

## CARDIOVASCULAR GENOMIC MEDICINE

# Circulating Endothelial Cells in Cardiovascular Disease

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Quantification of circulating endothelial cells (CECs) in peripheral blood is developing as a novel and reproducible method of assessing endothelial damage/dysfunction. The 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 endothelial progenitor cells (EPCs). The EPCs are nonleukocytes derived from the bone marrow that are believed to have proliferative potential and may be important in vascular regeneration. Currently accepted methods of CEC quantification include the use of immunomagnetic bead separation (with cell counting under fluorescence microscopy) and flow cytometry. Several recent studies have shown increased numbers of CECs in cardiovascular disease and its risk factors, such as unstable angina, acute myocardial infarction, stroke, diabetes mellitus, and critical limb ischemia, but no change in stable intermittent claudication, essential hypertension, or atrial fibrillation. Furthermore, CEC quantification at 48 h after acute myocardial infarction has been shown to be an accurate predictor of major adverse coronary events and death at both 1 month and 1 year. This article presents an overview of the pathophysiology of CECs in the setting of cardiovascular disease and a brief comparison with EPCs. (J Am Coll Cardiol 2006;48:1538–47) © 2006 by the American College of Cardiology Foundation

Cardiovascular disease (CVD) is the leading cause of death and disability in the developed world, and is projected to soon overtake infectious disease as the preeminent cause of death worldwide. Accounting for 49% of all deaths in Europe and 30% of all deaths before the age of 65 years, it contributes substantially to the escalating cost of health care (1–3). The reasons for this generally reflect an increasing prevalence of several known CVD risk factors: obesity and insulin resistance, diabetes, smoking, hypertension, poor diet, and an increasing ageing population (2,4). The spectrum of CVD is wide and includes coronary artery disease (CAD), peripheral vascular disease (PVD), congestive heart failure, and atrial fibrillation (AF), stroke, in addition to the major risk factors of hypertension, hypercholesterolemia, smoking, and diabetes mellitus. A commonality between these conditions is the loss of appropriate endothelial physiology (damage/injury, leading to dysfunction) that is paramount to the maintenance of vascular hemostasis and blood pressure control (5).

However, it is now recognized that up to half of patients presenting with a clinical manifestation of CVD do not possess any of the traditional risk factors (4). Hence, there is growing interest in the identification of other risk factors or markers (such as for assessing vascular injury) that may facilitate improved measures for the primary and secondary prevention of CVD (5). Methods for assessing endothelial function include the quantification of changes in coronary and brachial artery blood flow (the latter generally referred

to as flow-mediated dilatation [FMD]) and abnormal release of plasma markers such as von Willebrand factor (vWf) (6–8). More recently, the measurement of immunologically defined circulating endothelial cells (CECs) in the peripheral blood is gaining ground as an important and novel technique for assessment of endothelial injury. The CECs are part of a family of blood-borne endothelioid cells that include endothelial progenitor cells (EPCs).

In this article we present a comprehensive overview of the pathophysiology of CECs and the value of CEC quantification as an important marker for CVD, and briefly compare them with EPCs. To do this we performed an on-line search of the publication databases Cochrane Reviews, PubMed, Medline, and Embase, using the key words circulating endothelial cells and endothelial progenitor cells.

### ORIGINS OF CECs

Endothelial cells line the vascular tree and adhere to a basement membrane. In health, these cells would be expected to remain in this location, with perhaps a very low level of cell loss into the blood, with consequent clearance by the reticuloendothelial system. It would seem logical that pathological processes that cause damage to the endothelium might also cause endothelial cell detachment, resulting in increased numbers of CECs within the bloodstream (9). The mechanism of CEC detachment is complex and involves many factors, such as mechanical injury, the classic risk factors for atherosclerosis, alteration of endothelial/subendothelium cellular adhesion molecules, defective binding to anchoring matrix proteins, and cellular apoptosis with decreased survival of cytoskeletal proteins (9–11).

