Circulating Endothelial Cells in Coronary Artery Disease and Acute Coronary Syndrome

David E. Schmidt[a], Marco Manca[b,c], Imo E. Hoefer[d, e]

[a] University Medical Center Utrecht, The Netherlands, [b] Experimental Vascular Pathology, Department of Pathology, Maastricht University Medical Center, The Netherlands, [c] CERN European Organization for Nuclear Research, Meyrin, Switzerland, [d] Department of Experimental Cardiology, University Medical Center Utrecht, The Netherlands, [e] Laboratory of Clinical Chemistry and Hematology, University Medical Center Utrecht, The Netherlands.

Corresponding author:

Imo Hoefer, MD, PhD, FESC

UMC Utrecht

Laboratory of Clinical Chemistry and Hematology, G03.550

Heidelberglaan 100,3584CX Utrecht, Netherlands

Email: i.hoefer@umcutrecht.nl

Phone: +31 88 755 8654

Fax: +31 30 252 2693

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Abstract

Circulating endothelial cells (CEC) have been put forward as a promising biomarker for diagnosis and prognosis of coronary artery disease and acute coronary syndromes. This review entails current insights into the physiology and pathobiology of CEC, including their relationship with circulating endothelial progenitor cells and endothelial microparticles. Additionally, we present a comprehensive overview of the diagnostic and prognostic value of CEC quantification, as well as possibilities for improvement, for example by inclusion of CEC morphology, transcriptomics, and proteomics. The current stand of knowledge calls out for improved counting methods and consensus on a validated cell definition. Finally, our review accentuates the importance of large, well-designed population-based prospective studies that will have to show the clinical value of CEC as cardiovascular biomarker.

Introduction

Ischemic heart disease is the leading cause of death worldwide (1). Acute coronary syndromes (ACS), caused by atherosclerotic plaque rupture and superimposed thrombosis, lead to myocardial ischemia and infarction. The most effective therapy with proven long-term outcome improvement is timely revascularization: 'time is muscle'. The diagnosis of ST-elevation myocardial infarction (STEMI) based on typical symptoms and ECG changes is straightforward and usually does not require additional measurements. Non-ST elevation myocardial infarction (NSTEMI) or unstable angina symptoms are more diverse and the clinical diagnosis often depends on the combination of symptoms together with a rise of cardiac injury markers in the blood. Particularly in patients with more complex and/or atypical clinical presentation, such as diabetics, elderly, and women, novel markers are needed to facilitate rapid diagnosis. Ideally, such biomarkers could moreover be applied to guide personalized primary and secondary prevention of cardiovascular disease, especially since the prognostic value of traditional cardiovascular risk factors is limited in patients with symptomatic cardiovascular disease (2).

Circulating endothelial cells (CEC) might serve as a biomarker that answers to both diagnostic and prognostic needs. Upon desquamation from the vascular wall, for example during atherosclerotic plaque rupture, CEC are released into the bloodstream, where they can be detected in low numbers (3). Therefore, CEC are considered to reflect the occurrence of endothelial defects and vascular disruption (4). Because of the close temporal relationship with vascular injury, CEC might be superior to classic cardiac markers like CK-MB or cardiac troponin, which show a delayed rise after the actual coronary event.

CEC numbers have been shown to be elevated in coronary artery disease (CAD) and ACS, but also in other states of vascular remodeling and injury, such as pregnancy, hypertension, stroke, heart failure, vasculitis, systemic lupus erythematosus, sickle cell disease, septic shock, and systemic infections (4-6). Together, these conditions are relevant to the analysis of CEC profiles for diagnosis of ACS, as their co-existence may induce external variation in CEC counts.

Circulating endothelial progenitor cells (EPC) are similarly rare circulating cells, but are believed to have an opposite function. EPC have a role in the maintenance and promotion of vascular healthy, and are negatively correlated with cardiovascular risk (7), reviewed elsewhere(8) .

This literature review aims to give an overview on the current stand of knowledge on CEC, including insights into their pathobiology and use for diagnosis and prognosis of CAD and ACS. We also assess the current obstacles to the introduction of CEC in clinical cardiovascular medicine, including the cells' molecular characterization.

Nature and Pathobiology of Circulating Endothelial Cells

Circulating endothelial cells are nucleated cells with a size of 10-50 μm. A combination of molecular markers is used to identify and distinguish these cells (Table 1). CEC are present in peripheral blood in very low numbers (~10/mL) (5). However, the actual count can vary significantly and highly depends on the method of isolation and quantification (4). To differentiate between a hematopoietic or vascular origin of CEC, Lin and colleagues elegantly studied CEC in bone marrow transplant recipients with gender-mismatched transplants (9), where, more than 5 months after transplantation, ~95% of CEC still were of recipient origin. Culturing of endothelial cells from peripheral blood showed that these recipient CEC have much lower proliferation capabilities than bone marrow derived donor circulating endothelial cells, indicating that mature CEC originate from the vessel wall in contrast to EPC, which are released from the bone marrow.

The detachment of CEC from the vascular wall can be induced by various factors (Figure 1). First, mechanical injury to the vascular wall induces CEC detachment, as demonstrated after arterial- and venipuncture, coronary stent placement, and acute plaque rupture (10). Prolonged inflammation and ROS activity together with cytokines and tissue proteases that disrupt integrin integrity and cadherin adhesion of endothelial cells may also induce endothelial cell detachment from the vasculature (11). (12,13). Finally, certain drugs, for example calcineurin inhibitors and cyclosporine A, have been shown to induce endothelial cell desquamation (14).

Expression of surface markers by CEC depends on their origin and can help locating the cells' origin. Microvascular endothelial cells express CD36 in contrast to macrovascular endothelium (15).

Hence, changes in the ratio of CD36 positive and negative CEC suggests a shift towards either macro- or microvascular origin (4). In myocardial infarction and heart failure patients, less than 10% of CEC were CD36 positive, pointing towards a macrovascular origin (16,17). Conversely, certain microvascular endothelial cells may be negative for CD36 (18), which puts this concept in question.

Another reason for endothelial cells to detach is when undergoing apoptosis. Up to 10% of all CEC may be apoptotic with evident nuclear DNA fragmentation (17). Even though the mechanisms of CEC release are increasingly elucidated, it remains to be investigated whether CEC have intrinsic biological functions, e.g. induction of thrombosis and hemostasis. Apoptotic CEC and endothelial particle derivatives promote thrombin generation and hold intrinsic procoagulant activity through plasma membrane expression of phosphatidylserine, thromboxane release, and suppression of anticoagulant effector molecules (19-23). Another suggested mechanism is loss of endothelial cell protein receptor (ECPR) expression in CEC and subsequent impediment inhibition of endothelial thrombomodulin-protein C activation, which also promotes thrombosis (4).

