Endothelial progenitor cells in chronic obstructive pulmonary disease

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Abstract

Introduction
Chronic obstructive pulmonary disease is a degenerative, life-threatening lung disease defined by a persistent poor airflow. Pulmonary vascular endothelial dysfunction is a characteristic pathological finding of chronic obstructive pulmonary disease. Endothelial progenitor cells are a population of adult stem cells mobilised from the bone marrow into the circulation, with the function of maintaining vascular homeostasis. Decreasing number of endothelial progenitor cells has been established as a prognostic risk factor associated with endothelial dysfunction and high cardiovascular risk. In chronic obstructive pulmonary disease, few studies to date have investigated the number of endothelial progenitor cells with conflicting results. Quantification of endothelial progenitor cells is affected by high individual variability. Age, gender, smoking habit, cardiovascular comorbidities, different cell populations examined, different methodologies used or the sample size of the study, all are variables that can influence endothelial progenitor cell numbers. This review tries to comprehend why these discrepancies in endothelial progenitor cell numbers occur and sets strategies to encourage greater confidence in the reproducibility of the results, a basis for future stem cell therapy studies.

Conclusion
Greater confidence in the reproducibility of the quantification of EPCs taking into account all possible variables is necessary before using them as the basis for detailed mechanistic investigations and stem cell therapy.

Introduction
Chronic obstructive pulmonary disease
Chronic obstructive pulmonary disease (COPD) is a degenerative, life-threatening lung disease, affecting an estimated 329 million people and accounting for 5% of all deaths globally per year. Mortality is expected to increase due to a rise in smoking rates and an aging population in many countries. The World Health Organization predicts that COPD will become the third leading cause of death worldwide by 2030. Unfortunately, COPD is not curable and cannot be reversed. Drug treatment can improve symptoms and minimise further damage, but does not alter the underlying progression of this disease. Thus, COPD is a major public health problem with high prevalence, increasing incidence and elevated associated socioeconomic costs.

Post-bronchodilator spirometry is required for the diagnosis and assessment of severity of COPD. Is defined by a persistent poor airflow (FEV₁/FVC < 0.70), which results from an inflammatory process affecting the airways and lung parenchyma usually in the form of emphysema. Chronic and acute inflammation induced by the inhalation of noxious particles, mainly cigarette smoking (CS), is the primary pathogenesis of COPD. Genetic factors and aging effects are also involved in the disease progression.

Vascular change and angiogenesis is an integral part of the pulmonary inflammatory response. The lung is exposed to external challenges such as inhaled particles, toxic gases or infections every day; the defence against these external injuries depends on the immune system and the efficiency of the system to remove and replace apoptotic injured cells. CS not only causes inflammation and direct injury to lung tissue but also inhibits compensatory angiogenesis and thus impairs repair and adaptive mechanisms in the lung. The aim of this review was to discuss endothelial progenitor cells (EPCs) in COPD.

Discussion
COPD and endothelial dysfunction
Pulmonary vascular endothelial dysfunction is a characteristic pathological finding of COPD. Studies have shown alterations in the vessel structure and an abnormal functional associated with an atypical control of endothelial cellular growth and resistance to apoptosis in the endothelium of patients suffering from COPD. Genetic factors and aging effects are also involved in the disease progression. Growing evidence suggests that the endothelial cell damage and dysfunction found in pulmonary vessels of these patients is an initial and important triggering factor that promotes pulmonary vasculature remodelling and, hence, pulmonary hypertension in COPD. It has been recognised that patients with COPD showed an endothelial impairment in both pulmonary and systemic circulation at early stages of the disease. In addition, scanning electron microscopy of the endothelial surface...
of pulmonary arteries demonstrated the presence of an irregular endothelial surface and large areas of denudation in those obtained from COPD patients. Whilst the mechanism of endothelial repair/regeneration in COPD is not fully understood, it is believed that the upregulation of vascular endothelial growth factors (VEGFs) along with an increased number of bone marrow-derived EPCs plays an important role. An understanding of the relevance of vascular homeostasis, in particular EPC, to the pathophysiology of COPD is important, given their potential to contribute to endothelial repair and combat vascular remodelling in the emphysematous lung.