Current consensus supports the view that CECs represent a different population of cells from EPCs. The latter

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#### Abbreviations and Acronyms

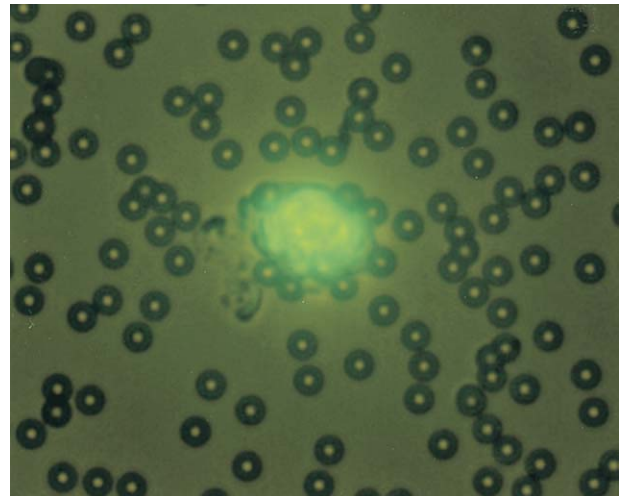
AF	= atrial fibrillation
CAD	= coronary artery disease
CEC	= circulating endothelial cell
CVD	= cardiovascular disease
EPC	= endothelial progenitor cell
FMD	= flow-mediated dilatation
MI	= myocardial infarction
PVD	= peripheral vascular disease
VEGFR	= vascular endothelial growth factor receptor
vWf	= von Willebrand factor

seem to be a heterologous population of largely bone marrow–derived large nonleukocyte cells with properties similar to those of embryonal angioblasts, at different stages of maturation, from early (vascular endothelial growth factor receptor [VEGFR]/CD133<sup>+</sup>) to a more mature (VEGFR/CD34<sup>+</sup>) phenotype. The EPCs have many properties that distinguish them from CECs (i.e., viability, forming colonies in vitro [12,13], and having the capacity to differentiate into mature endothelial cells [14–16]). Hence, EPCs represent a subset of cells at varying stages of development present in the peripheral bloodstream. In contrast, it is uncertain whether CECs are all truly viable because they are less likely to form colonies (if at all), some appearing in the circulation as irregular carcasses, as clumps of cells, or as filamentous cell remnants (9).

Broadly speaking, EPCs and CECs can be separated by identification of their surface markers: EPCs bear immaturity surface markers, such as CD34 and CD133, whereas CECs are positive for CD146 and negative for CD34 and CD133 (15–18). The CD146 molecule has clearly evolved as the most popular marker for the identification of CECs. This adhesion molecule is concentrated at the endothelial junction, where it plays a key role in the control of cell–cell cohesion, permeability, and signalization (19,20).

#### ISOLATION OF CECs

Although the precise quantification of CECs is difficult (partly because of the low numbers present in the circulation and because of their differing morphologic appearances), their detection has been improved by cell enrichment techniques and cell labeling using relatively endothelial-specific markers. The CECs are counted in whole blood using either immunomagnetic separation or flow cytometry. The former method [full details of which are available elsewhere (9,17,21)] involves the use of 4.5- $\mu$ m ferrous beads bound to an anti-CD146 monoclonal antibody. Briefly, these coated beads are mixed with venous blood in a head-over-head mixer for 30 min at 4°C. The anti-CD146–coated beads and blood/buffer mixture are placed in front of a magnet. The anti-CD146–coated beads (typically  $5 \times 10^7$ /ml of blood) bind to the CD146 epitope on the CECs and the magnet is then used to separate the bead-coated CECs from the other blood constituents. The