CEC desquamation with elevation of CEC counts has been shown to correlate with other indicators of endothelial damage and dysfunction: increased CEC counts in cardio- and cerebrovascular disease patients are associated with increased biomarker levels like von Willebrand Factor (vWF), IL-6, tissue plasminogen activator (tPA), tissue factor, soluble thrombomodulin and soluble E-selectin (sCD62E) (24-31). Similarly, non-invasive testing of endothelial function by flow mediated dilation has shown increased CEC levels in relation to *in vivo* endothelial dysfunction (24,32,33). Furthermore, a correlation between CEC count and carotid intima media thickness has been proposed by Gao Y and colleagues (34). However though, in their study, CEC were counted in patients with acute cerebral vascular thrombosis within 48 hours after hospital admission, and confounding of CEC numbers by the precedent vascular injury is likely.

The endovascular harvesting of endothelial cells by a guide wire, called endothelial biopsy, is a novel, invasive technique that may offer insights into the state of the vascular endothelium (35,36). This technique has also been combined with CD146-positive immunomagnetic enrichment, similar to the method of CEC isolation, but a direct comparison of both techniques has not been described yet.

It is a tempting idea to culture CEC from peripheral blood and perform further analyses on these cells. For example, gene expression and proteomic studies could reveal specific characteristics of CEC in disease states, and function assays could indicate the extent of *in vivo* endothelial dysfunction or provide measures to tailor treatment for individual patients. Some CEC captured during venipuncture are actually still viable (37). Although a small proportion of CD146+ cells CEC may be positive for the hematopoetic marker CD34 (38), mature CEC generally have poor proliferative capacities (4). Endothelial cell cultures derived from peripheral blood mononuclear cells predominantly reflect outgrowth from circulating angioblasts and EPC (9,39).

Endothelial Progenitor Cells

Whilst CEC are remnants of vascular decay and injury, circulating endothelial progenitor cells (EPC) on the contrary are believed to provide a source for vascular regeneration and renewal (Figure 1). EPC are nucleated bone-marrow derived cells, which can be mobilized by various factors (e.g. VEGF, plasmin, MMP-9) (40-43). There is no clear consensus on the identification of EPC. Hence, their definition differs considerably among studies. Frequently used markers include $CD146^-$, $CD45^-$, $CD133^+$, $CD31^+$, $VEGFR2^+$ (KDR), $VEGFR1^-$, and the hematopoietic stem cell marker $CD34^+$ (4,6,44). EPC contribute to neovascularization and vascular renewal through recruitment and migration into the vessel wall, and subsequent differentiation into mature endothelial cells (Figure 1) (45,46). Additionally, EPC exert pro-angiogenic effects through immediate cell-to-cell or paracrine crosstalk with local endothelial cells, leukocytes, and platelets.

An inverse relationship between EPC counts and the occurrence of cardiovascular events has been established, supporting their vasculoprotective role (47,48). Furthermore, a persistent negative correlation with risk factors for cardio- and cerebrovascular disease has become evident, e.g. in diabetes mellitus and dyslipidemia (6). Interestingly, EPC counts correlate negatively with CEC count (49), in line with their proposed contrasting functions. Furthermore, CEC have been suggested to attenuate EPC function (50).

Circulating Endothelial Microparticles

Circulating endothelial microparticles are subcellular fragments that express endothelial cell surface markers like CD31, CD51, CD54, CD62E, CD105, CD144 and CD146, but lack a nucleus (51-53). Their diameter ranges from 0.1-1.0 μm, thus being slightly bigger than cellular vesicles, but still smaller than apoptotic bodies and are considered to be cell remnants from vascular endothelial cells or CEC (Figure 1) (52).

The amount of circulating endothelial microparticles in peripheral blood has been associated with increased risk for cardiovascular disease (54,55), as well as coronary artery disease, and acute coronary syndrome (51,56,57). In STEMI patients with LAD infarction, the number of endothelial microparticles was correlated to the relative myocardium at risk, as well as increased troponin T. Moreover, the endothelial microparticle count was inversely associated with left ventricular ejection fraction (58). Whether microparticles are purposefully expelled by their host cells is controversial (59,60), and their definite nature and biology of these fragments as well as the mechanisms underlying their formation remain to be fully elucidated. Nonetheless, it has been demonstrated that microparticles can promote endothelial TF presentation and phosphatidylserine-dependent thrombin generation, which may promote thrombosis following vascular injury (19,61).

Prognostic and Diagnostic Potential of CEC

The relationship between CEC and risk for future cardiovascular events has exceedingly been established. In hypertensive patients, increased CEC counts correlated weakly with predicted Pockock scores, an estimator of the 5-year risk of death (62). Moreover, in ACS, an elevated CEC count was associated with higher TIMI, PURSUIT and GRACE risk scores (63).

CEC counts and their association with MACE and cardiovascular death have been studied in prospective ACS cohorts. A high CEC count correlated with MACE and cardiovascular death at a median follow-up of 338 days (26). However, correction for confounders showed contrasting results. Another study showed an association between high CEC levels and 30-day risk for MACE and cardiovascular death by multivariate logistic regression (29). The partly different results of these two studies could be reflective of different patient populations and definitions of events, while both studies

utilized similar isolation techniques. Long-term prognostic studies with a broad population base remain to be conducted to shed more light upon the prognostic value of CEC.

In addition to their use as prognostic marker in ACS patients, CEC counts correlate with the severity of acute coronary syndromes, increasing step-wise with UAP<NSTEMI<STEMI, suggesting a potential use for diagnostic purposes (Table 2). In contrast, one isolated study did not find an association between CEC count and STEMI (64). This may be explained by a different set of markers that had been employed for the identification of CEC, not including the traditionally used CD146 marker. The variation in absolute CEC levels between different studies can generally be attributed to other methods of analysis, varying cut-off values, and the applied marker sets. Furthermore, CEC counts seem to gradually decrease over time after a vascular event, as was observed when repeated measures were performed with a delay of some hours (65), days (49), and up to 30 days of follow up (66). This introduces another important variable that may explain variations between studies.

High sensitivity and specificity are crucial for the diagnostic value of a novel test. The sensitivity of CEC counts for NSTEMI diagnosis was found to be slightly lower than cardiac troponin I, with 53% and 63%, respectively (67). The specificity of both markers in this study was 100%. The diagnostic value of CEC in otherwise healthy individuals might have been underestimated in this report, as the control population consisted of patients undergoing preoperative screening prior to valve repair, a condition that is associated with endothelial dysfunction and hence may have influenced the CEC counts (68). Based on their limited sensitivity, CEC elevation had a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 59% in this study (67). This was supported by a PPV of 91% and NPV of 54% for STEMI/NSTEMI (63) and a PPV of 71% and NPV of 65% for unstable angina. An increased CEC count is therefore highly indicative of ACS, while a negative test should not be used as a rule-out criterion.

