Circulating Endothelial Cells in Coronary Artery Disease and Acute Coronary Syndrome

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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 hematopoietic 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.

Acknowledgements

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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.
TF Collagen Platelets Coagulation Vascular Progenitor Stem Cell

- EPC CD133+ CD146- DNA+
- migrate integrate differentiate

**Maintenance of Vasculature**

**Endothelial Cell Desquamation**

- Endothelial Cell Microparticles
- loss of adhesion
- Mechanical Stretch
- Proteases Cytokines ROS Necrosis Apoptosis

**Promotion of Thrombosis and Hemostasis**

- CEC CD146+ CD133- DNA+
- CEC PS+
- vWF TF IL-6
- Primary and Secondary Hemostasis Platelets Coagulation
- TF Collagen Subendothelial Extracellular Matrix
Table 1: Overview of markers and isolation methodology used for CEC enumeration in publications regarding CEC in ACS and CAD.

<table>
<thead>
<tr>
<th>Publication</th>
<th>CEC markers</th>
<th>Technique</th>
</tr>
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<tbody>
<tr>
<td>Bethel et al. 2014 (70)</td>
<td>CD146+, CD45+, CD105+, DAPI+</td>
<td>IB/FM (CS)</td>
</tr>
<tr>
<td></td>
<td>CD146+, CD45+, vWF+, DAPI+</td>
<td>fluid phase biopsy</td>
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<tr>
<td>Bonello et al. 2006 (49)</td>
<td>CD146+</td>
<td>IB</td>
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<tr>
<td>Boos et al. 2007 (10)</td>
<td>CD146+, UEL, size 10-50 µm</td>
<td>IB/FM</td>
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<td>Boos et al. 2007 (27)</td>
<td>CD146+, UEL, size 10-50 µm</td>
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<tr>
<td>Boos et al. 2008 (26)</td>
<td>CD146+, UEL, size 10-50 µm</td>
<td>IB/FM</td>
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<tr>
<td>Dignat – George et al. 1992 (79)</td>
<td>CD146+</td>
<td>IB</td>
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<tr>
<td>Freestone et al. 2005 (25)</td>
<td>CD146+, size &gt; 20 µm</td>
<td>IB/FM</td>
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<tr>
<td>Hladovec et al. 1978 (80)</td>
<td>size and shape</td>
<td>serial centrifugation</td>
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<tr>
<td>Lampka et al. 2010 (81)</td>
<td>CD146+, CD45+, CD31+</td>
<td>flow cytometry</td>
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<tr>
<td>Lee et al. 2005 (29)</td>
<td>CD146+, size 20-50 µm</td>
<td>IB/FM</td>
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<tr>
<td>Lee et al. 2006 (32)</td>
<td>CD146+, size 20-50 µm</td>
<td>IB/FM</td>
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<tr>
<td>Li et al. 2013 (66)</td>
<td>CD146+, CD45+, Hoechst 33342+, CD31+, CD133+</td>
<td>flow cytometry</td>
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<tr>
<td>Makin et al. 2004 (24)</td>
<td>CD146+, size &gt; 20 µm</td>
<td>IB/FM</td>
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<tr>
<td>Mutin et al. 1999 (17)</td>
<td>CD146+, size 20-50 µm</td>
<td>IB/FM</td>
</tr>
<tr>
<td>Mourino-Alvarez et al. 2012 (64)</td>
<td>CD45+, CD31bright+, CD34+, CD 133, FWD/SSC.</td>
<td>flow cytometry</td>
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<tr>
<td>Quilici et al. 2004 (67)</td>
<td>CD146+, size 20-50 µm</td>
<td>IB/FM</td>
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<tr>
<td>Vargova et al. 2008 (82)</td>
<td>CD146+, size 20-50 µm</td>
<td>IB/FM</td>
</tr>
<tr>
<td>Wang et al. 2005 (83)</td>
<td>CD146+, CD31+, vWF+, size 20-50 µm.</td>
<td>IB/EM</td>
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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 side scatter.
<table>
<thead>
<tr>
<th>Publication</th>
<th>Number of Subjects and Controls</th>
<th>Main Outcomes</th>
<th>Limitations</th>
</tr>
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<tbody>
<tr>
<td>Bethel 2014</td>
<td>AMI, n=79 Vascular surgery, n=6</td>
<td>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.</td>
<td>No distinction between STEMI/NSTEMI. Extramural transport of samples to research facility. Different anticoagulants used during sampling.</td>
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<td>Healthy controls, n=34</td>
<td></td>
<td></td>
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<tr>
<td>Bonello 2006 (49)</td>
<td>Elective PCI in stable CAD, n=15</td>
<td>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.</td>