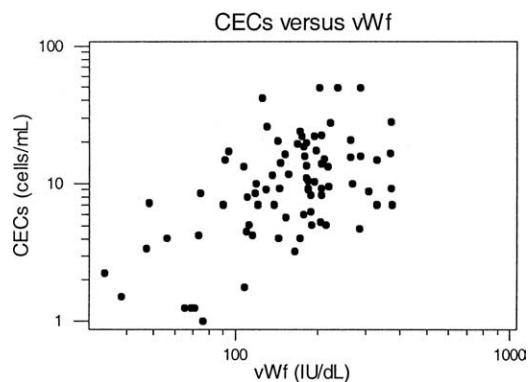


**Figure 1.** A circulating endothelial cell (stained bright green with Ulex europaeus lectin) forming a rosette with at least 10 immunomagnetic beads. Photomicrograph in epifluorescence (Zeiss), wet preparation, magnification  $\times 40$ . For scale, the large number of residual immunomagnetic beads each have a diameter of 4.5  $\mu$ m.

unbound cells are washed away with buffer, and bound cells are retained on the magnet. After additional wash cycles, the cells are resuspended in buffer and labeled (e.g., with acridine orange) before manual counting in a glass counting chamber under a fluorescent microscope. The use of an Fc-blocking agent (to prevent nonspecific leukocyte binding) and relatively endothelial-specific Ulex europaeus lectin 1 has improved the specificity of this technique (Fig. 1). A CEC is defined as an event of appropriate size that binds (forms a rosette with) 4 or more beads (21). The endothelial (and nonleukocyte) origin of CD146–defined CECs has been amply shown by co-marking with, for example, vWf, endothelial nitric oxide synthase, and E-selectin; however, a notable caveat (especially in cancer) is that CD146 may also be found on trophoblasts, mesenchymal stem cells, periodontal tissue, and malignant (prostatic, melanoma) tissues (9).

#### THE RELATIONSHIP BETWEEN CECs, CVD, AND ENDOTHELIAL DYSFUNCTION

Current well-accepted methods used to quantify the severity of endothelial damage/injury include vascular marker quantification (e.g., vWf, soluble E-selectin, soluble thrombomodulin), reduced FMD, and impaired skin blood flow response using laser Doppler flowmetry. There is a clearly shown association between almost all types of CVD (CAD [22,23], PVD [24], hypertension [25,26], stroke [27], heart failure [28,29], AF [30–32], and diabetes [33,34]) and the endothelial dysfunction that occurs after damage/injury. As indicated, the endothelial origin of CECs has been confirmed by the positive labeling for vWf, endothelial nitric oxide synthase, and E-selectin, but also for receptors for VEGF, the receptor of acetylated low-density lipoprotein cholesterol, thrombomodulin, and Ulex europaeus lectin



**Figure 2.** Relationship of von Willebrand factor (vWf) and circulating endothelial cells (CECs) in heart failure. Spearman correlation,  $r = 0.463$ ;  $p < 0.0001$ . Reprinted, with permission, from Chong et al. (37).

1 and by the identification of Weibel-Palade bodies (17,18,21,35).

There is increasing evidence to support the relationship between CECs and endothelial dysfunction. For example, an inverse correlation between CECs and FMD (a surrogate physiological marker of a disturbed endothelium) has been shown (36,37). In addition, a strong correlation between CECs and several plasma markers of endothelial injury (vWf, tissue plasminogen activator, soluble E-selectin) have also been shown (36–40). Figure 2 shows such a correlation between CECs and vWf in heart failure (39).

## CECs AND DISEASE

The CECs are rarely found in normal healthy individuals, in the order of  $<3$  cells/ml. Elevated numbers of CECs have been identified in a wide array of disease processes such as chronic venous insufficiency (41), aortitis (42), pulmonary hypertension (43), Behçet's disease (44), septic shock (45), breast cancer (46), acute sickle cell crises (47), and thrombotic thrombocytopenia (48). Elevated CECs have also been shown to correlate with disease severity in a variety of diseases, including Mediterranean spotted fever (49), inflammatory vasculitis (18,50), Kawasaki disease (51), systemic lupus erythematosus (52), and in systemic sclerosis (53).