For NSTEMI patients, CEC counts at admission and after 4 and 8 hours had superior, similar, and inferior diagnostic value compared to cardiac troponin (Table 3). Boos et al. (63) were the first to evaluate CEC counts for the whole spectrum of ACS. The calculated receiver operating characteristics area-under-the-curve (ROC-AUC) for diagnosis of ACS was 0.82, with 0.75 for unstable angina, 0.88

for STEMI, 0.82 for NSTEMI, and 0.85 for STEMI/NSTEMI together. Both groups used manual immunobead isolation and fluorescence microscopy for CEC enumeration. More recent studies reported an increased diagnostic value when making use of the semiautomated CellSearch® platform (ROC – AUC, 0.93 and 0.95, in 2 studies) or the fluid phase biopsy technique (ROC – AUC, 0.95) (69,70).

Taken together, the high specificity of CEC in the studied populations makes CEC quantification an interesting rule-in test for ACS. At admission, it provides better diagnostic value than troponin measurements. Consequently, these findings need to be confirmed on a broader population basis. Furthermore, it remains a challenge to develop more rapid and easy-to-use tests that are required for clinically applicable CEC quantification.

Clinically Important Confounders

Apart from ACS, the peripheral blood CEC count can be increased by non-cardiovascular conditions, including sepsis, infections, as well as rheumatologic and hematologic disorders (4,6). Furthermore, exercise training has been shown to increase markers of endothelial stress, including CEC count (28). Similar CEC increases can be found during menstruation, possibly as a result of vascular remodeling during endometrium proliferation and secretion (71). Moreover, malignancies can affect CEC counts, most likely due to tumor-associated angiogenesis and vascular remodeling (44,71). As such, CEC counts are also affected by anti-angiogenic therapies (72), and monitoring of CEC counts to predict treatment response has been suggested.

Morphological, Genomic and Proteomic Changes of CEC in Acute Coronary Syndrome

Circulating endothelial cells are morphologically similar to mature endothelial cells (73), but may change their morphological appearance depending on their functional state, i.e. activated (CD62E, CD106), apoptotic (phosphatidylserine exposing, Annexin A5-positive), or necrotic (karyolytic, disrupted plasma membrane, cellular degradation) (4). In patients with ST-elevation acute myocardial infarction cellular and nuclear size can differ significantly (17), as well as their shape, which suggests

that the morphology of CEC changes during acute vascular events (69). Additionally, CEC have been shown to form aggregates after STEMI (69,70).

Advances in molecular biomedical methodologies make a broad range of analytical techniques available for the investigation of CEC. To our knowledge, there is no study describing (epi-)genomic changes in CEC in cardiovascular disease. However, the CEC transcriptome was investigated in ACS by whole genome expression analysis (69), demonstrating pronounced expression of endothelin and vWF in STEMI patients. A recent study compared CEC and EPC proteomic profiles in healthy subjects and ACS patients by subsequent flow cytometry and mass spectrometry (64). Here, protein and protein class analysis revealed distinct CEC and EPC proteins and pathways that are exclusively activated in controls or ACS patients, indicating that there may be changes on proteomic and functional level during acute vascular events that may be investigated for clinical use. Ultimately, only longitudinal studies in individual patients will be able to reveal specific molecular changes in CEC during acute vascular events.

The Controversial Identity of CEC

The comparability of studies conducted in the field of CEC research is clearly limited by the variation of the profiling of CEC between studies (Table 1). There is currently no scientific consensus on the markers that identify CEC. Only the CD146, the most traditional CEC marker, can be considered to be generally accepted.

Similar challenges arise when CEC studies in animal models are considered. CEC have been investigated in a range of species including dogs (e.g. (74)) and mice (e.g. (66,75,76)), and methods are available for isolation in canine (77). However, varying sets of markers CEC are used to identify amongst the different species, which complicates the translation of findings from these studies to the human setting.

Importantly, the CD146-defined concept of CEC itself is currently being questioned. Tropea and colleagues phenotyped the circulating CD45-, CD34 bright/dim cell population (78). This cell population expressed the endothelial cell markers CD31, CD34, CD62E, CD105, CD141, CD144 and vWF, but lacked surface expression of CD146. Nonetheless, CD146 was detectable as a cellular

membrane protein, which could indicate that the extracellular domain of CD146 is released from the plasma membrane when endothelial cells detach and enter the bloodstream. This study highlights the capital importance of validating the molecular signature of CEC, and reaching a consensus among researchers in this field.

As long as the proposed set of CEC markers allows for non-variant, comparable, accurate, and reproducible results, the present CEC definition can enable diagnostic use or stratification of patient prognosis. However, if the cells would be uniquely identified, this might generate a subset of better-characterized cell markers, which in turn could improve the diagnostic and prognostic value of CEC. Moreover, the lack of methodological comparability of data from different studies limits progress in the field, which compromises the accumulation of a body of evidence sufficient for clinical translation.

Conclusion

The development of improved, time-effective methodologies for the quantification of CEC imposes a challenge and presents a serious bottleneck for the further research into the translation of CEC towards clinical practice in cardiovascular medicine. The costs of CEC enumeration is considerable, given the amount of antibodies that is needed, and the operating costs of flow cytometers. While fluorescence microscopy is relatively inexpensive, the wage costs for manual analysis may be relevant. Lastly, time-to-result is an utmost relevant parameter in the diagnostic setting, as CEC enumeration or characterization will need to be available before high-sensitivity tests for blood-borne necrotic markers during ACS. A feasible diagnostic method with standardized protocols would provide a critical basis for larger prospective population-based studies that are needed to ensure progress in the field.

At the same time, a better validation of the CEC phenotype is needed to understand their full physiology and pathobiology, and would provide additional information to improve the value of CEC for clinical applications. Additionally, the investigation of the cells' morphology, as well as changes in the transcriptome and proteome may provide improved diagnostic value as compared with the cells' enumeration alone.

In summary, CEC are promising markers for the diagnosis and prognosis of ACS and CAD, supporting the need for development of a feasible clinical methodology for the enumeration of rare blood cells.

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References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet. Elsevier; 2012 Dec 15;380(9859):2095–128.

2. Futterman LG, Lemberg L. Fifty percent of patients with coronary artery disease do not have any of the conventional risk factors. Am J Crit Care. 1998 Sep 10.

3. Dignat-George F, Sampol J. Circulating endothelial cells in vascular disorders: new insights into an old concept. Eur J Haematol. 2000 Oct;65(4):215–20.

4. Blann AD, Woywodt A, Bertolini F, Bull TM, Buyon JP, Clancy RM, et al. Circulating endothelial cells. Biomarker of vascular disease. Thromb Haemost. 2005 Feb;93(2):228–35.

5. Boos CJ, Lip GYH, Blann AD. Circulating endothelial cells in cardiovascular disease. J Am Coll Cardiol. 2006 Oct 17;48(8):1538–47.

6. Sabatier F, Camoin-Jau L, Anfosso F, Sampol J, Dignat-George F. Circulating endothelial cells, microparticles and progenitors: key players towards the definition of vascular competence. J Cell Mol Med. 2009 Mar;13(3):454–71.

7. Richardson MR, Yoder MC. Endothelial progenitor cells: quo vadis? J Mol Cell Cardiol. 2011 Feb;50(2):266–72.

8. Donahue M, Quintavalle C, Chiariello GA, Condorelli G, Briguori C. Endothelial progenitor cells in coronary artery disease. Biol Chem. 2013 Oct;394(10):1241–52.

9. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest. 2000 Jan 1;105(1):71–7.

10. Boos CJ, Balakrishnan B, Jessani S, Blann AD, Lip GYH. Effects of percutaneous coronary intervention on peripheral venous blood circulating endothelial cells and plasma indices of endothelial damage/dysfunction. Chest. 2007 Dec;132(6):1920–6.

11. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007 Mar 13;115(10):1285–95.

12. Rüegg C, Yilmaz A, Bieler G, Bamat J, Chaubert P, Lejeune FJ. Evidence for the involvement of endothelial cell integrin alphaVbeta3 in the disruption of the tumor vasculature induced by TNF and IFN-gamma. Nature Medicine. 1998 Apr;4(4):408–14.

13. Woywodt A. Circulating endothelial cells: life, death, detachment and repair of the endothelial cell layer. Nephrology Dialysis Transplantation. 2002 Oct 1;17(10):1728–30.

14. Bombeli T, Müller M, Straub PW, Haeberli A. Cyclosporine-induced detachment of vascular endothelial cells initiates the intrinsic coagulation system in plasma and whole blood. J Lab Clin Med. 1996 Jun;127(6):621–34.

15. Swerlick RA, Lee KH, Wick TM, Lawley TJ. Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. The Journal of Immunology. 1992 Jan 1;148(1):78–83.

16. Chong AY, Lip GYH, Freestone B, Blann AD. Increased circulating endothelial cells in acute

heart failure: Comparison with von Willebrand factor and soluble E-selectin. European Journal of Heart Failure. 2006 Sep 9;8(2):167–72.

17. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. Blood. 1999 May 1;93(9):2951–8.

18. Petzelbauer P, Bender JR, Wilson J, Pober JS. Heterogeneity of dermal microvascular endothelial cell antigen expression and cytokine responsiveness in situ and in cell culture. The Journal of Immunology. 1993 Nov 1;151(9):5062–72.

19. Freyssinet J-M, Toti F. Formation of procoagulant microparticles and properties. Thromb Res. 2010 Apr;125:S46–8.

20. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation. 2000 Feb 29;101(8):841–3.

21. Solh El AA, Akinnusi ME, Berim IG, Peter AM, Paasch LL, Szarpa KR. Hemostatic implications of endothelial cell apoptosis in obstructive sleep apnea. Sleep Breath. 2008 Nov;12(4):331–7.

22. Bombeli T, Karsan A, Tait JF, Harlan JM. Apoptotic vascular endothelial cells become procoagulant. Blood. 1997 Apr 1;89(7):2429–42.

23. Berckmans RJ, Nieuwland R, Böing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. Thromb Haemost. 2001 Apr;85(4):639–46.

24. Makin AJ, Blann AD, Chung NAY, Silverman SH, Lip GYH. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von Willebrand factor and tissue factor. European Heart Journal. 2004 Mar;25(5):371–6.

25. Freestone B, Lip GYH, Chong AY, Nadar S, Lee KW, Blann AD. Circulating endothelial cells in atrial fibrillation with and without acute cardiovascular disease. Thromb Haemost. 2005 Sep 9.

26. Boos CJ, Balakrishnan B, Blann AD, Lip GYH. The relationship of circulating endothelial cells to plasma indices of endothelial damage/dysfunction and apoptosis in acute coronary syndromes: implications for prognosis. J Thromb Haemost. 2008 Nov;6(11):1841–50.

27. Boos CJ, Jaumdally RJ, MacFadyen RJ, Varma C, Lip GYH. Circulating endothelial cells and von Willebrand factor as indices of endothelial damage/dysfunction in coronary artery disease: a comparison of central vs. peripheral levels and effects of coronary angioplasty. J Thromb Haemost. 2007 Mar;5(3):630–2.

28. Boos CJ, Balakrishnan B, Lip GYH. The effects of exercise stress testing on soluble E-selectin, von Willebrand factor, and circulating endothelial cells as indices of endothelial damage/dysfunction. Ann Med. 2008;40(1):66–73.

29. Lee KW, Lip GYH, Tayebjee M, Foster W, Blann AD. Circulating endothelial cells, von Willebrand factor, interleukin-6, and prognosis in patients with acute coronary syndromes. Blood. 2005 Jan 15;105(2):526–32.

30. Martínez-Sales V, Sánchez-Lázaro I, Vila V, Almenar L, Contreras T, Reganon E. Circulating endothelial cells in patients with heart failure and left ventricular dysfunction. Dis Markers. 2011;31(2):75–82.

31. Nadar SK, Lip GYH, Lee KW, Blann AD. Circulating endothelial cells in acute ischaemic stroke. Thromb Haemost. 2005 Sep 9.

32. Lee KW, Blann AD, Lip GYH. Inter-relationships of indices of endothelial damage/dysfunction [circulating endothelial cells, von Willebrand factor and flow-mediated dilatation] to tissue factor and interleukin-6 in acute coronary syndromes. International Journal of Cardiology. 2006 Aug;111(2):302–8.

33. Chong AY, Blann AD, Patel J, Freestone B, Hughes E, Lip GYH. Endothelial dysfunction and damage in congestive heart failure: relation of flow-mediated dilation to circulating endothelial cells, plasma indexes of endothelial damage, and brain natriuretic peptide. Circulation. 2004 Sep 28;110(13):1794–8.

34. Gao Y, Liu C, Zhang X, Gao J, Yang C. Circulating endothelial cells as potential markers of atherosclerosis. Can J Neurol Sci. 2008 Nov;35(5):638–42.

35. Onat D, Brillon D, Colombo PC, Schmidt AM. Human Vascular Endothelial Cells: A Model System for Studying Vascular Inflammation in Diabetes and Atherosclerosis. Endothelial cell high-enrichment from endovascular biopsy sample by laser capture microdissection and fluorescence activated cell sorting. 2011 Feb 22;11(3):193–202.

36. Fadini GP, Avogaro A. Cell-based methods for ex vivo evaluation of human endothelial biology. Cardiovascular Research. The Oxford University Press; 2010 Jul 1;87(1):12–21.

37. Solovey A, Gui L, Key NS, Hebbel RP. Tissue factor expression by endothelial cells in sickle cell anemia. J Clin Invest. 1998 May 1;101(9):1899–904.

38. Mutunga M, Fulton B, Bullock R, Batchelor A, Gascoigne A, Gillespie JI, et al. Circulating endothelial cells in patients with septic shock. Am J Respir Crit Care Med. 2001 Jan;163(1):195–200.

39. Campioni D, Zauli G, Gambetti S, Campo G, Cuneo A, Ferrari R, et al. In Vitro Characterization of Circulating Endothelial Progenitor Cells Isolated from Patients with Acute Coronary Syndrome. PLoS ONE. Public Library of Science; 2013 Feb 11;8(2):e56377.

