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REVIEW

Endothelial progenitor cells and erectile dysfunction: a brief review on diagnostic significance and summary of our experience

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Abstract

The article provides a brief review of the literature concerning the diagnostic use of endothelial progenitor cells in patients with erectile dysfunction. In particular, patients with arterial erectile dysfunction could benefit from the use of this diagnostic marker, which in clinical practice can be used together with more conventional methods such as the penile Doppler. It is very important to acquire diagnostic tools for the diagnosis of sub clinical form of endothelial dysfunction in these patients, in particular when the erectile dysfunction is associated with cardiovascular risk factors.

Keywords

Diagnostic value, endothelial progenitor cells, erectile dysfunction

History

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Introduction

The endothelial progenitor cells (EPCs) are bone marrow-derived endothelial cells with the capacity to circulate, proliferate and differentiate into mature endothelial cells [1], which represent main functional elements of postnatal vasculogenesis. Initially, the presence of EPCs was believed to be limited to the embryonic stage; however, it has since been demonstrated that their *in situ* differentiation can also occur in adults. These cells represent the product of transdifferentiation of mononuclear cells in peripheral blood; however, they are also isolated in the bone marrow and in the vessel wall [2,3].

The early detailed descriptions of EPCs [1] have established that markers that characterize these cells at the earliest stages of differentiation are also observed in hematopoietic stem cells, CD34+ cells, CD133+ cells and vascular endothelial (VE) growth factor receptor-2 or Flt-1-positve cells.

The use of EPCs has been prevalent for the diagnosis of ED; however, their low regeneration capacity from bone marrow is considered an additional risk factor, particularly in patients with comorbid cardiovascular risk factors (diabetes and hypertension). However, we have recently demonstrated an increase in serum concentrations of an original late phenotype of ECPs (CD45neg/CD34pos/CD144pos) in patients with arterial ED, most likely due to a more advanced phase of cell differentiation, compared to early phenotypes [4].

In our opinion, the use of these markers is valuable because these changes precede the structural alterations resulting from vascular damage in these patients, such as carotid atherosclerosis, which is evaluated with ultrasound [5].

EPCs have peculiar characteristics making their transplantation a promising potential treatment strategy for ischemic vascular disease. Gou et al. showed that intracavernosal injection of EPCs transfected with the *VEGF165* gene can restore erectile function in an experimental model. In particular, the authors suggest the following mechanisms to explain this result: (1) an increase in the expression of the VEGF165 protein, (2) synergy between *VEGF165* and EPCs that enhances neovascularization and (3) improved function of ECs supported by the incorporation of EPCs [6].

Recently, several groups demonstrated that reduced levels of EPCs could be reversed with the administration of phosphodiesterase-5 (PDE5) inhibitors in ED patients, showing effective vasculoprotection and prevention of the progression of endothelial damage. Another possible hypothesis for the mechanism involves the inhibition of PDE5 in bone marrow that may enhance the local effects of nitric oxide, thereby leading to the mobilization of EPCs [7].

In summary, the link between EPCs and ED improvement is both direct and indirect. It is direct because there is evidence of a possible therapeutic application [6] and it is

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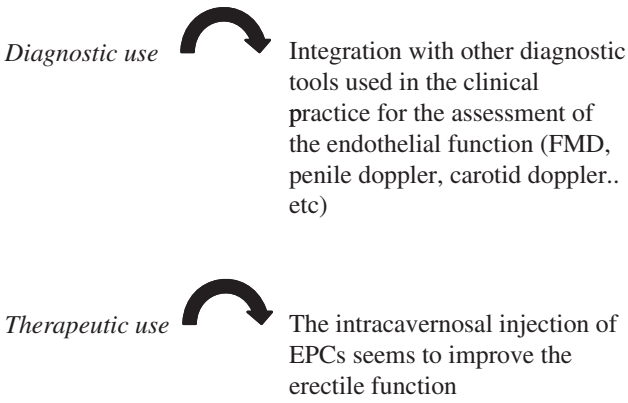

Figure 1. Endothelial progenitor cells and erectile dysfunction clinical applications.

indirect because the concentrations of circulating EPCs reflect the quality of systemic endothelial dysfunction (flow-mediated dilatation) [7] and the vascular penile parameters (dynamic penile Doppler) [4] (Figure 1).

Diagnostic use of EPCs

In patients with cardiovascular disease (CVD) risk factors, a reduction of blood circulating EPCs has been reported [8]. The study was conducted on 28 patients (35–50 years old) with ED diagnosed by means of the abbreviated version of the international index of erectile function (IIEF-5) and NPTRM. Out of 28 patients, 13 patients had cardiovascular risk factors (smoking, hypertension and hypercholesterolemia), whereas the remaining 15 patients had no cardiovascular risk factors. The control group was made up of men with a normal erectile function (IEEF-5 >21) and without cardiovascular risk factors. A sample of peripheral blood was taken from every participant and the number of circulating blood EPCs was measured by flow cytometry, using monoclonal antibodies anti-CD34 and anti-CD133. The results of this study showed that the number of EPCs in peripheral blood of patients affected by ED was significantly reduced with respected to controls and that mean EPCs values were not different between ED patients with and without cardiovascular risk factors.