<td>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.</td>
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<td>Electrocardiography, n=15</td>
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<td>Healthy controls, n=39</td>
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<tr>
<td>Boos 2007</td>
<td>Elective PCI in stable CAD, n=38</td>
<td>Baseline CEC count was similar amongst groups. PCI resulted in an increase in CEC count, while angiography alone did not.</td>
<td>Limited longitudinal information because only 15min and 24h postprocedural samples were obtained. Small coronary angiography group.</td>
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<td>Diagnostic coronary angiography,</td>
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<td></td>
<td>n=15</td>
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<tr>
<td></td>
<td>Healthy controls, n=39</td>
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<tr>
<td>Boos 2007</td>
<td>Hypertension, n=65</td>
<td>CEC count followed the patient groups: malignant hypertension &gt; hypertension &gt; controls. CEC levels predicted Pocock score in patients.</td>
<td>No longitudinal follow up; instead use of a risk score to predict long-term risk for cardiovascular events.</td>
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<td></td>
<td>Malignant hypertension, n=43</td>
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<tr>
<td></td>
<td>Healthy controls, n=63</td>
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</tbody>
</table>
| 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. |
<table>
<thead>
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<tbody>
<tr>
<td>Freestone 2005 (25)</td>
<td>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</td>
<td>CEC were stepwise with CVA + AF &gt; LVF + AF &gt; 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.</td>
<td>Temporal spread in blood sampling up to 48h after admission. Possible confounding by varying presence of hypertension.</td>
</tr>
<tr>
<td>Hladovec 1978 (80)</td>
<td>Transmural AMI, n=23 Non-transmural AMI, n=22 Severe rest angina, n=14 Mild angina, n=22 Controls, n=24 (heterogeneous)</td>
<td>CEC count was elevated in patients with acute myocardial infarction and severe angina, while controls and patients with mild angina had comparable counts.</td>
<td>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.</td>
</tr>
<tr>
<td>Lampka 2010 (81)</td>
<td>AMI, n=23 Stable angina, n=25 Healthy controls, n=20</td>
<td>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%.</td>
<td>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.</td>
</tr>
<tr>
<td>Lee 2005 (29)</td>
<td>ACS, n=156 Stable CAD, n=36 Healthy controls, n=40</td>
<td>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.</td>
<td>No distinction between STEMI/NSTEMI.</td>
</tr>
<tr>
<td>Lee 2006 (32)</td>
<td>AMI, n=80 Unstable angina, n=40 Stable angina, n=40 Healthy controls, n=40</td>
<td>CEC count was increased between groups with AMI &gt; UA &gt; SA or controls.</td>
<td>No distinction between STEMI/NSTEMI.</td>
</tr>
<tr>
<td>Publication</td>
<td>Number of Subjects and Controls</td>
<td>Main Outcomes</td>
<td>Limitations</td>
</tr>
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</tbody>
</table>
| 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. |
<table>
<thead>
<tr>
<th>Publication</th>
<th>Number of Subjects and Controls</th>
<th>Main Outcomes</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vargova 2008</td>
<td>STEMI, n=23</td>
<td>Baseline CEC count was higher in the STEMI group compared to stable angina groups. PCI and coronary angiography produced small increases in CEC levels.</td>
<td>Spread in event-to-balloon time with 25% of patients sampled less than 3h or more than 6h after onset of symptoms.</td>
</tr>
<tr>
<td></td>
<td>Stable angina with PCI, n=23</td>
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<td>Stable angina, with coronary angiography only, n=23</td>
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</tr>
<tr>
<td>Wang 2005</td>
<td>AMI, n=37</td>
<td>CEC count was higher in the AMI group than in controls.</td>
<td>Patients presented without any previous interventions to the coronary care unit 24-48h after onset of chest pain.</td>
</tr>
<tr>
<td></td>
<td>Healthy controls, n=42</td>
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</tr>
</tbody>
</table>

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.

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

*, Two different techniques for CEC enumeration were used.