40. Lee DY, Cho T-J, Kim JA, Lee HR, Yoo WJ, Chung CY, et al. Mobilization of endothelial progenitor cells in fracture healing and distraction osteogenesis. Bone. 2008 May;42(5):932–41.

41. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell. 2002 May 31;109(5):625–37.

42. Teraa M, Sprengers RW, Westerweel PE, Gremmels H, Goumans M-JTH, Teerlink T, et al. Bone Marrow Alterations and Lower Endothelial Progenitor Cell Numbers in Critical Limb Ischemia Patients. Pesce M, editor. PLoS ONE. Public Library of Science; 2013 Jan 31;8(1):e55592.

43. Yip H-K, Sun C-K, Tsai T-H, Sheu J-J, Kao Y-H, Lin Y-C, et al. Tissue plasminogen activator enhances mobilization of endothelial progenitor cells and angiogenesis in murine limb ischemia. International Journal of Cardiology. Elsevier; 2012 Oct.

44. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. Nat Rev Cancer. 2006 Nov;6(11):835–45.

45. George AL, Bangalore-Prakash P, Rajoria S, Suriano R, Shanmugam A, Mittelman A, et al. Endothelial progenitor cell biology in disease and tissue regeneration. J Hematol Oncol. BioMed Central Ltd; 2011;4(1):24.

46. Rafii S. Circulating endothelial precursors: mystery, reality, and promise. J Clin Invest. American Society for Clinical Investigation; 2000 Jan 1;105(1):17–9.

47. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. New England Journal of Medicine. 2005 Sep 8;353(10):999–1007.

48. Hill JM, Zalos G, Halcox JPJ, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. New England Journal of Medicine. 2003 Feb 13;348(7):593–600.

49. Bonello L, BASIRE A, Sabatier F, Paganelli F, Dignat-George F. Endothelial injury induced by coronary angioplasty triggers mobilization of endothelial progenitor cells in patients with stable coronary artery disease1. J Thromb Haemost. 2006 May;4(5):979–81.

50. Woywodt A, Erdbruegger U, Haubitz M. Circulating endothelial cells and endothelial progenitor cells after angioplasty: news from the endothelial rescue squad. J Thromb Haemost. 2006 May;4(5):976–8.

51. Nomura S, Ozaki Y, Ikeda Y. Function and role of microparticles in various clinical settings. Thromb Res. 2008 Jan;123(1):8–23.

52. Burger D, Touyz RM. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. Journal of the American Society of Hypertension. 2012 Mar;6(2):85–99.

53. Lanuti P, Santilli F, Marchisio M, Pierdomenico L, Vitacolonna E, Santavenere E, et al. A novel flow cytometric approach to distinguish circulating endothelial cells from endothelial microparticles: Relevance for the evaluation of endothelial dysfunction. Journal of Immunological methods. 2012 Mar 31.

54. Lee S-T, Chu K, Jung K-H, Kim J-M, Moon H-J, Bahn J-J, et al. Circulating CD62E+ microparticles and cardiovascular outcomes. PLoS ONE. 2012;7(4):e35713.

55. Sinning J-M, Losch J, Walenta K, Böhm M, Nickenig G, Werner N. Circulating CD31+/Annexin V+ microparticles correlate with cardiovascular outcomes. European Heart Journal. 2011 Aug;32(16):2034–41.

56. Bernal-Mizrachi L, Jy W, Jimenez JJ, Pastor J, Mauro LM, Horstman LL, et al. High levels of circulating endothelial microparticles in patients with acute coronary syndromes. American Heart Journal. 2003 Jun;145(6):962–70.

57. Zielińska M, Koniarek W, Goch JH, Cebula B, Tybura M, Robak T, et al. Circulating endothelial microparticles in patients with acute myocardial infarction. Kardiol Pol. 2005 Jun;62(6):531–42–discussion543–4.

58. Jung C, Sörensson P, Saleh N, Arheden H, Rydén L, Pernow J. Circulating endothelial and platelet derived microparticles reflect the size of myocardium at risk in patients with ST-

elevation myocardial infarction. Atherosclerosis. 2012 Mar;221(1):226–31.

59. Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. Nature Publishing Group; 2009 Jun 5;9(8):581–93.

60. Montoro-García S, Shantsila E, Marín F, Blann A, Lip GYH. Circulating microparticles: new insights into the biochemical basis of microparticle release and activity. Basic Res Cardiol. Springer-Verlag; 2011 Jun 21;106(6):911–23.

61. Collier MEW, Mah P-M, Xiao Y, Maraveyas A, Ettelaie C. Microparticle-associated tissue factor is recycled by endothelial cells resulting in enhanced surface tissue factor activity. Thromb Haemost. 2013 Nov;110(5):966–76.

62. Boos CJ, Lane DA, Karpha M, Beevers DG, Haynes R, Lip GYH. Circulating endothelial cells, arterial stiffness, and cardiovascular risk stratification in hypertension. Chest. 2007 Nov;132(5):1540–7.

63. Boos CJ, Soor SK, Kang D, Lip GYH. Relationship between circulating endothelial cells and the predicted risk of cardiovascular events in acute coronary syndromes. European Heart Journal. 2007 May;28(9):1092–101.

64. Mourino-Alvarez L, Calvo E, Moreau J, Padial LR, Lopez JA, Barderas MG, et al. Proteomic Characterization of EPCs and CECs “in vivo” from Acute Coronary Syndrome patients and Control subjects. Biochimica et Biophysica Acta (BBA) - General Subjects. 2012 Dec.

65. Boos CJ, Lane DA, Kang D, Goon PKY, Blann AD, Lip GYH. Temporal and venepuncture-related decline in circulating endothelial cell capture from mixed venous blood. J Thromb Thrombolysis. Kluwer Academic Publishers; 2006 Oct;22(2):125–31.

66. Li C, Wu Q, Liu B, Yao Y, Chen Y, Zhang H, et al. Detection and Validation of Circulating Endothelial Cells, a Blood-based Diagnostic Marker of Acute Myocardial Infarction. PLoS ONE. Public Library of Science; 2013 Mar 4;8(3):e58478.

67. Quilici J, Banzet N, Paule P, Meynard J-B, Mutin M, Bonnet J-L, et al. Circulating endothelial cell count as a diagnostic marker for non-ST-elevation acute coronary syndromes. Circulation [Internet]. 2004 Sep 21;110(12):1586–91. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=15364807&retmode=ref&cmd=prlinks

68. Leask RL, Jain N, Butany J. Endothelium and valvular diseases of the heart. Microsc Res Tech. 2003 Jan 21;60(2):129–37.

69. Damani S, Bacconi A, Libiger O, Chourasia AH, Serry R, Gollapudi R, et al. Characterization of Circulating Endothelial Cells in Acute Myocardial Infarction. Science Translational Medicine. 2012 Mar 21;4(126):126ra33–3.

70. Bethel K, Luttgen MS, Damani S, Kolatkar A, Lamy R, Sabouri-Ghomi M, et al. Fluid phase biopsy for detection and characterization of circulating endothelial cells in myocardial infarction. Phys Biol. IOP Publishing; 2014 Jan 9;11(1):016002.

71. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood. 2001 Jun 1;97(11):3658–61.

72. Kraan J, Sleijfer S, Foekens JA, Gratama JW. Clinical value of circulating endothelial cell

detection in oncology. Drug Discov Today. 2012 Jan 24.

73. Blann AD, Pretorius A. Circulating endothelial cells and endothelial progenitor cells: two sides of the same coin, or two different coins? Atherosclerosis. 2006 Sep;188(1):12–8.

74. Rusk A, McKeegan E, Haviv F, Majest S, Henkin J, Khanna C. Preclinical Evaluation of Antiangiogenic Thrombospondin-1 Peptide Mimetics, ABT-526 and ABT-510, in Companion Dogs with Naturally Occurring Cancers. Clinical Cancer Research. 2006 Dec 22;12(24):7444–55.

75. Monestiroli S, Mancuso P, Burlini A, Pruneri G, Dell'Agnola C, Gobbi A, et al. Kinetics and viability of circulating endothelial cells as surrogate angiogenesis marker in an animal model of human lymphoma. Cancer Res. 2001 Jun 1;61(11):4341–4.

76. BERTONI G, RAMPOLDI V. Activation of coagulation system during air travel. The Lancet. 2006 Jul 7;368(9529):25–6.

77. Wills TB, Heaney AM, Jane Wardrop K, Haldorson GJ. Immunomagnetic isolation of canine circulating endothelial and endothelial progenitor cells. Veterinary Clinical Pathology. 2009 Dec;38(4):437–42.

78. Tropea MM, Harper BJA, Graninger GM, Phillips TM, Ferreyra G, Mostowski HS, et al. Isolation of a circulating CD45-, CD34dim cell population and validation of their endothelial phenotype. Thromb Haemost. 2014 Jul 24;112(4).

79. Dignat-George F, Brisson C, Poncelet P, Laurent JC, Massot O, Arnoux D, et al. Rapid isolation of human endothelial cells from whole blood using S-Endo1 monoclonal antibody coupled to immuno-magnetic beads: demonstration of endothelial injury after angioplasty. Thromb Haemost. 1992 Jan 23;67(1):147–53.

80. Hladovec J, Přerovský I, Staněk V, Fabián J. Endotheliale Zellen im zirkulierenden Blut bei akutem Myokardinfarkt und Angina pectoris. Klinische Wochenschrift. Springer-Verlag; 1978 Oct;56(20):1033–6.

81. Lampka M, Grąbczewska Z, Jendryczka-Maćkiewicz E, Hołyńska-Iwan I, Sukiennik A, Kubica J, et al. Circulating endothelial cells in coronary artery disease. Kardiol Pol. 2010 Oct;68(10):1100–5.

82. Vargová K, Toth-Zsamboki E, Beres BJ, Bencze J, Kerecsen G, Gulacsi-Bardos P, et al. Circulating endothelial cell count, plasma vWF and soluble ICAM-1 levels following primary or elective percutaneous coronary intervention. Atherosclerosis. 2008 Jun;198(2):366–72.

83. Wang C, Li H, Fu P, Zhang S, Xiu R. Serum C-reactive protein and circulating endothelial cells in patients with acute myocardial infarction. Clin Hemorheol Microcirc. 2005;32(4):287–96.

Figure Legends

Figure 1: Summary of CEC, EPC and endothelial microparticle physiology and pathology, including mechanisms of release, and mechanisms by which cellular components contribute to local vascular thrombosis.

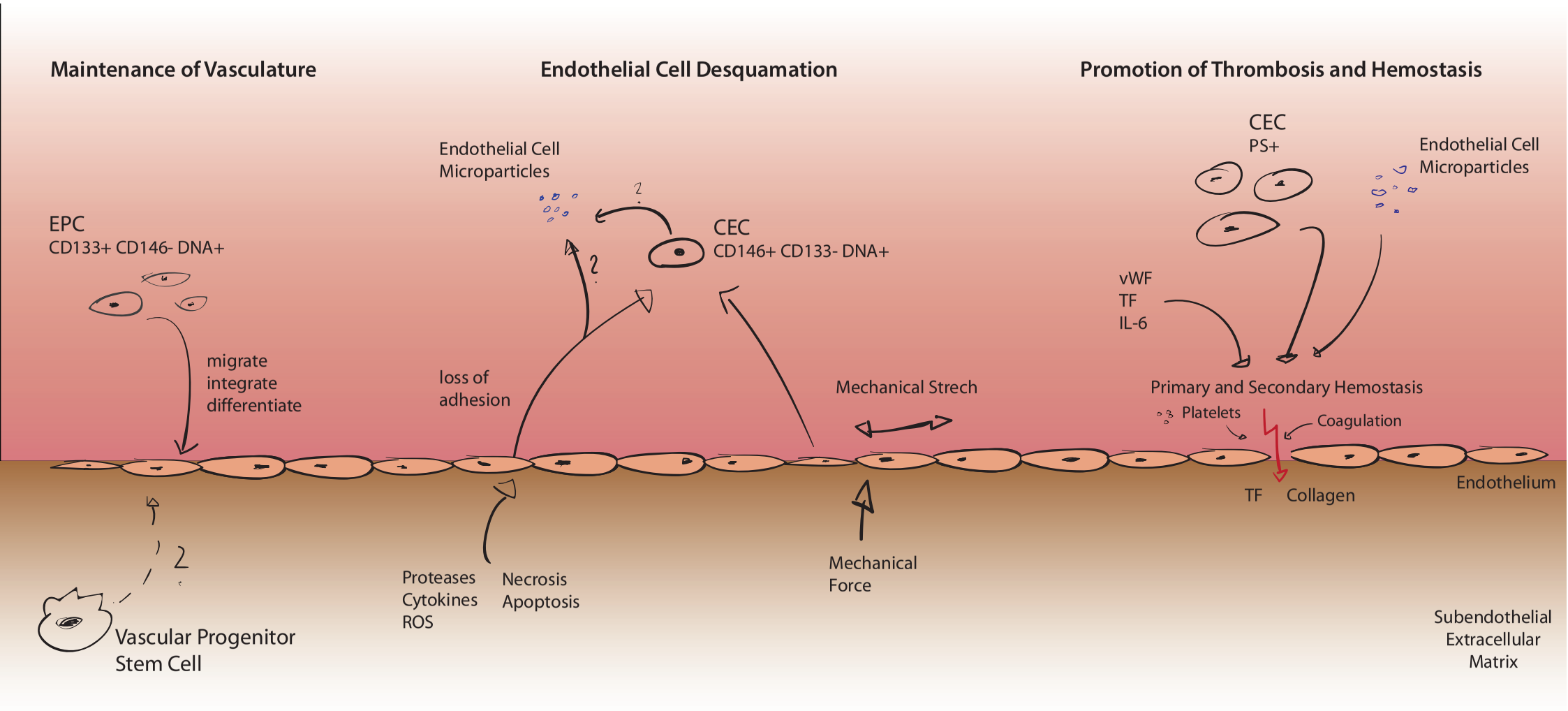
Maintenance of Vasculature
Endothelial Cell Desquamation
Promotion of Thrombosis and Hemostasis
EPC
CD133+ CD146- DNA+
migrate
integrate
differentiate
Vascular Progenitor
Stem Cell
Endothelial Cell
Microparticles
CEC
CD146+ CD133- DNA+
loss of
adhesion
Proteases
Cytokines
ROS
Necrosis
Apoptosis
Mechanical Strech
Mechanical
Force
CEC
PS+
Endothelial Cell
Microparticles
vWF
TF
IL-6
Primary and Secondary Hemostasis
Platelets
Coagulation
TF
Collagen
Endothelium
Subendothelial
Extracellular
Matrix

Table 1: Overview of markers and isolation methodology used for CEC enumeration in publications regarding CEC in ACS and CAD.

