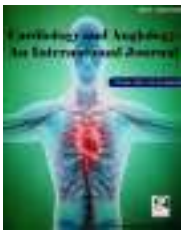

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Endothelial Progenitor Cells in Coronary Artery Disease: The 5-Year Experience at a Single Center

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Authors' contributions

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Review Article

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ABSTRACT

Endothelial progenitor cells (EPCs) are a heterologous population of bone marrow-derived cells that play a key role in maintaining homeostasis of the endothelium, as they home to areas of endothelial injury, replace damaged endothelium, and participate in neovascularisation. The relationship between EPCs number and the severity of atherosclerosis is still a matter of debate. Abnormalities in EPCs have been associated with coronary artery disease, as experimental investigations have shown that a decrease in the endogenous pool of EPCs may accelerate the course of atherosclerosis, and the number of EPCs has been reported to be reduced in patients with atherosclerosis and in apparently healthy subjects without overt disease. On the opposite, other studies have found that the number of EPCs in the blood is increased in patients with angiographically significant coronary artery disease. The potential exists that EPCs constitute a therapeutic target, because persistent stimulation of EPCs by pharmacological intervention may, at least theoretically, repair endothelial injury and prevent the progression of atherosclerosis in patients at risk. Indeed, experimental and clinical studies have revealed that the number of EPCs can be increased by several pharmacological interventions such as

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hormones, statins, recombinant human EPO, and blockage of the angiotensin converting enzyme system. This review addresses the clinical correlates and prognostic significance of EPCs in a large cohort of patients with coronary artery disease that has been evaluated at a single Academic center in Italy.

Keywords: Endothelial progenitor cells; atherosclerosis; coronary artery disease; drugs.

1. INTRODUCTION

Maintenance of endothelial integrity and functioning is vital to the preservation of a healthy vasculature [1], and the inability of the endothelial cell monolayer to recover is believed to be a critical factor during the initiaton and progression of atherosclerosis [2]. Indeed, endothelial damage/dysfunction has been proved to be involved in the pathogenesis of atherothrombotic vascular disease, with important prognostic and therapeutic implications [3-5].

Research on stem cells has identified a population of bone marrow–derived cells, called circulating endothelial progenitor cells (EPCs) that incorporate into sites of neovascularization and are home to sites of endothelial denudation thus contributing to the maintenance of vascular homeostasis [6]. Although extensive work has been conducted to verify if EPCs impairment plays a key role in coronary atherogenesis, it is still matter of debate if the extension and severity of coronary artery disease are associated with reduced or increased numbers of EPCs [7,8], as it remains unclear if these cells exert favorable or unfavorable effects at sites of percutaneous coronary intervention (PCI) [9].

Most investigations have been hampered by discordant definitions of EPCs and by different timing of EPCs sampling, thus determining much uncertainty on the role of EPCs in atherosclerosis progression and cardiac diseases [9]. Nowadays, there is general agreement that EPCs are immature BM-derived cells that must be defined and enumerated by flow cytometry using sequential gating strategies that conform to International Society of Hematotherapy and Graft Engineering criteria issued in 1996 [10]. Accordingly, cells are first gated on the CD45 area and then gated to identify the coexpression of stemness antigens and endothelial markers. In brief, circulating EPCs are depicted by the lack of expression of CD45 (i.e., a leukocyte/monocyte antigen), and by the simultaneous expression of KDR (i.e., a marker of endothelial lineage) and CD34 (i.e., a hematopoietic stem cell marker that is also expressed on mature microvascular endothelial cells) or CD133 (i.e., a more immature hematopoietic stem cell marker that is absent on mature endothelial cells and monocytic cells. Also, analytical gates are used to enumerate subsets of circulating CD45–/CD34– cells that express the antigen CD105 (i.e. CD105+/CD45-/CD34– cells). Finally, circulating CD45+ leukocytes that express the monocytic marker CD14 can be assessed [6]. These cells are derived from monocytes/macrophages and cannot be considered early EPCs [6]. They are positive for both the leukocyte antigens CD45 and CD14 but are negative for the antigens CD34 and CD133. Noteworthy, they have no proliferation potential but play an important role in angiogenesis and arteriogenesis via a paracrine effect [9].

This review addresses the clinical correlates and prognostic significance of the different subtypes of EPCs in a large cohort of patients with coronary artery disease that has been evaluated at a single Academic center in Italy over a 5-year period.

2. EPC AND AGEING

Physiological factors and conventional risk factors for atherosclerosis are associated with variations of the number and activity of endothelial progenitors and may be the bridge linking EPCs to common cardiovascular disorders such as coronary artery disease (CAD), myocardial infarction and heart failure.