EPCs and COPD

EPCs are a population of rare pre-differentiated adult stem cells that circulate in the blood with the ability to proliferate and differentiate into mature endothelial cells. It is not presently clear how EPCs contribute to vascular disease, but it is thought that, under pathophysiological conditions, EPCs are mobilised from the bone marrow into the circulation maintaining the vascular homeostasis of the lung endothelium. EPCs are believed to be essential in maintenance of the endothelium and restoration of the normal function, by replacing terminally differentiated cells lost as a consequence of physiological cellular turnover or tissue damage due to injury. Therefore, EPCs are considered to be a biomarker of vascular dysfunction, and their presence in the peripheral circulation offers the opportunity to monitor their number and function as a marker of disease state or response to therapy.

Decreasing number of EPCs has been established as an independent prognostic risk factor associated with endothelial dysfunction and high cardiovascular risk. Patients with cardiovascular risk factors have shown a reduced number of circulating progenitors and an impaired endothelial function in systemic arteries. Both changes are associated with shorter survival and increased number of detrimental cardiovascular events.

Cardiovascular events are common in COPD patients. Recent studies imply that in COPD patients circulating progenitors are decreased and systemic arteries dysfunctional. CS and COPD are associated with reduced numbers of circulating EPCs. This reduction in circulating progenitor cells in COPD patients might lead to a lower repair capacity and consequently to an altered vascular function. Following cessation of smoking, EPC number and functionality are known to increase rapidly.

In COPD, few studies to date have investigated the number of circulating progenitor cells. While most of the studies indicate lower levels of EPCs in COPD patients compared with controls, others do not find a significant difference. This discrepancy between results could be due to significant methodological differences and failure to reliably match for cardiovascular risk factors, gender and age.

In 2006, Palange et al. were the first to report a significant decrease of circulating CD34+ cells in COPD (n = 18) compared to healthy age-matched controls (n = 12). Dual labelling experiments with anti-CD34 and anti-CD38 or anti-HLA-DR monoclonal antibodies were performed to allow the identification of immature (CD34+CD38- and CD34+HLA-DR-) and mature (CD34+CD38+ and CD34+HLA-DR+) haematopoietic progenitor cells (HSCs). All cellular subsets were consistently decreased in COPD patients compared to controls without significant differences between immature or mature HSCs. Moreover, COPD patients displayed a significantly lower number of haematopoietic colony-forming units than control individuals. This was the first study to state that circulating HSCs were greatly decreased in COPD patients. However, as the authors only used markers of HSCs and did not use any specific endothelial marker such as kinase-insert domain receptor (KDR), they could not infer whether EPCs along with HSCs were reduced in COPD patients. In the same year, a cross-sectional study by Fadini et al. showed that EPCs defined by flow cytometry as CD34+CD133+KDR+ cells were reduced in COPD patients (n = 15) compared to controls (n = 15). This reduction was especially evident in patients with severe end-stage disease. The number of EPCs strongly correlated with disease severity in both the above studies.

In 2010, Sala et al. were the first to study the response of circulating EPCs (CD34+KDR+) in COPD patients during episodes of exacerbation (ECOPD). The results showed that the levels of EPC were higher in ECOPD (n = 35) than patients with stable COPD (n = 44) and healthy controls (n = 20). They also found a relationship between the number of EPCs and the plasma concentration of VEGF during ECOPD. Importantly, and in contrast to the two above controlled studies, differences in EPC between patients with stable COPD and controls did not reach statistical significance.

