and EMPs chosen as markers of endothelial damage. In particular, the reliability is demonstrated by the significant simultaneous increases of their blood concentrations in patients affected by aED and severe atherosclerosis compared with patients with isolated aED and/or associated mild peripheral atherosclerosis.

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