The relationship between the number of blood EPCs, endothelial dysfunction and ED was further evaluated by Baumhäkel et al. [9]. To accomplish this, patients of the EPCAD study (circulating EPCs and cardiovascular morbidity and mortality in patients with coronary artery disease study) were assessed with regards to sexual and erectile function within the EROSS Programme (evaluation of role of sexual dysfunction in the saarland program). A total of 119 patients (32–86 years old) with coronary artery disease (CAD), diagnosed by angiography and classified according to the level of stenosis and the number of coronary arteries affected, were enrolled in this study. ED was documented by the KEED questionnaire, while EPCs were measured in peripheral blood withdrew from the femoral artery before catheterization. The results allowed the establish a positive relationship between hypertension, diabetes and low levels of high-density lipoproteins, while age and body mass index resulted to be independent risk factors for ED. Cigarette smoke, a well-known life habit which damages the endothelial function, did not play a significant role in this study. This is likely due to the fact that the prevalence of smokers among the participants was low. Finally, a more severe ED was not significantly associated with strokes or myocardial infarction. Furthermore, some of the drugs used for CAD treatment influence erectile function; diuretics worsen and statins improve ED. As far as EPCs, the study showed their reduction in patients with cardiovascular risk factors, heart failure or ED, suggesting that EPCs may play an important role in atherosclerosis and CAD. In patients with ED, the number of EPCs was also significantly reduced in patients without cardiovascular risk factors. This finding suggests that EPCs may be an independent risk factor of ED. In addition, EPCs may be useful from a therapeutic point of view to restore erectile function, through the regeneration of the endothelial layer of the vessels of the corpora cavernosa.

However, Fadini et al. [10] criticized Baumhäkel's study [9] from the methodological perspective since the flow cytometry description did not specify which labeling method or which antibodies were used. In addition, it was underlined that the correlation between EPCs and ED was only found with $CD133_{pos}$ cells and not with $CD134_{pos}$ or KDR_{pos} cells, to date the most widely accepted phenotype of EPCs. In replying to this critique, Baumhäkel and colleagues quoted an article [11] which states that endothelial cells, *in vitro*, are more vasculogenic when differentiated from $CD34_{neg}/CD133_{pos}$ than from $CD34_{pos}/CD133_{pos}$ cells. In addition, these authors suggested that $CD34_{neg}/CD133_{pos}$ cells are precursors of $CD34_{pos}/CD133_{pos}$ cells. This debate points out the need to standardize the definition and the method for EPCs measurements.

In a review of the literature, Traish [12], taking into account the physiology of the erection, highlighted the fundamental role played by androgens in the maintenance of the endothelial function. In particular, experimental data showed that a decreased circulating androgen levels, obtained through castration or by 5α-reductase inhibitor administration, results in vascular endothelium damage, detectable by electron microscopy and testosterone replacement therapy (TRT) restored the endothelial structural integrity. In addition, serum total and free testosterone levels correlated with flow-mediated dilatation (FMD), independently from cardiovascular risk factors, suggesting a protective effect of testosterone on the endothelium.

Finally, it is noteworthy that patients with central hypogonadism have a low number of EPCs [13] and their number increases significantly after TRT, suggesting that testosterone serum concentration is associated with EPCs reduction and that TRT cause EPCs migration and proliferation [14]. A subsequent study was carried out to evaluate the effects of androgens at cardiovascular and penile levels [15]. At vascular level, androgens promote the survival of endothelial cells, reduce the occurrence of pro-inflammatory endothelial markers and inhibit the proliferation and migration of intimal vascular smooth muscle cells. At penile level, low levels of androgens are associated with endothelial and smooth muscle cells apoptosis. In addition, low levels of androgens will impact negatively on proliferation, migration and homing of EPCs and myogenic differentiation of mesenchymal

progenitor cells. Therefore, normal androgen levels are required for vascular and penile homeostasis, mainly through mechanisms involving endothelial cells and smooth muscle cells. On the other hand, low levels of these hormones are associated with disorders of such mechanisms, leading to vessel structural remodeling abnormalities.

In 2009, a study was carried out to evaluate circulating EPCs number in overweight men with or without ED [16]. Thirty overweight patients with symptomatic ED (present for at least 6 months) and 30 age- and weight-matched men without ED were studied. Erectile function was assessed by completing the IIEF-5 questionnaire. Seven EPCs populations were determined by flow cytometry on the basis of the expression of CD34, CD133 and KDR antigens. FMD was evaluated in the right brachial artery. The results showed that $CD34_{pos}/KDR_{pos}$ EPCs were significantly lower both in men with ED and in men without ED and direct correlations between circulating cells $CD34_{pos}/KDR_{pos}$ and IIEF-5 score or FMD was reported. FMD did not correlate with other EPC phenotypes.