More recently, in 2013, two studies have been published with contradictory results. Janssen et al. measured both EPCs and HSCs, defined as CD34+CD45+KDR+ and CD34+CD45+KDR+ respectively in patients with COPD (n = 61) and in control subjects (n = 32). Similar to the findings of Palange et al., they showed that COPD patients had lower numbers of HSCs than control subjects. HSC levels correlated with severity of obstruction and were lowest in subjects with severe emphysema. Further, the ability to form endothelial cell colony-forming units (CFC) was also reduced in subjects with COPD. However, contrary to the results obtained previously by Fadini et al., the circulating levels...
of EPCs were similar between the two groups. Finally, Brittan et al. reported that the number of EPCs defined as CD34⁺KDR⁺CD133⁺ was not reduced in COPD patients compared to carefully matched controls and that contrary to previous studies, CFU-EPC were higher in COPD patients than controls.

In recent years, the idea that the vasculature is capable of regeneration has raised the possibility that EPC-based therapies may provide an alternative to conventional treatments. Despite the growing enthusiasm for the use of EPC to slow progressive lung destruction and to potentially regenerate and restore lung function, it appears too premature to draw any definitive conclusion as to the utility of EPC in treatment of COPD. Additional evidence is required to better define and understand the role of EPC in COPD.

**Why do EPC numbers differ among studies involving COPD patients?**

The maintenance of endothelial homeostasis by EPC has been implicated in a range of pathological conditions. The levels of EPC in the adult circulation are believed to oscillate depending on individual circumstances. Studies have demonstrated that EPC levels vary with age, gender, smoking and certain conditions such as diabetes, hypertension and/or other cardiovascular diseases. Robb et al. demonstrated that EPC levels even vary during the menstrual cycle in a potential effort to repair the uterine endometrium. Thus, studies involving measurement of EPC are affected by high individual variability, and study design should aim to reduce this by taking into account the variables described below.

**EPC and aging**

Advanced age is itself a significant risk factor for cardiovascular disease. Aging is associated with reduced number and function of EPC which may contribute to greater cardiovascular risk and reduced angiogenic capacity in the elderly population. Kushner et al. have recently showed that increased apoptotic susceptibility of EPC in aging is associated with increased active intracellular caspase-3 concentrations compared with EPC from young men. The mechanisms responsible for this apparent pro-apoptotic EPC phenotype with aging are unclear. The prevalence of COPD increases gradually with age, with the highest prevalence among those over the age of 60. It is unclear whether age is the risk factor itself or whether is the sum of cumulative exposures throughout life which leads to COPD. Therefore, it is important that studies investigating changes in EPC levels between COPD patients and healthy controls have carefully age-matched populations.

Despite showing an inevitable slightly older population in the COPD group than control subjects, most of the studies reviewed above had groups with similar ages. Sala et al. showed a statistically significant increase in the age of COPD patients (mean = 68) compared to healthy smokers (mean = 60) or healthy non-smokers (mean = 62). Interestingly, the same study did not find any significant difference in EPC numbers between patients with stable COPD and controls. However, as older age should translate to a decreased number of EPC, higher numbers of EPC in COPD found in this study could not be explained by this age difference.

**EPC and gender**

In the past, studies have showed greater prevalence and mortality in COPD among men than women. Recently, it has been indicated that the prevalence of the COPD in developed countries between genders is almost equal. Some studies have even suggested that women are more susceptible to the effects of tobacco smoke than men. While some of the studies published to date matched the control and patient groups for gender, some do not or were not described. In future studies, gender matching should be taken into consideration to avoid inconclusive results due to gender and not to the disease itself.

**EPC and obesity**

It is well accepted that obesity is associated with decreased numbers of EPC. Tobler et al. in 2010 also stated that obese individuals not only had lower levels of circulating EPC; they also have EPC with functional deficiencies. All the studies reviewed above purposefully matched all subjects for their BMI without significant differences between COPD patients and healthy controls.