There is an age-related quantitative decline in bone marrow cells expressing endothelial progenitor markers. Jie et al. [11] analyzed the number of circulating CD34+/KDR+ EPCs in healthy subjects aged from 1 to 81 years old, and an inverse relationship with age was observed. The progressive reduction in different progenitor cell populations with age was also confirmed by Schaffer et al. [12], both in healthy donors and in patients with peripheral arterial disease.

These earlier findings have not been confirmed by subsequent studies. Pelliccia et al. [12] aimed at verifying whether the numbers of subpopulation of EPCs in peripheral blood are different in older and younger patients with coronary artery disease. They studied a consecutive series of 30 patients with coronary artery disease aged 65 to 82 years and 30 matched patients aged 41 to 64 years. Flow cytometric analsysis was used for stem cells assay and absolute number of CD34+/KDR+/CD45− cells, CD133+/KDR+/CD45− cells, CD105+/CD45-/CD34– cells, and CD14+/CD45+ cells were measured. Older and younger patients were similar with regard to age, sex, cardiovascular risk factors, clinical presentation, left ventricular function, renal function, and medical therapy at time of coronary angiography. No differences were noted among groups both in the significant and not significant lesions, as coronary anatomy, number of diseased vessels, number of significant coronary stenoses, lesion types, and stenoses characteristics were similar among the two groups. Specifically, there were no significant differences in lesion severity or lesion location among older and younger patients. Hematologic analysis showed that the two groups had similar white cell count, and mononuclear cells. Also, the absolute numbers of CD34+/KDR+/CD45− cells, CD133+/KDR+/CD45− cells, CD105+/CD45-/CD34– cells, and CD14+/CD45+ cells in older patients were not different from those recorded in younger patients and in the control group. Pelliccia et al. [13] concluded that subpopulations of circulating EPCs are not different between older and younger patients with coronary artery disease. Contrary to the current but unproven belief, aging “per se” is not associated with reductions in EPCs in ischemic patients.

3. EPC AND GENDER

CAD is characterized by gender-specific differences with respect to its development, course, and prognosis due to mechanisms that remain not fully elucidated. Recently, interest in circulating EPCs as a possible emerging vascular risk factor has arised, probably because their importance for endothelial function and atherosclerotic disease progression. It has been speculated that circulating EPCs are protective, and may account for the slower progression to endothelial dysfunction and atherosclerosis of postmenopausal women. This hypothesis, however, remains largely unproven, as limited information is available about the normal range of EPCs in women and men. Furthermore, no previous investigation has assessed whether numbers of EPCs are different between women and men with CAD, or between postmenopausal women with or without CAD.

Pelliccia et al [14] designed a study to test the hypothesis that CAD in women is associated with changes in concentrations of circulating EPCs cell subsets, i.e. those involved directly or indirectly in vascular repair. Accordingly, they compared subpopulations of EPCs among postmenopausal normal women, female patients with CAD, and age-matched men. Overall, the authors studied 71 consecutive middle-aged patients with stable CAD (30 postmenopausal women and 41 men), and 40 middle-aged normal controls (20 postmenopausal women and 20 men). Blood samples were drawn at time of coronary angiography and subpopulations of EPCs were measured by flow cytometry. Women and men with CAD had similar age, risk factors, clinical presentation, left ventricular function, extension of CAD, and medical therapy at time of coronary angiography. Hematologic analysis showed that men and women with CAD had similar white cell count, mononuclear cells, and subpopulations of EPCs. Postmenopausal normal women, conversely, had significantly higher absolute numbers of CD34+/KDR+/CD45− cells, CD133+/KDR+/CD45− cells, CD105+/CD45-/CD34– cells, and CD14+/CD45+ cells than other groups. Pelliccia et al [14] concluded that increased numbers of subpopulations of EPCs in normal postmenopausal women might contribute to the gender-specific difference of CAD in the middle-age. Lack of difference in EPCs between women and men with CAD suggests that stem cells become unable to play a protective role when the disease is clinically evident.

4. EPC AND HEART FAILURE

The link between EPCs and left ventricular (LV) dysfunction in patients with CAD, however, remains unclear, as earlier investigations have yielded opposite results. Possible explanations include the fact that many studies have been hampered by the lack of uniform criteria to precisely identify EPCs, and no earlier study has evaluated different subpopulations of EPCs in patients with CAD and heart failure.