Publication	CEC markers	Technique
Bethel et al. 2014 (70)	$CD146^+$, $CD45^-$, $CD105^+$, $DAPI^+$	IB/FM (CS)
	$CD146^+$, $CD45^-$, vWF^+, $DAPI^+$	fluid phase biopsy
Bonello et al. 2006 (49)	$CD146^+$	IB
Boos et al. 2007 (10)	$CD146^+$, UEL, size 10-50 µm	IB/FM
Boos et al. 2007 (27)	$CD146^+$, UEL, size 10-50 µm	IB/FM
Boos et al. 2007 (62)	$CD146^+$, UEL, size 10-50 µm	IB/FM
Boos et al. 2007 (63)	$CD146^+$, UEL, size 10-50 µm	IB/FM
Boos et al. 2008 (26)	$CD146^+$, UEL, size 10-50 µm	IB/FM
Damani et al. 2012 (69)	$CD146^+$, $CD45^-$, $CD105^+$, $DAPI^+$	IB/FM (CS)
Dignat – George et al. 1992 (79)	$CD146^+$	IB
Freestone et al. 2005 (25)	$CD146^+$, size > 20 µm	IB/FM
Hladovec et al. 1978 (80)	size and shape	serial centrifugation
Lampka et al. 2010 (81)	$CD146^+$, $CD45^-$, $CD31^+$	flow cytometry
Lee et al. 2005 (29)	$CD146^+$, size 20-50 µm	IB/FM
Lee et al. 2006 (32)	$CD146^+$, size 20-50 µm	IB/FM
Li et al. 2013 (66)	$CD146^+$, $CD45^-$, Hoechst 33342^+, $CD31^+$, $CD133^-$ FWD/SSC.	flow cytometry
Makin et al. 2004 (24)	$CD146^+$, size > 20 µm	IB/FM
Mutin et al. 1999 (17)	$CD146^+$, size 20-50 µm	IB/FM
Mourino-Alvarez et al. 2012 (64)	$CD45^-$, $CD31^{bright}$, $CD34^+$, $CD\ 133^-$, FWD/SSC.	flow cytometry
Quilici et al. 2004 (67)	$CD146^+$, size 20-50 µm.	IB/FM
Vargova et al. 2008 (82)	$CD146^+$, size 20-50 µm.	IB/FM
Wang et al. 2005 (83)	$CD146^+$, $CD31^+$, vWF^+, size 20-50 µm.	IB/EM

DAPI, nuclear staining of AT-rich regions; CS, CellSearch® platform; UEL, Ulex Europeus Lectin; IB, immunobead isolation; FM, fluorescence microscopy; EM, electron microscopy. FWD/SSC, size and density by forward- and sidescatter.

Table 2: Overview of studies that quantified circulating endothelial cells in the context of acute coronary syndrome and coronary artery disease, indicating study population and study outcomes.

Publication	Number of Subjects and Controls	Main Outcomes	Limitations
Bethel 2014 (70)	AMI, n=79 Vascular surgery, n=6 Healthy controls, n=34	CEC count was higher in AMI than in controls and patients that had undergone vascular surgery. CEC count between controls and vascular surgery patients did not differ. ROC-AUC was 93.3% and 95.4% for CS and fluid phase biopsy, respectively.	No distinction between STEMI/NSTEMI. Extramural transport of samples to research facility. Different anticoagulants used during sampling.
Bonello 2006 (49)	Elective PCI in stable CAD, n=15	Baseline CEC count was comparable to other healthy cohorts. CEC count increased after PCI with a peak at 6h post-PCI, and returned to baseline after 7 days. CEC peak value also correlated with post-procedural cardiac Troponin levels, which is related to worse outcomes.	Correlation of increased CEC count with troponin after PCI may be weaker in patients with more extensive preexistent coronary disease. No measurement of other plasma markers of endothelial injury, e.g. soluble E-selectin or vWF. Blood sampling technique not described.
Boos 2007 (10)	Elective PCI in stable CAD, n=38 Diagnostic coronary angiography, n=15 Healthy controls, n=39	Baseline CEC count was similar amongst groups. PCI resulted in an increase in CEC count, while angiography alone did not.	Limited longitudinal information because only 15min and 24h postprocedural samples were obtained. Small coronary angiography group.
Boos 2007 (62)	Hypertension, n=65 Malignant hypertension, n=43 Healthy controls, n=63	CEC count followed the patient groups: malignant hypertension > hypertension > controls. CEC levels predicted Pocock score in patients.	No longitudinal follow up; instead use of a risk score to predict long-term risk for cardiovascular events.

Publication	Number of Subjects and Controls	Main Outcomes	Limitations
Boos 2007 (63)	NSTEMI, n=84 STEMI, n=67 Unstable Angina, n=46 Healthy Controls, n=50	Baseline CEC count was increased in groups: STEMI > NSTEMI > UA as compared to controls. An increased CEC count correlated with higher calculated cardiovascular risk using the scores TIMI, PURSUIT and GRACE. Presence of heart failure was associated with increased CEC numbers. ROC – AUC was 85% for STEMI/NSTEMI. A high CEC count had a PPV of 91% and a NPV of 53.5% for diagnosis of myocardial infarction.	Blood sampling for determination of CEC count was occasionally performed after PCI/thrombolysis. The timing of blood sampling was spread until up to 24h after initial presentation. Risk scores were used instead of prospective follow up.
Boos 2007 (27)	Elective cardiac angiography, n=32 ACS, n=9 Of those total n=41, 26 had PCI	Pre-procedural CEC count was higher in ACS patients than in elective patients. Stent placement increased CEC count in samples from the coronary sinus, aortic root, and femoral vein.	Only 1 sampling point after PCI. Delay between sampling from arterial and venous sites.
Boos 2008 (26)	STEMI, n=72 NSTEMI, n=87 Unstable Angina, n=52 Stable CAD, n=45 Healthy controls, n=60	CEC count was increased in groups, following STEMI > NSTEMI > UA > controls. A high CEC count was associated with major adverse cardiac events (HR 2.4; 95% CI 1.2-4.1) and cardiovascular death (HR 2.95, 95% CI 1.01 – 8.81) during follow up. CEC count was a predictor of cardiac events, but not CV death (when applying Kaplan-Meier survival and multivariate analysis, respectively).	Significant spread in timing of venipuncture for CEC enumeration (2-23h after ACS presentation). 15% of samples were taken after PCI/thrombolysis.
Damani 2012 (69)	STEMI, n=50 Healthy controls, n=44	CEC count was increased in STEMI compared to controls. Elevated CECs did not correlate with CK-MB or cardiac Troponin. ROC AUC for STEMI was 95%.	Young age of healthy controls in comparison with STEMI cases. Timing of cardiac enzyme determination not reported. Extramural transport of samples.

