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Endothelial progenitor cells in cardiovascular disease and chronic inflammation: from biomarker to therapeutic agent

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Abstract

The discovery of endothelial progenitor cells in the 1990s challenged the paradigm of angiogenesis by showing that cells derived from hematopoietic stem cells are capable of forming new blood vessels even in the absence of a pre-existing vessel network, a process termed vasculogenesis. Since then, the majority of studies in the field have found a strong association between circulating endothelial progenitor cells and cardiovascular risk. Several studies have also reported that inflammation influences the mobilization and differentiation of endothelial progenitor cells. In this review, we discuss the emerging role of endothelial progenitor cells as biomarkers of cardiovascular disease as well as the interplay between inflammation and endothelial progenitor cell biology. We will also review the challenges in the field of endothelial progenitor cell-based therapy.

Keywords

biomarker; cardiovascular disease; endothelial progenitor cell; inflammation; vasculogenesis

Angiogenesis, the creation of new blood vessels, has attracted tremendous interest in the field of cardiology and cancer biology in the last decades. However, angiogenesis has always been a double-edged sword; in some diseases, such as coronary artery disease, it may benefit patients by increasing oxygen supply, whereas in others, such as cancer and inflammatory diseases, angiogenesis may lead to further disease progression and tumor growth. It was long believed that angiogenesis, the formation of new blood vessels in humans, could only occur by the new blood vessels sprouting out of pre-existing vessels [1]. This paradigm changed in the mid-1990s, when researchers detected endothelial cells of hematopoietic origin on the surface of left ventricular assist devices [2]. Earlier studies had also described endothelial-like cells within the peripheral blood stream [3]. In 1997, Asahara and colleagues described the so-called endothelial progenitor cells (EPCs) isolated from the circulation. EPCs are also capable of forming new blood vessels [4], mainly after their recruitment and migration into the ischemic tissue, a process later termed ‘vasculogenesis’.

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In contrast to angiogenesis, during vasculogenesis the formation of new blood vessels can occur in the absence of pre-existing blood vessels.

Since EPCs do not need pre-existing blood vessels to work, interest in EPC biology has been growing continuously since their discovery. EPCs are now regarded as biomarkers in cardiovascular disease and also as a potential therapeutic tool or target. In this article, we discuss the emerging role of EPCs as biomarkers of cardiovascular disease as well as the interplay between inflammation and EPC biology. We will also review the challenges in the field of EPC-based therapy.

Methodology

We conducted a search in PubMed® using the following search terms: endothelial progenitor (precursor) cells, coronary artery disease, myocardial infarction, stroke, heart failure, pulmonary hypertension, allograft vasculopathy and chronic inflammatory diseases. We selected articles with more than ten patients that analyzed the association or relationship between outcome and EPCs. Basic science papers on EPC biology were also reviewed. Furthermore, we screened and analyzed different reviews on the topic. However, because studies often referred to different characterization and quantification techniques for EPCs, the observations may not always be directly comparable.

Definition & characterization of EPCs

The exact definition and characterization of EPCs is still an ongoing and unresolved issue, although researchers have been trying to standardize the definitions of EPCs [5–8]. When quantifying and analyzing EPCs by flow cytometry, staining for the expression of CD34 is essential. Nevertheless, there is no clear consensus on what other markers should be mandatory. Other surface molecules, mainly CD45, have been added to the panel by some investigators to characterize EPCs [9] and may increase the specificity for EPCs. Different technical protocols have also been recommended to stain EPCs; some recommend staining for EPCs directly from full blood after erythrocyte lysis, whereas others use peripheral blood mononuclear cells after density centrifugation, but the latter was recently associated with a loss of EPCs [10].

The majority of outcome studies have quantified absolute or relative numbers of circulating EPCs within the peripheral blood. In these studies, the number of EPCs has been either expressed as an absolute number, or as a percentage of positive cells per certain volume or per acquired cells. Assays that assess and quantify the outgrowth of colonies have also emerged. It is essential to differentiate among three of the most commonly used assays – the so called ‘early outgrowth assay’ [11,12], the related ‘Hill assay’ [13], and the ‘late outgrowth assay’ [14] – because it is now evident that the first two and the latter exhibit two different cell types. In the early outgrowth assay and the Hill assay, cells staining positive for acetylated low-density lipoprotein and *Ulex europaeus* [12], or forming specific colonies [13] are of hematopoietic and monocytic origin and express CD31, different endothelial markers and von Willebrand factor-secreting proangiogenic factors [15–17]. In the late outgrowth assay, cells are cultivated for a longer time period of 14–21 days, do not express CD45, but do exhibit a clonal proliferative potential [14,18,19].