The CECs have been identified as a useful marker of endothelial damage and potential vascular rejection in kidney transplantation patients (54,55). Popa et al. (54) were able to show that the presence of donor CECs in recipient blood related to post-transplantation injury. More recently, Woywodt et al. (56) showed that in allogeneic hematopoietic stem cell transplantation, those patients who received reduced-intensity conditioning had significantly lower CEC numbers.

The endothelium plays an important role in the spread and propagation of cancer cells. It is actively involved in angiogenesis (new vessel formation) and in metastasis (57). Increased CECs have been identified in both breast cancer and lymphoma, and their numbers have been shown to significantly correlate with plasma vascular cell adhesion

molecule-1 and VEGF levels, although these molecules are not endothelial-specific (46,58,59).

In various diseases, the longitudinal quantification of CECs has shown that their levels vary according to the clinical condition/severity. Levels among patients who are acutely ill are higher than in those patients who are in clinical remission or in a recovery phase of the disease. From a pharmacologic point of view, several studies have also shown that CECs may be useful in monitoring therapeutic efficacy. For example, Woywodt et al. (50) showed a decrease in CECs during 6 months of successful treatment of anti-neutrophil cytoplasmic antibody-associated small-vessel vasculitis, whereas George et al. (60) showed a decrease in CECs with treatment of *Rickettsial conorii* infection in familial Mediterranean fever. In another study involving the treatment of disseminated human cytomegalovirus infection, there was a dramatic decrease in the CEC count from blood within just a few days of treatment by ganciclovir and foscarnet (61). In addition, CEC numbers are reported to be higher in those patients with progressive disease compared with those who have stable disease and with healthy controls (62).

The precise anatomic origin of CECs, i.e., arterial or venous, is unclear. However, several groups have used CD36 to show that in some circumstances, CECs may arise from the microvascular (47) or the macrovascular portion of the vascular tree (63).

## CECs IN CVD

**CAD.** There have been several studies quantifying CECs in myocardial infarction (MI) (17,39,63–66) (Table 1). Despite small numbers, results are consistent: acute MI and unstable angina are both associated with significantly increased CEC counts compared with healthy controls, and CEC counts are either normal or only minimally elevated in patients with stable angina. The CEC counts in patients with acute coronary syndromes correlate with endothelial dysfunction (by measurement of vWf levels) and with the prothrombotic state (measurement of tissue factor levels) (39). In the study by Wang et al. (66), the investigators showed that CEC counts correlated with levels of inflammatory marker C-reactive protein, even after adjustment for confounding factors.

Quilici et al. (64) showed that the combined use of CEC quantification with cardiac troponin measurement markedly improved diagnostic accuracy in patients presenting with non-ST-segment elevation MI. In addition, they were able to give some insight into the temporal pattern of CEC decrease. They showed that the highest CEC counts were obtained at the time of infarct presentation, with decreasing levels over the ensuing 8 h, with a steady decrease thereafter. However, this is in contrast to the much smaller study (only 5 patients were studied for temporal data) by Mutin et al. (63), in which maximal CEC levels in serial samples were found at 18 to 24 h after MI.