Biphasic changes of the number of CD34+ cells and EPCs have been observed in patients with heart failure. EPCs are significantly up-regulated in mild heart failure (NYHA class I-II) but their mobilization is severely depressed in patients with advanced heart failure (NYHA III-IV) irrespectively of the origin of the disease [15]. Depletion in EPCs has been correlated with high levels of TNF-α indicating that endothelial precursors arem additional 'victim' of excessive inflammation seen in heart failure [16]. These data are in accordance with observations in animal model that statin-induced prevention of left ventricular dysfunction was strongly associated the ability of statins to mobilize EPCs [17].

The proportion of CD34+ cells in hospitalized patients with congestive heart failure increases with the improvement of clinical status and is correlated with brain natriuretic peptide levels. Of note, Michowitz et al have recently demonstrated that the CFU-EC numbers, together with age and presence of diabetes were independent predictor of all-cause mortality in 107 patients with congestive heart failure [18].

Pelliccia et al. [19] performed a pilot study to verify the hypothesis that CAD patients with or without LV dysfunction have different number of the most common subpopulations of EPCs in peripheral blood. Sixty-eight consecutive patients (37 men, age 60±18 years) with CAD were studied. All patients underwent quantitative coronary angiography and flow cytometric analysis. Patients with LV ejection fraction <45% (n= 22) were compared with those with normal function (n=46). The two groups had similar age, sex, cardiovascular risk factors, medical therapy, LV dimension, and number of diseased vessels. Patients with LV dysfunction, by study design, were more symptomatic and had a lower LV ejection fraction. The two groups had similar white cell count and mononuclear cells. The absolute number of

CD34+/KDR+/CD45− cells and CD133+/KDR+/CD45− cells was significantly (P<0.05) higher in patients with LV dysfunction as compared with patients with normal function or healthy participants. In contrast, CD14+/CD45+ cells were significantly (P=0.005) lower in the former patients than in the latter, whereas no significant difference was noted in the number of CD105+/CD45-/CD34– cells among groups. Pelliccia et al concluded that cells positive for the endothelial markers CD34 and CD133 are increased and cells that promote vasculogenesis and microvascular development are significantly reduced in CAD patients with LV dysfunction.

5. EPC AND ATHEROSCLEROTIC DISEASE PROGRESSION

Reduced levels of circulating EPCs have been shown to be independent predictors of atherosclerotic disease progression. Indeed, endothelial integrity is a fine balance between endothelial damage and repair. Since atherosclerotic risk factors are associated with reduced numbers and function of circulating EPCs, it is possible that progression of atherosclerosis is 'driven' by an impairment of EPC repairing capacity [11].

In addition, disease processes that 'damage' the endothelium per se lead to endothelial cell detachment, resulting in increased levels of circulating endothelial cells (CECs) in the blood [20]. CECs are thought to be mature cells that have detached from the intimal monolayer in response to endothelial injury and are a different cell population to EPCs. There is increasing evidence to support a relation between endothelial damage/dysfunction and CECs. An inverse relationship has been found between EPCs and CECs, as elevated numbers of CECs have been seen in patients with CAD whereas a reduction in the number of circulating EPCs has been associated with CAD.

Pelliccia et al. [9] prospectively investigated the relationship of circulating endothelial progenitor cells at time of PCI to the subsequent development of in-stent restenosis or progression of coronary atherosclerosis. They studied 155 consecutive stable angina patients (92 men, age 60±11 years). All patients had flow cytometry the day before elective percutaneous coronary intervention in order to derive subpopulations of endothelial progenitor cells. A control group of 20 normal subjects was considered for comparison. At 8-month control angiography, 30 patients showed in-stent restenosis (restenosis group), 22 patients showed progression of coronary atherosclerosis (progression group), whereas the remaining 103 patients had neither in-stent restenosis nor progression of coronary atherosclerosis (stable group). Comparison of the 3 groups did not show any difference in risk factors, cardiac morphology and function, extension of coronary artery disease, and treatment. Absolute numbers of CD34/KDR/CD45– cells measured in the restenosis group were significantly higher than in the progression, stable, and control groups. Similarly, CD133/KDR/CD45– cells (i.e., progenitors of endothelial cells at an earlier stage) were significantly higher in the restenosis compared with progression, stable, and control groups. Also, numbers of CD14+/CD45+ cells (i.e., which have a role in angiogenesis via a paracrine effect) were significantly different among the restenosis, progression, stable, and control groups, whereas CD105+/CD45-/CD34– cells (i.e., which have a receptor for transforming growth factor-beta) were similar among groups. In conclusion, patients with restenosis have higher numbers of subpopulations of endothelial progenitor cells that incorporate into endothelial cells or play a role in arteriogenesis compared with controls and patients with either progression of coronary atherosclerosis or stable disease.