In addition to the EPC quantities and the potential to form colonies, the characterization of their functional capacities also include proliferation, migration, adhesion and *in vitro* vasculogenic capacity that can be assessed in various ways using distinct readout systems. The definition of EPCs has also undergone constant changes over the last years. Initially, EPCs were usually classified as a subtype of CD34⁺ hematopoietic stem and progenitor cells and characterized by the coexpression of CD133, also called prominin 1 [20]. In various

studies, EPCs were also characterized by the coexpression of KDR-1, also known as VEGF-R2. Later, accumulating evidence showed that a ‘true’ EPC, which by definition represents a cell that can differentiate into an endothelial cell, is not of hematopoietic origin, whereas the other so-called EPCs were linked to the mononuclear lineage, secreting proangiogenic factors and were referred to as EPCs due to their ability to form colonies (see above). Currently, there is evidence to suggest that ‘true’ EPCs are not of hematopoietic origin, but rather of an already differentiated endothelial origin with clonal proliferative potential, and therefore the term ‘endothelial colony forming cell’ (ECFC) has emerged [14,17,21]. Recently, Richardson and Yoder proposed to subdivide EPCs into mainly two groups, the proangiogenic hematopoietic cell and ECFCs [22].

These findings of distinct and different cell types also led to modifications in the flow cytometry staining protocols. Recently, staining for CD45^{dim} (the hematopoietic cell marker), CD34⁺ and KDR⁺ cells was recommended as a modified International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol, although there is still no clear consensus about a definite panel [9]. In another recent approach, both CD45 and KDR were excluded due to difficulties with titration [23]. Moreover, the scaling involved in gating for cells was considered to be very important to detect ECFCs. The constantly evolving flow cytometry staining and gating protocols, of course, are additional factors that complicate comparisons with older studies.

Taken together, both historically named EPCs, which were mostly of hematopoietic origin and the ‘new’ EPCs (now increasingly known as ECFCs) exhibit a potential to form, or at least increase and support the formation of, new vessels. A major problem for researchers is the lack of a unique marker for EPCs, leaving the field in limbo and complicating precise comparisons among different studies. This is, therefore, an important caveat to mention when reviewing the literature, because not all the papers refer to the same cell type. In this review, we will use the term EPCs as it was used in the studies that are referenced. Tables 1–4 highlight important studies and also refer to the methods used for EPC quantification.

EPCs as potential biomarkers of cardiovascular & inflammatory diseases

The value of EPCs as biomarkers for disease severity, prognosis and response to therapy has been the focus of several investigations. In patients at an increased risk of cardiovascular disease, including those with comorbid conditions such as diabetes mellitus [24,25], systemic hypertension [26,27] and chronic kidney disease [28–30], the peripheral EPC number is reduced while EPC function is often impaired. The reduction in EPC quantities is also observed in patients with established coronary artery disease [31] and stroke [32]. By contrast, an increased number of EPCs is often observed in patients who present with an acute coronary syndrome such as acute myocardial infarction [33] or unstable angina [34,35], suggesting the mobilization of EPCs during acute ischemic events (Figure 1).

Heart failure has been associated with increased EPC counts in the earlier, less severe stages of the disease, but EPC numbers tend to decrease with further disease progression [36]. In pulmonary hypertension, some studies have shown a decrease in EPCs [37–39], while others report normal levels or an increase [40,41]. The studies on EPCs and transplant allograft vasculopathy thus far have small sample sizes that limit the generalizability of their results [42–44]. Tables 1–4 summarize some of the important studies relating to EPCs and outcomes in distinct diseases. In this section, we expand on each of the disease entities.

EPCs & atherosclerosis

In their landmark study, Hill *et al.* reported that a lower number of circulating EPCs was associated with a higher Framingham risk score and endothelial dysfunction as measured by

flow-mediated brachial artery reactivity [13]. The relationship between EPCs and cardiovascular outcome was later confirmed by Werner and colleagues, who demonstrated that patients with coronary artery disease and a low baseline number of EPCs experience a higher likelihood of death from cardiovascular causes (Table 1 & 2) [31]. Moreover, EPC levels independently predict atherosclerotic disease progression even after adjustment for traditional cardiovascular risk factors [45]. Aging was also associated with a decline in the number of circulating EPCs [46].