**Table 1.** Studies Summarizing the Quantification of CECs in Coronary Artery Disease

Author	Year	Method of CEC Isolation	Patient Type	Number of Patients	CEC Counts (Cells/ml)	Adjusted Variables	Main Findings
George et al. (17)	1992	IB	Post-angioplasty Healthy controls		8-15 <3	Nil	There is a significant increase in CEC from baseline in both venous and arterial blood postangioplasty. There is no significant difference in CEC counts in arterial versus venous blood samples.
Mutin et al. (63)	1999	IB	Acute MI Unstable angina Stable CAD Healthy controls	26 33 13 14	7.5 (1.5-43.5) 4.5 (0.8-133) 0 0	Nil	CEC counts are increased in both acute MI and unstable angina compared with healthy controls and patients with stable CAD (p < 0.01).
Makin et al. (39)	2004	IB	Acute MI Healthy controls	20 20	4.9 (3.6-8.4) 1.0 (0.5-1.7)	Nil	CEC counts are significantly higher in patients with acute MI as compared with healthy controls.
Quilici et al. (64)	2004	IB	Non-ST-segment elevation ACS Control	60 40	8.5 (15-25.5) <3	Chest pain frequency and interval between chest pain and admission	Higher CEC counts with non-ST-segment elevation ACS compared with controls. The interval between chest pain and elevation was shorter for CEC than troponin I.
Lee et al. (65)	2005	IB	Acute MI Stable CAD Healthy controls	156 36 40	10.6 (5.8-14.5) 3.1 (2.3-4.0) 2.75 (1.6-3.9)	Nil	CEC counts elevated in acute MI compared with stable CAD and healthy controls. CEC counts were predictive of 30-day and 1-year MACE and death. CEC counts correlated with IL-6 and vWf levels.
Wang et al. (66)	2005	IB	Acute MI Healthy controls	37 42	52 (28-81.5) 10.5 (6-16.5)	Age, gender, serum cholesterol, hypertension, obesity, history of CVD, or smoking	CEC counts were significantly higher in patients with acute MI compared with healthy controls. There was a significant correlation between CEC counts and C-reactive protein.

Data are mean or median (interquartile range).

ACS = acute coronary syndromes; CAD = coronary artery disease; CEC = circulating endothelial cell; CVD = cardiovascular disease; IB = immunobead; IL-6 = interleukin-6; MACE = major adverse cardiovascular event; MI = myocardial infarction; vWf = von Willebrand factor.

George et al. (17) plotted the effects of coronary angioplasty on CEC counts in 10 patients listed for elective coronary angioplasty. All patients had normal CEC counts before the procedure, with a peak increase in CEC counts to 8 to 15 cells/ml at 4 h after the procedure. The CEC counts in peripheral venous and arterial blood taken at 4 h were similar. There was then a gradual decrease in CEC counts over the ensuing 20 h toward near-baseline CEC levels.

**PVD.** Peripheral vascular disease is a well-established predictor of cardiovascular risk in which disease severity relates to the level of vascular damage, prothrombotic markers, and endothelial dysfunction, all of which play a major role in atherosclerosis. There has been only 1 study to date designed to examine CEC counts in PVD severity (Table 2). Makin et al. (39) studied 4 patient groups, each of 20 patients, with lower limb ischemic rest pain, intermittent claudication, and acute MI, and healthy controls (the MI data has been presented above). They showed that CEC counts were elevated in patients with ischemic rest pain compared with normal levels among the patients with intermittent claudication and in healthy controls. Furthermore, levels of CECs correlated with plasma tissue factor (associated with a prothrombotic state) and vWf. Thus, increased CECs are present only in severe disease of the coronary (acute MI, unstable angina) and peripheral circulation (ischemic rest pain), not in relatively stable disease of those vessels (i.e., stable angina and intermittent claudication).

**Stroke, hypertension, and AF.** Because stroke is well known to be associated with endothelial abnormalities, it would be reasonable to speculate that CECs would also be increased in patients with this condition, and that they would correlate with other indices of endothelial dysfunction. There have been 2 studies investigating CEC quantification in stroke. Freestone et al. (67) looked at patients with AF and stroke as part of a broader study of CECs in AF. They found higher levels of CECs in patients with concurrent AF and a history of stroke compared with healthy controls in sinus rhythm, and that CECs correlated with vWf. Nadar et al. (68) studied 29 patients presenting with stroke and hypertension (but no AF), and compared them with 30 high-risk hypertensive patients (Table 2) and 30 normotensive controls. Compared with the other 2 groups, the patients with acute ischemic stroke had significantly higher numbers of CECs per milliliter in venous blood and higher levels of vWf and soluble E-selectin. In addition, the numbers of CECs correlated with both vWf and soluble E-selectin.