Publication	Number of Subjects and Controls	Main Outcomes	Limitations
Freestone 2005 (25)	AMI + atrial fibrillation, n=22 Left ventricular failure + AF, n=20 CVA + atrial fibrillation, n=21 Chronic atrial fibrillation, n=28 Healthy controls, n=20	CEC were stepwise with CVA + AF > LVF + AF > AMI + AF. All of those groups had increased CEC count relative to chronic AF and controls. There was no difference between patients with chronic atrial fibrillation and controls.	Temporal spread in blood sampling up to 48h after admission. Possible confounding by varying presence of hypertension.
Hladovec 1978 (80)	Transmural AMI, n=23 Non-transmural AMI, n=22 Severe rest angina, n=14 Mild angina, n=22 Controls, n=24 (heterogeneous)	CEC count was elevated in patients with acute myocardial infarction and severe angina, while controls and patients with mild angina had comparable counts.	CEC definition only by shape/morphology, as assessed by microscopy. The article is inconclusive about the specific properties used for characterization of CEC. MI and angina pectoris defined by WHO criteria of 1978.
Lampka 2010 (81)	AMI, n=23 Stable angina, n=25 Healthy controls, n=20	CEC count in AMI was increased relative to stable angina patients and controls. Sensitivity of CEC count for AMI diagnosis was 39%. Combined CEC and Troponin I had a sensitivity of 73%.	Timing of blood sampling (for CEC enumeration and troponin) from presentation of symptoms not reported. Diagnostic criteria for AMI and SA not presented. No calculation of specificity, PPV and NPV.
Lee 2005 (29)	ACS, n=156 Stable CAD, n=36 Healthy controls, n=40	Baseline CEC count was higher in ACS patients than in stable CAD or in controls. On multivariate analysis, 48h CEC count was an independent predictor of major cardiac events at 30-days, as well as death at 30-days and 1-year follow-up.	No distinction between STEMI/NSTEMI.
Lee 2006 (32)	AMI, n=80 Unstable angina, n=40 Stable angina, n=40 Healthy controls, n=40	CEC count was increased between groups with AMI > UA > SA or controls.	No distinction between STEMI/NSTEMI.

Publication	Number of Subjects and Controls	Main Outcomes	Limitations
Li 2013 (66)	AMI, n=61 AMI, 1 month post-treatment, n=19 Healthy controls, n=45	CEC count was elevated in ACS relative to controls. At 1 month after treatment, the CEC count had decreased.	Timing of blood sampling not reported, unclear whether primary interventions/PCI were performed before. Unclear whether samples were from STEMI/NSTEMI patients. Relatively low mean of troponin and CK-MB in AMI group.
Makin 2004 (24)	Ischemic rest pain (PAD), n=20 Intermittend claudication (PAD), n=20 AMI, n=20 Healthy controls, n=20	CEC count in AMI was higher than in patients with peripheral artery disease or healthy controls.	Blood sampling anywhere within 24h period after admission to coronary care unit.
Mourino-Alvarez 2012 (64)	STEMI, n=29 Healthy controls, n=29	No significant difference in CEC count between STEMI and controls.	Only study in which CEC were not identified by CD146. May be underpowered (low number of subjects).
Mutin 1999 (17)	AMI, n=26 Unstable Angina, n=33 Effort Angina, n=13 Healthy controls, n=14	CECs were detectable in AMI and UA, but not in EA and controls.	AMI patients were hospitalized >6h after onset of symptoms (patients who presented earlier were excluded from the study). Four AMI patients were thrombolysed.
Quilici 2004 (67)	NSTEMI, n=60 Controls (valve repair), n=40	In the NSTEMI group, 32/60 cases had an elevated CEC count. CEC enumeration had a PPV of 100% and a NPV of 58.8%. The admission time CEC count ROC AUC was 78% compared with 67% for Troponin I. 4-hour Troponin ROC-AUC was 0.76, which was similar to that of the CEC count. Combined 4-hour CEC count – Troponin I was 86%.	Different timing of interventions across the patient group.

Publication	Number of Subjects and Controls	Main Outcomes	Limitations
Vargova 2008 (82)	STEMI, n=23 Stable angina with PCI, n=23 Stable angina, with coronary angiography only, n=23	Baseline CEC count was higher in the STEMI group compared to stable angina groups. PCI and coronary angiography produced small increases in CEC levels.	Spread in event-to-balloon time with 25% of patients sampled less than 3h or more than 6h after onset of symptoms.
Wang 2005 (83)	AMI, n=37 Healthy controls, n=42	CEC count was higher in the AMI group than in controls.	Patients presented without any previous interventions to the coronary care unit 24-48h after onset of chest pain.

Abbreviations: AMI, acute myocardial infarction; NSTEMI, non-ST segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PAD, peripheral artery disease; Pocock score, predicted 5 year risk of cardiovascular death in heart failure; TIMI risk score, estimates 14-day mortality for patients with unstable angina and NSTEMI; PURSUIT, estimates 30-day mortality for patients with unstable angina and NSTEMI; GRACE, estimates in-hospital mortality for patients with unstable angina, NSTEMI, and STEMI; CS, CellSearch® platform.

Table 3: Receiver operating characteristics area-under-the-curve results from studies that analyzed the diagnostic potential of CEC enumeration.

Publication	Diagnosis	Marker	ROC AUC	Patients and controls
Quilici et al. 2004 (67)	NSTEMI	Admission CEC	0.78	NSTEMI, n=60 Valvular disease patients, n=40
		CEC + troponin	0.82	
		4h CEC	0.76	
		4h CEC + troponin	0.87	
		8h CEC	0.67	
		8h CEC + troponin	0.85	
Boos et al. 2007 (63)	ACS	CEC	0.82	ACS patients, n=197 Healthy controls, n=50
	UA		0.75	n=46
	STEMI		0.88	n=67
	NSTEMI		0.82	n=84
	STEMI/NSTEMI		0.85	n=151
Damani et al. 2012 (69)	STEMI	CEC	0.95	STEMI patients, n=50 Healthy controls, n=44
Bethel et al. 2014 (70)	MI	CEC	0.93 and 0.95*	MI patients, n=79 Healthy controls, n=25

*, Two different techniques for CEC enumeration were used.