6. EPC AND RENIN ANGIOTENSIN SYSTEM

Angiotensin II subtype 1-receptor blockade increases the number of EPCs, an effect which seems to be common to all angiotensin II receptor antagonists [21,22]. Also, vascular endothelial growth factor appears to be involved in angiotensin receptor blocker-mediated EPC stimulation.

Min et al showed that treatment with ramipril 5 mg daily for 4 weeks in patients with CAD was associated with an approximately 1.5-fold increase in the number of circulating EPCs within 1 week of initiating treatment [23]. This trend persisted with increased levels to approximately 2.5-fold throughout the 4-week study period. In addition, increases in the functional activity of EPCs - as assessed by their proliferation, migration, adhesion and in vitro vasculogenesis capacity - was also seen. Broadly similar effects were also seen with enalapril, as shown in a study by Wang et al, whereby patients on enalapril displayed a significant increase in circulating EPCs in response to ischemic stress [24]. Enalapril also caused a 6-fold increase in the contribution of bone marrow-derived EPCs to the ischemia-induced neovascularization.

Angiotensin II accelerates the onset of EPC senescence, leading to impaired proliferation of EPCs; this seems to be inhibited by treatment with the angiotensin II type 1 receptor blocker, valsartan [24]. Ramipril also improves the proliferation and migration of EPCs, as well as *in vitro* vasculogenesis in patients with CAD [23].

These observations were confirmed in the Endothelial Progenitor Cells in Coronary Artery Disease (EPCAD) study, demonstrating that angiotensin-converting enzyme inhibitor treatment was associated with increased numbers and improved clonogenic potential of circulating EPCs, when compared with patients who were not taking angiotensin-converting enzyme inhibitors [22].

Pelliccia et al. [24] have recently performed a study to evaluate whether telmisartan, an angiotensin II receptor antagonist, can modify the number of subpopulations of EPCs and may in turn affect the endothelial function of normotensive patients with CAD. In a prospective double-blind parallel group study, 40 normotensive patients with CAD were randomly treated with telmisartan (80 mg) or placebo for 4 weeks at time of coronary angiography. Measurements of EPCs and assessment of flow-mediated dilatation of the brachial artery was performed before and after therapy. Absolute number of EPCs was similar at baseline in the telmisartan and placebo groups. After 4 weeks treatment, CD34+/KDR+/CD45− cells increased significantly in the telmisartan group but not in the placebo group. Similarly, CD133+/KDR+/CD45− cells raised significantly with telmisartan but not with placebo. Also, CD14+/CD45+ cells increased significantly with telmisartan and were unchanged with placebo: flow-mediated dilatation improved significantly in patients who received telmisartan but did not change in the placebo group. A significant positive correlation was found in the telmisartan group between the improvement in flow-mediated dilatation and the increase in CD34+/KDR+/CD45− cells and CD133+/KDR+/CD45− cells ($r=0.55$, $p<0.01$, and $r=0.49$, $p<0.05$, respectively). Based on these results, Pelliccia et al concluded that angiotensin II receptor antagonism with telmisartan increases the number of regenerative EPCs and improves endothelial function in normotensive patients with CAD. These novel effects are interrelated and can explain, at least in part, why telmisartan has beneficial cardiovascular effects independent of its blood pressure lowering action.