In animal models, EPCs were observed to restore carotid injury [47,48] by promoting differentiation into endothelial cells. In another study, however, ApoE^{-/-} mice infused with EPCs were found to have increased plaque size and decreased plaque stability, hence contributing to neointimal hyperplasia [49]. In another observation, hematopoietic stem and progenitor cells, which are considered to be the ancestors of EPCs, were observed to participate in the development of atherosclerosis [50] and have been detected in the atherosclerotic plaque [51]. However, recently, a Danish group analyzed transplanted EPCs within the endothelium of atherosclerotic plaques in a complex mouse model and determined that EPCs do not take part in this process [52].

Since these data provide conflicting evidence regarding the exact role of EPCs, especially in the early stages of atherosclerosis, therapeutic protocols that involve EPC mobilization with the goal of increasing vasculogenesis in ischemic tissues have to be carefully evaluated.

EPCs as a biomarker in diabetes mellitus

Peripheral levels of EPCs have been found to be significantly reduced and associated with complications of diabetes mellitus [53]. The macrovasculopathy as assessed by ankle brachial index or carotid narrowing was reportedly associated with reduced peripheral levels of EPCs [54,55], and EPC levels were also found to be reduced in Type 2 diabetes retinopathy patients [56]. Moreover, EPC counts were negatively correlated with the albumin excretion rates in Type 2 diabetes patients [57]. A recent study performed in 425 patients revealed that the reduction of EPCs can already be observed at the onset of Type 2 diabetes, but a further reduction is seen with longer lasting disease [58]. Taken together, there is tremendous evidence that circulating EPCs are impaired in diabetes and that the reduction of EPCs is also negatively associated with various complications in diabetes [53]. The correlations of peripheral EPC levels with clinical characteristics in both inflammatory diseases and Type 2 diabetes would make serial measurements of EPCs an ideal candidate, possibly for both monitoring the treatment of the disease and measuring the cardiovascular risk.

EPCs as a biomarker in heart failure & pulmonary arterial hypertension

In patients with heart failure, several studies have shown that in mildly symptomatic heart failure (New York Heart Association [NYHA] functional class I), the levels of EPCs are increased compared with matched controls and then decline progressively with increasing severity of heart failure [36]. In a small outcome study, the peripheral quantities of EPCs were an independent predictor of survival along with age and diabetes mellitus in heart failure [59]. In this study, however, diastolic parameters of heart function and pulmonary hypertension were not considered as covariates in the multivariate models. Interestingly, exercise was observed to be associated with an increase of peripheral EPCs in patients with NYHA class III heart failure [60].

EPCs were also studied in patients with pulmonary arterial hypertension, with conflicting results. Whereas some studies reported depleted peripheral EPC levels [37–39], other studies noted increased EPC levels [40,41]. EPCs have further been shown to take part in the

vascular remodeling in pulmonary arterial hypertension [61]. These different findings may be related to discrepancies regarding the methods of characterization and quantification of EPCs as well as assessment of patients with different etiologies or stages of pulmonary arterial hypertension [62].

EPCs in rheumatic & chronic inflammatory conditions

In many inflammatory rheumatic diseases, decreased peripheral levels and functionally altered EPCs have been described. Rheumatoid arthritis (RA) is the most common inflammatory joint disease that can lead to joint destruction and disability if insufficiently treated, and it is characterized by an increased cardiovascular mortality and morbidity [63] even after adjustment for traditional risk factors [64]. The increased cardiovascular risk is also true for other chronic inflammatory conditions [65,66], such as systemic lupus erythematosus (SLE), which exhibits up to a 50-fold increase in cardiovascular mortality [67,68].

In RA, the levels of peripheral EPCs are decreased compared with matched healthy controls [69–71], and an inverse relationship has been reported between peripheral EPC levels and disease activity as measured by the disease activity score 28 (DAS-28) [69,72]. EPCs were also observed to accumulate in the inflamed joints [73,74], where an increased blood vessel supply is needed, giving rise to the hypothesis that peripheral EPCs are trapped within the highly vascularized inflamed joints [75]. The trapped EPCs are suspected to contribute to disease progression by helping to maintain the inflammatory process via vasculogenesis, further facilitating the influx of immune cells [75].