More recently, on the basis that patients with CAD have increased EPC-osteocalcin (OCN) positive cells and that ED is often an expression of systemic vascular disease that may precede CAD by a few years, a correlation between levels of EPCs OCN_{pos} and atherosclerotic lesion at cavernous level in patients with ED was searched [17]. The study was conducted on 35 subjects, 20 patients with ED and 15 controls, who underwent to routine clinical examinations and high-resolution penile echocolor Doppler to evaluate intima-media thickness (IMT), before and after intracavernous injection of alprostadil. The number of circulating EPCs and the fraction of EPC-OCN_{pos} cells was also determined. A progressive reduction of EPCs correlated with the severity of cavernous arteries atherosclerosis. Conversely, circulating EPC-OCN_{pos} number increased significantly with the progression of the atherosclerotic process. Therefore, the levels of EPC-OCN_{pos}, combined with the echocolor Doppler ultrasound evaluation of cavernous vessels IMT, may be predictive of future CAD in patients with ED. This latter finding was confirmed by another study [18] which reiterates that in patients with ED and cardiovascular risk factors the number of EPCs decreases significantly. The authors conclude that ED may be a clinical indicator of CVD, not only because an endothelial dysfunction is present, but also because a reduction of the vessel diameter is evident. Indeed, ED manifests itself before any other clinical evidence. In fact, the diameter of the penile arteries (1–2 mm) is smaller than that of the coronary (3–4 mm), carotid (5–7 mm) or femoral (6–8 mm) arteries. Therefore, an atherosclerotic plaque of size sufficient to block the blood flow through the penile arteries would only produce a stenosis of 30–40% in the major arteries. Consequently, all patients above 25 years of age with ED should be carefully evaluated for cardiovascular risk. It is proven that the intervention on risk factors has itself a beneficial effect on sexual function. Finally, it is believed that chronic exposure to PDE5 inhibitors can improve endothelial function. Therefore, PDE5 inhibitors may probably prevent potentially fatal acute cardiovascular events. In particular, Foresta and colleagues showed that treatment with Tadalafil is associated with a significant increase in EPCs and FMD with respect to basal level, moreover the authors showed a positive correlation between basal FMD and EPCs and between basal FMD and EPCs increase after Tadalafil treatment [19]. In another study, the same authors evaluated the effect of a single dose of vardenafil 20 mg on the number of these cells in men with ED and various degree of vascular injury at the carotid artery level, showing that 4 h after vardenafil administration was observed an increase in the number of EPCs in all patients and controls [7].

A synthesis of our experience about this topic

The immunophenotype of the EPCs published by our group [20] is characterized by the simultaneous expression of monoclonal antibodies CD34 and CD144 on cells negative for CD45. CD34 is an antigen that is expressed in all lines of hematopoietic progenitor cells and is gradually lost when the progenitors differentiate into mature endothelial cells [20,21]. In addition, CD144, or VE-cadherin, is specifically localized in the cell junctions between endothelial cells [20,21] and appears to be involved in maintaining endothelial permeability, as the monolayer of cells transfected shown a reduced permeability to calcium-dependent. Finally, CD45 is expressed on the surface of all leukocytes and human EPCs do not express this antigen [20,21].

The phenotypic variation, detected by flow cytometry, during culture of EPCs showed that CD144 is acquired later, it is almost absent between the 5th and the 8th day of culture and is over-expressed between 30th and 32th day of culture, together with CD34 and CD31 [20,21]. These observations suggest that the phenotype CD45neg/CD34pos/CD144pos represents a group of late differentiation of EPCs, moreover no data have been reported on this phenotype of EPCs in patients with ED.

In the our studies the serum concentrations of this phenotype of EPCs were significantly higher in presence of classical cardiovascular risk factors, such as metabolic syndrome [22,23], hypogonadism [20,21], atherosclerosis [5] with a decrease after pharmacological intervention with androgens [21], PDE5 inhibitors [23] and physical activity [24] showing therefore a different kinetic from traditional phenotype.

This apparent discrepancy may be explained by the different phenotype that expresses a different functional state of the mechanism of endothelial repair. About it, recently has been shown the increased serum concentrations of EPCs in patients with chronic CAD [4,20–22].

In our opinion, the phenotype that we used has the following advantages:

- Easy to acquire in terms of methodology (sampling of peripheral blood).
- It examine a dynamic phase of the endothelial response. In fact in our studies is always associated with the increase of serum concentrations of endothelial microparticles (markers apoptosis of endothelial) where the increase of their serum concentration represents the stimulus for the final stage of EPCs differentiation.
- It examine a late differentiative phase of EPCs (generally poorly evaluated).

- This phenotype is potentially usable in clinical practice. In fact, in our study we evaluated their diagnostic accuracy and the use associated with penile Doppler in the evaluation of patients with ED [25,26].

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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