It is somewhat surprising that CEC counts were normal in high-risk hypertensive controls because these patients are known to have marked endothelial dysfunction (25,26,69). However, it would seem that perhaps, as in the case of chronic stable angina, a certain threshold level of endothelial injury is required to cause CEC detachment from the endothelium. Notably, Bull et al. (43) reported increased CECs in primary and secondary pulmonary hypertension.

**Heart failure.** Chong et al. (37) tested the hypothesis of a clear relationship between the endothelial dysfunction (defined by FMD, vWf, and soluble thrombomodulin) and CECs in patients with chronic HF. Among the patients with HF there was a 3-fold higher level of CECs, but no difference in soluble thrombomodulin, compared with controls. In addition, there was also a positive correlation between CECs and vWF, confirming the ability of CECs to predict endothelial dysfunction. They subsequently reported equally raised CECs in acute HF as in chronic HF (70).

**Diabetes.** McClung et al. (71) investigated the presence of CECs in the peripheral blood of 25 patients with diabetes mellitus and in 9 nondiabetic control donors (Table 2). They showed that patients with diabetes mellitus had an elevated number of CECs compared with healthy controls. However, CEC counts did not correlate with the levels of hemoglobin A<sub>1c</sub> and were independent of plasma glucose levels.

**CECs and the prediction of cardiovascular events.** Koc et al. (72) asked whether or not CEC counts would be predictive of cardiovascular events in hemodialysis subjects at increased risk for CVD. They studied 2 patient cohorts: in the first, CEC numbers were determined in 29 hemodialysis patients followed up for a mean of 470 days; in the second cohort of 44 hemodialysis patients, they analyzed the association between CEC counts with other markers of vascular inflammation. Seven of the 19 subjects with elevated CECs (defined as >19 cells/ml) had cardiovascular events during follow-up, compared with no events among the low CEC count population ( $p = 0.04$ ). Among the second cohort, there was a positive correlation between CEC counts and high-sensitivity C-reactive protein, interleukin-6, interleukin-10, monocyte chemoattractant protein-1, and soluble vascular cell adhesion molecule-1.

Lee et al. (65) asked the same question in patients admitted to a coronary care unit with the diagnosis of an acute coronary syndrome. Compared with interleukin-6 (a proinflammatory cytokine) and plasma vWf, high numbers of CECs 48 h after admission were the only predictor of both major adverse coronary events and death at 1 month and 1 year.

Although strictly speaking not CVD, when defining CECs by intracellular vWf and the expression of the VEGF receptor KDR, Mutunga et al. (73) reported high numbers of CECs in those patients who died of septic shock compared with those who had similar disease but survived.

## IS THERE A RELATIONSHIP BETWEEN CECs AND EPCs?

A current theory is that increased numbers of CECs reflect severe endothelial damage, and that therefore there are areas of the subendothelium that are denuded and that potentially may attract platelets (9). In parallel is the hypothesis that EPCs are restorative/regenerative cells, possibly destined to