SLE, a potentially life-threatening disease that can affect multiple organs, was found to be associated with depleted [76–80] or normal [81,82] peripheral EPC levels. Furthermore, SLE is also associated with impaired EPC differentiation into endothelial cells, depleted adhesion and migration capacity, as well as an increased susceptibility to apoptosis and a reduction of angiogenic growth factors [80,82].

Systemic sclerosis (or scleroderma) is a disease characterized by a suspected endothelial damage, causing fibrosis of the skin and other organs such as the lung and the kidney. Owing to the heterogeneity of the disease and the different surface markers used, the findings on EPCs have been conflicting. One study showed decreased peripheral EPC levels [83], but others found increased EPCs within the circulation when compared with controls [84,85] that correlated with disease activity [86]. These discrepant results may be caused by different markers, as well as the fact that the disease duration and the disease activity might affect the EPC quantities [87]. At present, there is no clear consensus regarding the exact role EPCs play in scleroderma. However, EPCs might develop into a therapeutic target in systemic sclerosis. There is evidence that the healing of digital ulcers, which are common in systemic sclerosis, can be improved by enhancing peripheral EPC levels with erythropoietin [88]. Moreover, treatment with intravenous cyclophosphamide for interstitial lung disease associated with systemic sclerosis was linked to an increase in peripheral EPC levels [89].

Kawasaki's disease is a rare vasculitis that mainly affects children in the Asian Pacific region [90]. Its hallmark is the occlusion of medium-sized vessels that can lead to life threatening events. Two publications indicated that the disease is characterized by lowered EPC levels [91] that were inversely correlated with serum TNF and C-reactive protein (CRP) levels [92]. In a follow-up study, successful treatment with intravenous immunoglobulins was associated with a reduction of TNF and high-sensitivity CRP levels, and restored the functional deficiencies of the EPCs in the Kawasaki patients [93].

Interplay between EPC biology, inflammation & immunity

Immune activation plays an important role in atherosclerosis and heart failure. Understanding the interplay between EPC biology and inflammation is important from both a biomarker and therapeutic perspective. Moreover, immune modulation of stem cell biology might be essential in the future to improve stem cell survival in the clinical setting. Both markers of inflammation and low numbers of EPCs have been associated with an increased risk of cardiovascular events [31,94–96]. Data published in recent years have convincingly shown that EPCs play a role and participate in inflammation, but are also themselves significantly affected by chronic inflammation and chronic inflammatory diseases [97]. Inflammation is associated with an increased secretion of cytokines by the various immune cell types. Amongst the relevant cytokines, TNF and IL-6 are considered to be key players [98] and have been shown to significantly affect EPC biology. The same is the case for CRP, one of the best surrogate parameters of systemic inflammation [99].

To understand the effects of inflammation on EPC biology, it is important to differentiate between the acute effects of a cytokine on stem cell mobilization and the effect of chronic cytokine activation and inflammatory milieu on EPC biology (Figure 1). Furthermore, recruitment of EPCs in an inflammatory milieu can lead to a reduction of the circulating EPCs.

Inflammation & EPC mobilization & function

CRP is one of the most common acute phase reactants. In 2004, Verma and colleagues showed that CRP inhibits EPC differentiation, survival and function *in vitro* [100], a finding that was further corroborated by an observation showing that even in healthy subjects, the colony-forming capacity is negatively correlated with the CRP serum levels [101]. CRP itself may impair EPC antioxidant defenses, and may promote EPC sensitivity toward oxidant-mediated apoptosis and telomerase inactivation [102]. This effect might be due to the CRP-induced upregulation of the receptor for advanced glycation end products, leading to an increased EPC sensitivity and oxidative stress-mediated apoptosis [103].

By contrast, CRP release as a consequence of an ischemic event or endothelial injury was shown to lead to rapid EPC mobilization [34,35,104,105]. Together, these findings suggest a dual role of CRP in EPC biology (Figure 1), depending on the cause and duration of CRP secretion. Another possible explanation for this phenomenon was recently published by Ahrens and coworkers, who found somewhat controversial results when studying the effects of native, monomeric, and pentameric CRP on EPCs in a tube formation assay [106]. It turned out that the two types of CRP induced an opposing gene expression profile. Interestingly, the gene expression pattern of monomeric CRP-treated EPCs was related to the one found in patients suffering from SLE. Moreover, pentameric CRP was associated with increased apoptosis and lowered tube formation of EPCs *in vitro* [106].