7. CONCLUSION

Since the discovery of EPCs, there has been a rapid proliferation of research data on the relation of EPC to cardiovascular risk, pathology and treatment [25]. So far, EPCs have been implicated in the whole cardiovascular disease process, and many conventional therapies have been shown to alter EPC number and function. More recently, attempts to utilize the clinical potential of EPCs such as in the form of CD34-antibody coated stents, has been attempted. Further challenges will be to develop simple techniques to measure EPCs numbers and function accurately and quickly, as these cells may help determine cardiac risk and outcomes for patients with heart disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dong C, Goldschmidt-Clermont PJ. Endothelial progenitor cells: a promising therapeutic alternative for cardiovascular disease. J Interv Cardiol. 2007;20:93-9.
2. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340:115-25.
3. Padfield GJ, Tura-Ceide O, Freyer E, Barclay GR, Turner M, Newby DE, Mills NL. Endothelial progenitor cells, atheroma burden and clinical outcome in patients with coronary artery disease. Heart. 2013 Feb 6.
4. Moon JH, Chae MK, Kim KJ, Kim HM, Cha BS, Lee HC, et al. Decreased endothelial progenitor cells and increased serum glycated albumin are independently correlated with plaque-forming carotid artery atherosclerosis in type 2 diabetes patients without documented ischemic disease. Circ J. 2012;76:2273-9
5. França CN, Pinheiro LF, Izar MC, Brunialti MK, Salomão R, Bianco HT, et al. Endothelial progenitor cell mobilization and platelet microparticle release are influenced by clopidogrel plasma levels in stable coronary artery disease. Circ J. 2012;76:729-36.
6. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res. 1999;85:221-8.
7. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al. Number and migratory capacity of circulating Endothelial Progenitor Cells inversely correlate with risk factors for coronary artery disease. Circ Res. 2001;89:1-7.
8. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med. 2003;348:593-600.
9. Pelliccia F, Cianfrocca C, Rosano G, Mercuro G, Speciale G, Pasceri V. Role of endothelial progenitor cells in restenosis and progression of coronary atherosclerosis after percutaneous coronary intervention: a prospective study. JACC Cardiovasc Interv. 2010;3:78-86.
10. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry, International Society of Hematotherapy and Graft Engineering. J Hematother. 1996;5:213–6.
11. Jie KE, Goossens MH, van Oostrom O, Lilien MR, Verhaar MC. Circulating endothelial progenitor cell levels are higher during childhood than in adult life. Atherosclerosis. 2009;202:345-7.

12. Schaffer RG, Greene S, Arshi A, Supple G, Bantly A, Moore JS, et al. Effect of acute exercise on endothelial progenitor cells in patients with peripheral arterial disease. Vasc Med. 2006;11:219-26.
13. Pelliccia F, Pasceri V, Meoni G, Pristipino C, Cianfrocca C, Li X, et al. Numbers of endothelial progenitor cells in peripheral blood are similar in younger and older patients with coronary artery disease. Int J Cardiol. 2009;133:277-9.
14. Pelliccia F, Pasceri V, Cianfrocca C, Vitale C, Meoni G, Pristipino C, et al. Circulating endothelial progenitor cells in postmenopausal women with and without coronary artery disease. Climacteric. 2009;12:259-65.
15. 14-Valgimigli M, Rigolin GM, Fucili A, Porta MD, Soukhomovskaia O, Malagutti P, et al. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. Circulation. 2004;110:1209-12.
16. Nonaka-Sarukawa M, Yamamoto K, Aoki H, Nishimura Y, Tomizawa H, Ichida M, et al. Circulating endothelial progenitor cells in congestive heart failure. Int J Cardiol. 2007;119:344-8.
17. Sethi R, Lee CH. Endothelial progenitor cell capture stent: safety and effectiveness. J Interv Cardiol. 2012;25:493-500.
18. Michowitz Y, Goldstein E, Wexler D, Sheps D, Keren G, George J. Circulating endothelial progenitor cells and clinical outcome in patients with congestive heart failure. Heart. 2007;93:1046-50.
19. Pelliccia F, Pasceri V, Cianfrocca C, Vitale C, Pristipino C, G. Speciale G, et al. Endothelial progenitor cells in patients with coronary artery disease and left ventricular dysfunction. Coron Artery Dis. 2009;20:303-8.
20. 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. Circulation. 2006;114:I114-9.
21. M, Sevá Pessôa B, Roks AJ. Clin Sci (Lond). The renin-angiotensin system, bone marrow and progenitor cells. Durik. 2012;123(4):205-23.
22. Thum T, Tsikas D, Stein S, Schultheiss M, Eigenthaler M, Anker SD, et al. Suppression of Endothelial Progenitor Cells in Human Coronary Artery Disease by the Endogenous Nitric Oxide Synthase Inhibitor Asymmetric Dimethylarginine. J Am Coll Cardiol. 2005;46:1693-701.
23. Min TQ, Zhu CJ, Xiang WX, Hui ZJ, Peng SY. Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. Cardiovasc Drugs Ther. 2004;18:203-9.
24. Pelliccia F, Pasceri V, Cianfrocca C, Vitale C, Speciale G, Gaudio C, et al. Angiotensin II receptor antagonism with telmisartan increases number of endothelial progenitor cells in normotensive patients with coronary artery disease: A randomized, double-blind, placebo-controlled study. Atherosclerosis. 2010;210;510-5.
25. Hoetzer GL, Van Guilder Gary GP, Irmiger HM, Keith RS. Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. J Appl Physiol. 2007;102:847-52.

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