**Table 2.** CEC Quantification in Other Cardiovascular Disease States

Author	Year	Method of CEC Isolation	Patient Type	Number of Patients	Median/Mean ± SD CEC Counts in Active Group in Cells/ml (Range)	Adjusted Variables	Main Findings
Peripheral artery disease Makin et al. (39)	2004	IB	Acute MI	26	7.6 (1.5–43.5)	Nil, but groups well matched	Patients with ischemic rest pain have significantly elevated CEC counts, but not as high as for acute MI. CEC counts correlated with vWf and tissue factor levels.
			Ischemic rest pain	20	3.5 (2.0–5.8)		
			IC	20	1.1 (0.6–2.9)		
			Healthy controls	20	1.0 (0.5–1.7)		
Stroke and high blood pressure (HBP) Nadar et al. (68)	2005	IB	Stroke and HBP	29	15.5 (10.8–20.7)	Age, gender, glucose, creatinine, clinical group, systolic blood pressure, vWf, E-selectin	Acute ischemic stroke is associated with increased numbers of CECs compared with both healthy and hypertensive controls ( $p < 0.001$ ). CECs significantly correlated with soluble E-selectin and vWf.
			HBP controls	30	3.1 (1.6–4.8)		
			Healthy controls	30	2.7 (1.6–3.7)		
Atrial fibrillation (AF) Freestone et al. (67)	2005	IB	AF patients with:			Smoking	No increased CEC levels with chronic stable AF. CEC counts are increased in AF when associated with MI, stroke, or heart failure. CEC counts correlated with vWf levels.
			Acute MI	22	9 (6–15)		
			Acute stroke	28	15 (11–33)		
			Acute LVF	20	10 (6–19)		
			Chronic stable AF	28	5 (3–10)		
Heart failure Chong et al. (37)	2004	IB	Healthy controls in SR	20	4.5 (1.6–7.2)	None for CEC quantification, but groups well matched	CEC counts significantly higher in patients with LVF versus healthy controls ( $p < 0.001$ ). CEC counts were inversely correlated with BNP and positively correlated with vWf.
			Chronic LVF	30	11.8 (4.6–18.4)		
			Healthy controls	20	3.7 (1.3–7.2)		
Diabetes mellitus McClung et al. (71)	2005	IB	DM	25	69 ± 30 (35–126)	Gender, age, BMI, HbA1C	CEC counts in DM are significantly higher than healthy controls.
			Healthy controls	9	10 ± 5 (3–18)		
Pulmonary hypertension (PHT) Bull et al. (43)	2003	IB	Primary PHT	5	33.1 ± 1.9	Unclear	CECs correlated strongly with SBP, DBP, and mean arterial pressure, not with cardiac output or pulmonary vascular resistance.
			Secondary PHT	11	27.2 ± 6.9		
			Normal controls	12	3.5 ± 1.3		

AF = atrial fibrillation; BMI = body mass index; BNP = brain natriuretic peptide; DBP = diastolic blood pressure; DM = diabetes mellitus; HbA1C = haemoglobin A1C; IC = intermittent claudication; LVF = left ventricular failure; SBP = systolic blood pressure; SR = sinus rhythm; vWf = von Willebrand factor; other abbreviations as in Table 1.

replace/renew damaged areas of the intima (15,74-77). Are these theories related?

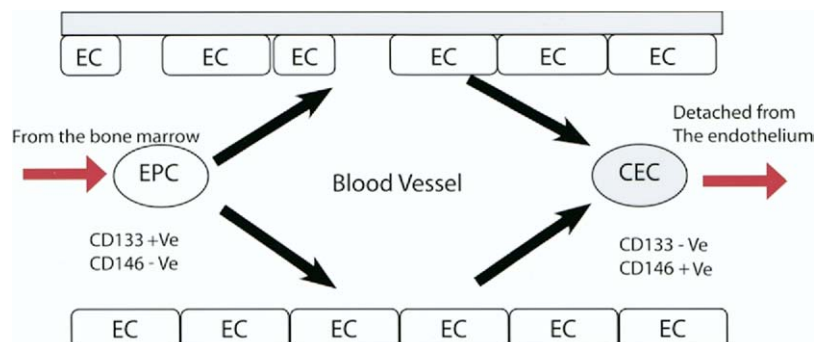
**Are numbers of CECs and EPCs inversely related?** The current literature has largely shown elevated numbers of CECs, yet reduced numbers of EPCs, among a broad spectrum of patients with CVD and its risk factors, raising the suggestion of an inverse relationship (37,39,63,71,76-81). It may be that the disease process that drives mural endothelial cells into the blood to become CECs also influences (reduces) levels of EPCs. However, the reality is likely to be far more complex than merely a reciprocal situation. For example, in acute MI elevated numbers of both CECs and EPCs have been shown (17,39,63-66,82-84). Furthermore, stimulatory/therapeutic factors (e.g., statins and endurance training) among patients with CAD leads to both improved endothelial function and increased EPC counts, although the relationship between therapeutic factors and decreasing CEC counts has not been studied for CVD (85-87). In addition, there have been no studies to date that have actually attempted to quantify both CECs and EPCs simultaneously in the same patient population.

**Are CECs and EPCs phylogenetically related?** However, much of the above presupposes that CECs and EPCs are entirely different cells. This view may be premature, and the 2 cells types may share a common cell biology. Although ultimately defined by different laboratory techniques (immunomagnetic beads or flow cytometry for CECs alongside flow cytometry and/or tissue culture for EPCs), the initial problem is with the distribution of the particular molecules purported to define each cell type (i.e., CD146 for CECs and any combination of CD34, CD133, VEGFR-2, and so on, for EPCs) (12-17). It seems likely that under certain conditions, a small number of cells bear both CD146 and CD34 (53,88-90), possibly leading to difficulties in interpretation, especially as both CECs and EPCs are present in very small numbers. Notably, for example, Nakatani et al. (91) estimated that 0% to 18% of CD146-defined CECs from patients with Kawasaki disease were also positive for CD133. Although much development work has been performed in vitro (92) (e.g., changes in proportions of cells bearing CD34 and CD133) (90), a parallel with the clinical situation cannot necessarily be assumed.

Some of these issues may be partially resolved with additional phenotyping. The endothelioid nature of CECs and EPCs is generally presumed by staining for vWf, lectins (e.g., *Ulex europaeus*), endothelial nitric oxide synthase, and acetylated low-density lipoprotein cholesterol (9,90). However, the anatomical origin of CECs can be traced with the use of CD36, a microvascular marker (40,43,47,58,63,65), and others have used E-selectin and intracellular adhesion molecule-1 expression to quantify activation (40,43,53,63). Although it is known that atherosclerosis is an arterial disease, it is not known whether or not CECs arise from the arterial or the venous circulation, although the use of CD45 to exclude a leukocyte origin for these cells is common (53,58,65).

**The heterogenous nature of EPCs.** As mentioned, a problem is the definition of an EPC, because many markers are available (CD34, CD133, VEGFR-2) (12-16,90). A further growing issue with EPCs is the question of a monocytic origin for these cells. Current data tend to suggest that under angiogenic stimulation, monocytes (bearing CD14) have the capacity to develop an endothelial phenotype with expression of specific surface markers and even form cord- and tubular-like structures in vitro (93,94). The relationship between monocytes and EPCs is complex; there are clearly EPCs that are nonmonocytic, but there also seem to be monocytes that possess both functional characteristics and, perhaps, surface markers (in lower numbers) for CECs and EPCs, and vice versa (95-97). However, despite differences in their derivation, EPCs possess the commonality of being capable of augmenting revascularization and endothelial regeneration. This problem of heterogeneity is compounded by the different names (endothelial outgrowth cells [98], circulating angiogenic cells [99], endothelial-like cells [100]) given to possibly overlapping cell types.

**Conclusions.** The measurement of immunologically defined CECs and EPCs in venous blood now represents an important and novel technique for assessment of endothelial injury and repair (Fig. 3). Circulating endothelial cells are biomarkers of damage, and high levels predict a poor outcome (9,65). Endothelial progenitor cells are biomarkers of repair with therapeutic potential, and low levels predict a



**Figure 3.** Simplified discrimination of endothelial progenitor cells from circulating endothelial cells. CEC = circulating endothelial cell, EC = mural endothelial cell, EPC = endothelial progenitor cell.

poor outcome (76,101). It may be that damage to mural endothelial cells that lead to increased CECs is physiologically restored by EPCs. Levels of CECs in the bloodstream have been shown to significantly correlate with several well-established plasma and physiological markers of endothelial function, are technically simple to measure (102), are elevated in a wide spectrum of conditions, and are predictive of major adverse cardiovascular end points and death in patients with acute coronary syndromes. As such, they represent one of the first specific cellular markers to provide a direct link with the pathophysiology of CVD. Bonello et al. (103) have shown increased CECs and mobilization of EPCs after coronary angioplasty.

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