The production of CRP in the liver is mainly induced and perpetuated by the proinflammatory cytokines IL-6, -1 and -17 [107]. In recent years, it has become evident that IL-6 plays a key role in inflammation of chronic inflammatory disorders. This cytokine is produced by various cells contributing to inflammatory reactions, with the vast majority believed to be secreted from macrophages and other lymphocytes [108]. In RA, IL-6 is systemically elevated, and recently its blockade with an IL-6 receptor antagonist was shown to be a successful treatment option for this crippling disease and is now a routinely administered drug [109]. Herbrig and coworkers first described that higher levels of serum IL-6 levels are associated with lower EPC numbers while studying these cells in RA patients and healthy controls [71], suggesting a potential role for this cytokine in EPC biology.

Experimental studies also suggest that an acute increase in IL-6 can lead to EPC mobilization, whereas chronic IL-6 secretion is associated with a reduction in peripheral EPCs [110] (Figure 1). In the study by Fan *et al.*, IL-6 improved EPC proliferation, migration and tube formation [110]. This observation is further corroborated by the fact that exercise-induced EPC mobilization was linked to IL-6 secretion in healthy individuals [104,111]. Thus, it is likely that IL-6 plays a promoting role that could be regarded as physiological, whereas chronically increased systemic IL-6 levels might directly and/or indirectly impair EPC function and number.

TNF is another pivotal proinflammatory cytokine that is highly upregulated in many inflammatory diseases. TNF and its relation to EPCs have been investigated in various diseases, and this proinflammatory cytokine indeed has significant effects on EPC biology, mobilization and differentiation. In RA, increased TNF serum levels were shown to be associated with reduced peripheral EPC numbers, and patients treated with antibodies blocking TNF showed either normal EPC levels [69] or an increase of EPCs after drug administration [112]. However, the decreased peripheral levels of EPCs in active RA were reversible after treating the patients with medium-dose glucocorticoids for one week [70], an effect that was partly dependent on TNF and glucocorticoid, as shown by *in vitro* experiments.

Moreover, in Type 1 diabetes, in line with the results described in RA, an inverse relationship between peripheral EPC levels and TNF serum levels has been demonstrated [113]. Investigating the effects of TNF on EPCs *in vitro*, Chen and coworkers showed that addition of TNF to EPCs isolated from healthy controls led to a dose-dependent reduction of proliferation, migration, adhesion and tube formation capacity [114]. In addition, the presence of TNF increased the EPC apoptosis rate, and augmented the expression of proinflammatory adhesion molecules and paracrine factors in EPCs. The TNF-induced reduction of EPCs, however, could be reduced or reverted in the presence of statins [115] or resveratrol found in red wine [116]. TNF was also further shown to induce apoptosis of EPCs, an effect that could also be inhibited by the addition of simvastatin [115].

Recruitment of EPCs in the inflammatory milieu

Inflamed tissue is usually characterized by an increased demand for new vessels to sufficiently supply the tissue with oxygen and immune-competent cells. Moreover, inflammation is characterized by hypoxia, which has been shown to be a very strong stimulus for EPC recruitment in order to enhance vasculogenesis [117], which is at least partly driven by hypoxia-inducible factor-1 α . In RA, there is strong evidence that EPCs are selectively recruited into the inflamed joint by vascular adhesion molecule-1 [74]. Accumulation of EPCs in inflamed tissues might trigger local synovial inflammation by promoting the process of inflammation via vasculogenesis [75]. As discussed above, inflammatory diseases are mainly associated with increased levels of CRP and reduced number of peripheral EPCs; both of these factors can contribute to the progression of cardiovascular risk. Therefore, the increase of EPCs after successful treatment, might also be a consequence of modulation of both systemic and local inflammation.

EPCs as a therapeutic agent or target

Given the hard evidence that an enhancement of the EPC pool and an increase of peripheral EPCs might be beneficial for and/or preventive of cardiovascular disease modalities, increasing the number or improving the function of EPCs may be promising in the treatment of atherosclerotic disease or heart failure. The goal of increasing circulating EPCs to facilitate vasculogenesis in ischemic tissues can be reached by different methods, either by enhanced mobilization from the bone marrow or autologous application of EPCs.