**Concurrent cardiovascular diseases. Cardiovascular risk factors**

Number and functional activity of EPC are reduced in the setting of several cardiovascular risk factors. (i) **EPC in diabetes**. Diabetes can be characterised by a chronic systemic inflammatory state disturbing the function of multiple vital body systems, in particular the vasculature. Levels of EPC are significantly reduced in patients with diabetes type I or type II compared to non-diabetic controls. Moreover, Reinhard et al. recently showed that EPC levels return to control levels after treatment. These recent findings suggest that EPC levels are altered in diabetes. Tepper et al. showed that EPC derived from diabetic patients were functionally abnormal with lower migration, tube formation and adhesion capacity than healthy EPC, likely as a consequence of hyperglycaemia. Moreover, a recent study by Fadini et al. showed that the number of circulating EPC inversely correlates with the severity of peripheral vascular complications of patients with type II diabetes, further supporting a role for EPC dysfunction in the pathogenesis of ischemic vascular disease.

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(ii) EPC in hypertension: Similar deficiencies in number and function have been attributed to EPC derived from patients with hypertension and have been associated with several cardiovascular risk factors\textsuperscript{13}. Hypertension is a well-known cardiovascular risk factor and one of the initial steps in the development of atherosclerosis\textsuperscript{44}. It has been shown that EPCs of patients with coronary artery disease are less frequent and have reduced capacity for migration, the latter being mainly influenced by hypertension\textsuperscript{44}. Recently, Oliveras et al. showed that the concentration of circulating EPC is significantly reduced in patients with hypertension as compared to healthy subjects\textsuperscript{44}. This is the first study to indicate that there is a clear relationship between EPC numbers and hypertension, independent of age or other factors. In addition, Giannotti et al. in 2010 showed a deficient \textit{in vivo} repair capacity and an increase senescence of EPC in patients diagnosed with hypertension\textsuperscript{20}.

Some of the studies reviewed here did not clearly describe whether patients with cardiovascular risk factors were excluded or not\textsuperscript{28}. Others clearly stated that patients with primary cardiovascular diseases such as hypertension, cardiomyopathy, hypercholesterolemia, acute myocardial infarction as well as any family history of cardiovascular disease were excluded\textsuperscript{23,24,31}. Finally others did not exclude but matched risk factors in both patient and control groups\textsuperscript{28}. In the study of Sala et al., both COPD and EPCOPD patients had significantly higher cardiovascular risk factors when compared to controls\textsuperscript{20}. Although they showed that circulating EPC do not appear to be influenced by the presence or absence of cardiovascular risk factors between COPD and EPOC, they did not use the same assessment to compare patients versus controls. Thus, we cannot rule out the possibility that this might have influenced the outcome of the results. To attribute the changes in EPC number to COPD or any other disease \textit{per se}, studies should reliably match for cardiovascular comorbidities and risk factors. Otherwise, they can notably affect the end result and generate erroneous conclusions. Other cardiovascular risk factors such as hyperlipidaemia also need to be taken into consideration. Hyperlipidaemia can lead to endothelial injury, therefore compromising the capabilities of EPC in maintaining vascular function and homeostasis\textsuperscript{21}.

(iii) EPC and smoking: CS plays a key role in endothelial dysfunction, which is present in both healthy smokers and COPD patients and is associated with an increased risk of cardiovascular disease. There is an inverse correlation between the number of cigarettes smoked and frequency of EPC, the number of which is known to increase rapidly following smoking cessation\textsuperscript{28,52}. EPC isolated from healthy smokers were reported to have reduced proliferative, migratory and adhesive capacities\textsuperscript{53}. A failure to match for CS in patients with COPD and controls prevents a definitive conclusion as to whether COPD is associated with a reduction in circulating EPCs or whether these findings are due to the established effects of CS\textsuperscript{28}.