Effects of exercise & drug therapy on EPC mobilization

Exercise has been shown to enhance mobilization of EPCs in both healthy controls and patients with heart failure [118–121]. This provides another mechanistic explanation for why exercise is regarded as beneficial in patients with cardiovascular disease. Dietary and lifestyle factors have also been shown to increase EPC mobilization, including green tea consumption [122], Mediterranean diet [123] and moderate red wine consumption [116]. By contrast, obesity [124] and smoking are related to lower EPC counts [125]. Moreover, several drugs have been shown to be associated with either enhanced peripheral EPC levels or with an improvement of EPC function. Amongst them are various statins [12,126], erythropoietin [127,128], glitazones [129,130] and antihypertensive drugs such as angiotensin-converting enzyme inhibitors [131] and angiotensin II receptor blockers [132,133].

EPC-based therapies

Given the strong association between EPCs and cardiovascular outcomes, there has been a growing interest in investigating the therapeutic role of EPCs. However, several obstacles exist before large scale use of EPCs can commence. For instance, the relatively rare cells must be expanded in sufficient numbers from peripheral blood, and possible changes in phenotype may create a risk of cell senescence after *in vitro* enumeration of progenitor cells. The clinical application of EPC-based therapy is, therefore, still in very early stages, as critical questions regarding EPC survival, timing of administration, and phase- or activity-dependent efficacy of the disease still need to be resolved.

The available clinical studies on stem cell administration and outcome in cardiovascular disease are mainly based on the administration of CD34⁺ cells, with only a few studies investigating the role of CD34⁺/CD133⁺ cells. In the study by Stamm *et al.*, intracoronary infusion of CD133⁺ cells after acute myocardial infarction resulted in an early improvement of left ventricular ejection fraction [134], but later was associated with luminal narrowing and failure in remodeling [135]. Another study reported an improvement in left ventricular function following the transepical injection of CD133⁺ cells into the myocardial border zone [136,137]. One multicenter double-blind and randomized placebo-controlled trial with 167 patients who underwent transendocardial application (NOGA® mapping) of mobilized, autologously transplanted CD34⁺ cells reported a lower weekly rate of angina in the low-cell-dose-treated patients and a significantly greater improvement in exercise response [138]. In patients with dilated cardiomyopathy, Vrovec *et al.* have shown that administration of autologously transplanted CD34⁺ cells led to an improvement in left ventricular ejection fraction and was associated with more clinical stability [139].

Another therapeutic approach, besides increasing mobilization and transplantation of EPCs, is to aim for a better homing of these cells into a 'target organ'. Data from animal and *in vitro* experiments showed that blockade of C-X-C chemokine receptor type 4 was sufficient to mobilize EPCs, increase recruitment to the neovasculature and reduce mortality [140]. However, these options of therapeutic interventions in humans remain to be further validated.

Future perspective

EPCs (both ECFCs and hematopoietic cells with angiogenic properties) are emerging as useful biomarkers in cardiovascular disease. They may improve risk stratification, and offer novel tools for monitoring disease progression and response to therapy. However, the search for a unique marker allowing a generalized protocol for quantification is still ongoing. Validation of the findings on the predictive role of EPCs on outcome requires larger sample sizes and appropriate adjustment for covariates of disease such as age, diastolic left heart

function and pulmonary hypertension. Future studies will also help define the role of EPC-based therapy and answer challenging questions on patient selection, EPC expansion, EPC survival and EPC administration. Modulation of EPC recruitment in inflamed tissues could also be a promising area of research, because blocking their influx into the inflamed area might reduce local inflammation and progression.

Acknowledgments

Due to space limitations, we are unable to include all of the important papers relevant to biomarkers, endothelial cells and cardiovascular disease; we apologize to those investigators whose work we omitted here.

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▪▪ of considerable interest

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Executive summary

Definition of endothelial progenitor cells

- Endothelial progenitor cells (EPCs) were first described in 1997 as bone marrow-derived cells capable of neovascularization. EPCs were previously thought to differentiate out of the hematopoietic lineage, but there is evidence that the 'earlier' termed EPCs can be mainly subdivided into endothelial colony-forming cells and hematopoietic cells with angiogenic properties.
- Owing to the lack of a unique marker, the exact definition of EPCs is still being discussed. For flow cytometry, the hematopoietic marker CD45 has been described to be critical in recent recommendations. However, the variations in the methods used in different studies make direct comparisons difficult.
- EPCs are decreased in the majority of cardiovascular diseases.
- The majority of studies investigating EPCs in diverse disease states report decreased levels of EPCs in chronic cardiovascular disorders, whereas acute ischemic events are associated with an increased mobilization of EPCs. These results reveal that EPCs are potential biomarkers for monitoring the 'cardiovascular health status'.

Interplay between the immune system & EPCs

- Proinflammatory mediators such as cytokines and growth factors are significantly involved in EPC biology and underlie the interplay between the immune system and the cardiovascular system.

EPCs as therapeutics

- Increasing the pool of peripheral (or, in ischemic tissue, local) EPCs may improve vascular repair. Several drugs, exercise and dietary factors have been found to enhance EPC levels and improve function. Autologous transplantation of EPCs is another potentially promising avenue.

Figure 1. Endothelial progenitor cells in acute ischemia and chronic inflammation

Acute vascular events lead to a mobilization of EPCs from the bone marrow and an enhancement in the peripheral blood by a variety of factors, including TNF, IL-6 and CRP for contribution in vascular repair. Persistent systemic inflammation, however, is believed to reduce mobilization of EPCs from the bone marrow, reducing their quantities within the circulation. In addition, EPCs have been found to accumulate in chronically inflamed tissue, perpetuating vasculogenesis and further decreasing the peripheral EPC levels. Therefore, the quantities of EPCs taking part in vascular repair might be limited via this mechanism in chronic inflammatory diseases. CRP: C-reactive protein; EPC: Endothelial progenitor cell.

Selected outcome and characterization studies on endothelial progenitor cells in patients at risk of cardiovascular disease.

Table 1

Author	Population	Study design	n	Methods	Main finding	Ref.
Hill <i>et al.</i> (2003)	General population	Cross-sectional	45	Hill outgrowth assay	Number of EPCs correlated with Framingham risk score and was a better correlate of endothelial function	[13]
Xiao <i>et al.</i> (2007)	At risk of CVD	Cross-sectional	571	Early outgrowth assay, Hill assay	EPC number declined with age. EPC number was higher with increasing Framingham risk score (contrasting previous studies)	[46]
Tepper <i>et al.</i> (2002)	Diabetes mellitus (Type 2)	Cross-sectional	20	FACS, proliferation assay, adhesion assay, Matrigel™ tubule assay	EPCs were decreased in Type 2 diabetes mellitus and inversely related to HbA1c levels	[25]
Loomans <i>et al.</i> (2004)	Diabetes mellitus (Type 1)	Cross-sectional	20	FACS, early outgrowth assay, <i>in vitro</i> angiogenesis assay	EPCs were 44% lower in Type 1 diabetic patients and inversely related to HbA1c levels	[24]
Lorenzen <i>et al.</i> (2010)	Chronic kidney disease (stage V)	Prospective	265	FACS, early outgrowth assay	Number of functional active EPCs and age were independently associated with cardiovascular events	[30]

CVD: Cardiovascular disease; EPC: Endothelial progenitor cell; FACS: Fluorescence-activated cell sorting.

Selected outcome and characterization studies on endothelial progenitor cells in patients with coronary artery disease, acute coronary syndrome and stroke.

Table 2

Author	Population	Study design	n	Methods	Main finding	Ref.
Stable CAD						
Werner <i>et al.</i> (2005)	CAD	Prospective	519	FACS, Hill assay	Lower EPC levels were associated with a higher risk of 1-year mortality of cardiovascular cause or major cardiovascular event	[31]
Schmidt-Lucke <i>et al.</i> (2005)	CAD ACS	Prospective	44 33	FACS	Reduced levels of circulating EPCs independently predicted atherosclerotic disease progression even after adjustment for traditional cardiovascular risk factors	[45]
ACS						
George <i>et al.</i> (2004)	Unstable angina	Prospective	29	FACS, Hill assay, adhesion assay	Increase in circulating EPCs in patients with unstable angina without necrosis	[34]
Guyen <i>et al.</i> (2006)	Patients referred for angiography	Cross-sectional	48	FACS, early outgrowth assay	In patients referred for angiography, EPCs were found to be higher in patients with significant CAD and correlated with maximum stenosis	[35]
In-stent restenosis						
Pelliccia <i>et al.</i> (2010)	CAD post-bare metal stent	Prospective	155	FACS	Higher levels of subpopulations of EPCs in patients with restenosis compared with those without	[141]
den Dekker <i>et al.</i> (2011) (HEALING 2B study)	Bioengineered CD34 antibody coated stents	Prospective	100	FACS	Study suggests that statin therapy combined with the EPC capture stent is not contributing to a reduction of in-stent restenosis formation for the treatment of <i>de novo</i> CAD	[142]
Stroke						
Ghani <i>et al.</i> (2005)	Cerebrovascular disease	Prospective	88	Hill assay	Number of EPCs is lower in patients with acute and subacute cerebrovascular disease than in healthy controls	[32]
Sobrino <i>et al.</i> (2007)	Acute ischemic stroke	Prospective	48	Hill assay	EPC levels increased during the first week after stroke and were associated with good functional outcome at 3 months	[143]
CAV						
Simper <i>et al.</i> (2003)	Heart transplantation	Prospective	15	Early outgrowth assay, long-term outgrowth assay	Suggests an association between a reduction of EPCs and CAV	[144]
Thomas <i>et al.</i> (2009)	Heart transplantation	Cross-sectional	64	FACS	Compared with matched patients without CAV, no difference was noted in EPC quantities in patients with CAV	[42]
Osto <i>et al.</i> (2011)	Heart transplantation	Cross-sectional	29	FACS, immunohistochemistry	Lower levels of EPCs correlated with microvascular dysfunction	[44]