Sala et al. had a significantly higher number of smokers in the COPD patient group than in that of healthy controls\textsuperscript{30}. We cannot rule out the possibility that the lower number of EPCs found in COPD patients was due to their smoking status and not to the disease itself\textsuperscript{28}. Takahashi et al. showed a reduction of EPC colonies in COPD patients compared to healthy controls\textsuperscript{31}. Although a significantly higher number of COPD patients were smokers compared to controls, linear regression analysis did not indicate that smoking \textit{per se} was responsible for this decrease in EPC colony formation. Some studies did not include current smokers\textsuperscript{23,24}, whereas others included smokers and were well matched with the control population\textsuperscript{22,28}. Fadini et al. concluded that the EPC reduction observed in the COPD group might have been attributed mainly to patients who smoked, as when smokers were excluded from the analysis, the difference in EPC was no longer significant\textsuperscript{22}. These results are in agreement with recent findings of Brittan et al.\textsuperscript{28}. As smoking clearly appears to influence EPC number and function, future studies must reliably match for smoking history in order to achieve better consistent results.

Severity of disease

The severity of disease could also account for variability in the number of EPCs. COPD is a complex heterogeneous disease whose clinical and functional presentation varies greatly from patient to patient\textsuperscript{54}. Patients are commonly classified upon the degree of airflow limitation into mild-to-moderate airflow limitation (Gold I and II) and into patients with severe or very severe airflow limitation (Gold III, IV)\textsuperscript{2}. Some studies have indicated that a high severity score and high number of vascular lesions were associated with low EPC counts\textsuperscript{55}. Patients in Gold III, IV have higher exacerbations rates which are associated with an accelerated loss of FEV\textsubscript{1} and a greater risk of hospital admission and death\textsuperscript{56}. As the stage of disease severity could influence the number of circulating EPCs in COPD patients, studies should separate patients with mild-to-moderate from those with severe or very severe disease score. In the studies reviewed here, Liu et al. included moderate-to-severe COPD patients (Gold II-III)\textsuperscript{21}; Palange et al. used COPD patients in Gold II\textsuperscript{22}, and other studies did not specify\textsuperscript{22,28,30}. Finally, Takahashi et al. included COPD and non-COPD patients with pathological stage I and II lung cancer who underwent lung resection\textsuperscript{31}. It would be significant...
if further studies aiming to investigate changes in EPC number in COPD separate the patients according to their disease severity and/or other comorbidities.

Sample size
It is possible that the discrepancy between findings in previous reports could also have been influenced by the differences in the number of patients and healthy controls recruited in the study. The sample size of the reviewed studies varied. Initial studies had smaller sample size. Palange et al. included 18 COPD patients and 12 healthy volunteers.14,15 Fadini et al. analysed 15 COPD patients and 15 controls.16 Sala et al. studied 44 COPD patients and 20 controls and Takahashi et al. used 30 COPD patients and 30 controls.17 More recently, Brittan et al. analysed 37 COPD patients and 19 healthy controls.18 Jansen et al. had 61 COPD patients and 32 controls.19 A large sample size is more representative of the population, limiting the influence of outliers. Larger samples have more statistical power, allowing more accurate conclusions to be drawn from results. An n = 30 should be a good starting point when designing future studies in EPC and COPD patients. Inferior sample size could lead to erroneous conclusions.

Definition of EPC. State of the art
The observation in 1997 by Asahara and colleagues that peripheral blood contains a population of circulating cells capable of forming mature endothelial cells in vitro and contributing to the formation of new blood vessels in vivo suggested the presence of a circulating EPC in an adult (a process named neo-vascularogenesis).20 This concept challenged the traditional dogma that postnatal blood vessel growth occurred exclusively from the outgrowth of pre-existing vasculature (a process named angiogenesis). This discovery proposed an EPC as a potential candidate for cell-based therapeutic angiogenesis. The development of therapies employing EPC progressed rapidly through preclinical studies to early clinical studies. It was shown that such procedures were safe and showed modest benefit in the treatment of myocardial and peripheral ischemia.21,22 However, it was recognized that therapeutic angiogenesis is more complex than previously thought, and continuing studies of therapeutic angiogenesis by EPC in cardiac ischemia and peripheral ischemia have not shown consistent clinical efficacy. This lack of obvious clinical benefit has led to calls for a better understanding of the identities and roles of cells participating in angiogenesis, so that cell-based therapies could be designed to be more beneficial.

The main problem remains that there is not a standardised definition of what is an EPC and what is the best methodology to isolate it. A decade of research has sought to identify the origin and phenotype of EPC and to harness their potential for cardiovascular regeneration, but ambiguity over the precise definition of an EPC continues to cause problems in the field.23 The phenotypic and functional overlap between EPC, HSCs and mature endothelial cells add to this uncertainty.

Thus, since the identity of EPCs has so far been confusing, there cannot be confidence that previous pre-clinical or clinical studies have employed the most appropriate therapeutic cells. For example, these studies have employed different cell sources autologous bone marrow or mobilised peripheral blood CD34+ cells as the therapeutic source, used either as unfractonated mononuclear cells (MNCs) or as enriched CD34+ or CD133+ MNC following selection using these stem cell markers.

To date, three methods have been used to isolate and culture EPC.24 Method A: Circulating EPCs have been identified and enumerated by flow cytometry by the expression of progenitor (CD34, CD133) and VEGFR2 cell markers (original description).25 Method B: Alternatively, EPCs have been identified and quantified by the culture of blood MNCs as early outgrowth EPC (CFU-EPC) (now known of monocyte origin).26 Method C: Culture of late outgrowth endothelial cells (EOCs), also called endothelial colony-forming cells.27 EOCs are thought to originate in the bone marrow together with CFU-EPC, although EOCs differ in that they demonstrate robust proliferative potential, express endothelial markers, but not hematopoietic or monocyte markers, and form de novo blood vessels when transplanted into immunodeficient mice.

While the literature points to the existence of more than one population of circulating cells, supporting vascular repair and angiogenesis,28,29 it has been suggested that only EOCs are true EPCs with clonogenic and proliferative potential.

Using a well-established mouse model of angiogenesis, which allows investigation of angiogenesis in vivo, we have recently carried out a systematic comparison under the same conditions of the effects of different numbers of the proposed sources of EPC and their fractions on new vessel formation. In this model, a control implanted sponge is spontaneously encapsulated and vascularised by the host, whilst cells of interest loaded into another sponge allows direct comparison of their capacity for vascularisation.30,31 Our study showed that the EPC definitions described different cell types which had different angiogenic properties.

Notably, the strongest, most significant pro-angiogenic effect on host cells came from CD34+ enriched cells. Implantation of unfractonated MNC or CFU-EPC had no apparent pro-angiogenic effect on host-derived vessels. Finally while EOC did not enhance intrinsic vascularisation by host cells, they were the only cell
type implanted which had the ability to form new blood vessels in vivo. Thus, this model of angiogenic potency appears to comprise two distinct activities mediated by different cells: (i) true endothelial progenitor activity with cell incorporation into vessel walls provided exclusively by EOC and (ii) pro-angiogenic amplification of vascularisation provided by CD34+ cells. Other cells, such as monocytes or mesenchymal stromal cells, appeared to be irrelevant in this model.

These results reveal that angiogenic potency is not simply dependent proportionally on EPC numbers, as proposed in many prevailing interpretations, but is complex and may involve multiple cell types with different roles, as has been reviewed. This shows that the number of different proposed sources and sub-populations of cells for therapeutic vascular repair are not equivalent. Further studies are needed to determine whether true EPCs have a role in therapeutic vascular repair, and to elucidate the nature of the differences in pro-angiogenic potency between different EPC sources.

**EPC and COPD**

To date, EPC is the all-encompassing term used to define a bone marrow-derived cell population of distinct phenotypes, which shares the ability to differentiate into mature and functionally competent endothelial cells. The use of CD34 or CD133 expression alone used in some EPC studies is not sufficient to distinguish EPCs from other circulating progenitors such as HSCs. A more comprehensive approach to define the EPC is necessary. Initial studies such as the one from Palange and Liu et al. identify EPCs as cells positive for CD34 and CD133. As discussed, this phenotype does not uniquely identify an EPC and includes other circulating progenitor cells which could have no role in angiogenesis. In other studies such as the ones from Fadini, Takahashi, Sala and Brittan, EPCs were better defined by the expression of CD34 and CD133 progenitor cell marker but also by the expression of KDR, a receptor of vascular endothelial growth factor. Finally, the recent study published by Jansen et al. introduces a new EPC definition CD34+CD45−, which further complicates EPC standardisation. Differences in study methodology and the criteria used to define an EPC may also have been major contributors to this disparity between studies.

In addition, some studies also quantified the number of CFU-EPC. CFU-EPC is a standardised colony assay discovered in 2003 by Hill et al., and originally employed to overcome difficulties associated with accurately quantifying small numbers of circulating cells by flow cytometry. Functional CFU-EPC are reduced in people with cardiovascular risk factors. It is now accepted that CFU-EPC are derived from cells of monocyte origin (angiogenic monocytes). Whist CFU-EPC express endothelial markers and its quantification potentially provides an accurate measure of the activity of circulating mononuclear cells to form endothelial cells, these colonies are unrelated to the circulating CD34+ or CD133+ stem cells. Our group recently showed dissociation between the number of peripheral blood CD34+ cells and functional EPC (CFU-EPC) in patients undergoing percutaneous coronary intervention.

In 2011, Takahashi et al. found an impaired mobilisation and proliferation capacity of CFU-EPC in COPD patients compared to non-COPD patients. Recently, Brittan et al., contrary to the previous study, found that CFU-EPC were fourfold higher in COPD patients than controls. The size of the sample between the two studies was similar. However, while in the study of Brittan et al. all patients were matched for smoking history, in Takahashi’s et al., they were not.

This, and patient-associated diseases such as lung cancer in Takahashi’s et al.’s study could have influenced the difference of the results.

It is believed that an increase of CFU-EPC in COPD patients could reflect increased systemic inflammation in response to a vascular perturbation. As CFU-EPC are generated by a cell of a monocyte origin, one could presume that an increase in leukocyte or monocyte number would translate into a higher number of CFU-EPC. However, not all CD14+ monocyte cells are responsible for the formation of the CFU-EPC. A circulating monocyte population may be divided into different subsets. The two primary subpopulations originally described are the inflammatory/classical (CD14++CD16−) (more than 85% of monocytes) and the non-classical/resident monocytes (CD14+CD16−) suggested to have a pro-angiogenic activity. Non-classical monocytes could be envisaged to be the monocyte subset responsible for the generation of CFU-EPC. COPD patients commonly present higher number of circulating monocytes than non-COPD controls. However, only an increase of CD14+CD16− cells and not a solely increase of monocytes would imply an increase of CFU-EPC. Further studies are required to define the angiogenic role of CFU-EPC in COPD patients. Moreover, as Yoder et al. have suggested, as long as there is no unique marker to identify the circulating EPCs, it might be helpful to avoid the term EPC and refer instead to the specific cell population under investigation.

**Conclusion**

Greater confidence in the reproducibility of the quantification of EPCs is necessary before using them as the basis for detailed mechanistic investigations and stem cell therapy. As seen in this review, circulating EPC levels are highly dependent on individual variability. Age, gender, smoking habit, cardiovascular comorbidities, different
cell populations examined, different methodologies used or the sample size of the study can influence the quantification of EPC. Future studies involving measurement of EPC should take all these variable factors into consideration when designing the study to avoid confusing or contradictory results.

Acknowledgements

The author would like to thank Dr. Mills, Dr. Barberà, Kay Samuel and Dr. Peinado for the endless skilful support. Funding: Project Grant from the Chief Scientist Office, Scotland (CZB/4/812); BHF Project Grant (PG/06/051); BIOTRACK-IDIBAPS Programme co-funded by the 7th Framework Programme from the European Commission.

Abbreviations list

COPD, chronic obstructive pulmonary disease; CS, cigarette smoking; EOC, outgrowth endothelial cell; EPC, endothelial progenitor cell; HSC, haematopoietic progenitor cell; KDR, kinase-insert domain receptor; MNC, mononuclear cell; VEGF, vascular endothelial growth factor.

References