Selected outcome and characterization studies on endothelial progenitor cells in patients with heart failure and pulmonary hypertension.

Table 3

Author	Population	Study design	n	Methods	Main finding	Ref.
Heart failure						
Valgimigli <i>et al.</i> (2004)	Heart failure	Cross-sectional	91	FACS, Hill assay	Compared with controls, EPCs were increased in class I heart failure with numbers progressively decreasing with increased severity of disease	[36]
Michowitz <i>et al.</i> (2007)	Heart failure NYHA class II–IV	Prospective	107	Hill assay	EPC levels, age and diabetes mellitus were independent predictors of all-cause mortality	[59]
Erbs <i>et al.</i> (2010)	Heart Failure, NYHA class III	Exercise-based trial	18 (exercise) 19 (controls)	FACS	Exercise increased circulating EPCs, endothelial function, left ventricular ejection fraction and maximal oxygen consumption	[60]
PAH						
Diller <i>et al.</i> (2008)	Idiopathic PAH Eisenmenger	Cross-sectional	55 41	FACS	Circulating EPC numbers were found to be reduced in idiopathic PAH and Eisenmenger, which also showed increased levels of inflammatory mediators	[37]
Junhui <i>et al.</i> (2008)	Idiopathic PAH	Cross-sectional	20	FACS, early outgrowth assay, migration assay, adhesion assay	EPC number and function impaired compared with healthy controls	[39]
Asosingh <i>et al.</i> (2008)	Idiopathic PAH	Cross-sectional	16	FACS, late outgrowth assay, Hill assay	Patients with idiopathic PAH have higher numbers of circulating proangiogenic precursors	[41]

EPC: Endothelial progenitor cell; FACS: Fluorescence-activated cell sorting; NYHA: New York Heart Association; PAH: Pulmonary arterial hypertension.

Table 4

Selected outcome and characterization studies on endothelial progenitor cells in patients with rheumatic diseases.

Author	Population	Study design	n	Methods	Main finding	Ref.
Grisar <i>et al.</i> (2005)	RA: active and nonactive	Cross-sectional	52	FACS, Hill assay, adhesion assay, migration assay	EPCs are reduced in RA, inverse correlation of EPC levels with disease activity, high serum TNF levels are associated with low EPC counts	[69]
Herbrig <i>et al.</i> (2006)	RA	Cross-sectional	15	FACS, early outgrowth assay, Hill assay	Reduced EPC levels in RA, inverse correlation of EPC levels and IL-6 levels	[71]
Grisar <i>et al.</i> (2007)	RA with intermediate and high disease activity	Prospective	36	FACS, early outgrowth assay, Hill assay	Reduced EPC levels in RA were normalized after 1 week of glucocorticoid therapy	[70]
Westerweel <i>et al.</i> (2007)	SLE	Cross-sectional	13	FACS, outgrowth assay, apoptosis assay, functional assay	Decreased peripheral levels of EPC in SLE and increased apoptosis rate	[77]
Grisar <i>et al.</i> (2008)	SLE	Cross-sectional	31	FACS, early outgrowth assay, functional assay	Normal peripheral EPC quantities but reduced adhesion and migration capacity	[82]
Kuwana <i>et al.</i> (2004)	Systemic sclerosis	Cross-sectional	11	FACS	Reduced EPC levels compared with healthy controls and RA patients	[83]

EPC: Endothelial progenitor cell; FACS: Fluorescence-activated cell sorting; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus.