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Can the Number of Endothelial Progenitor Cells Help Predict Future Cardiovascular Events?

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Abstract
Background: Despite advances in treatment and risk factor management, coronary artery disease (CAD) remains the largest cause of mortality worldwide. Much has been made of the role of endothelial dysfunction in CAD, but tools to measure and augment its progression are lacking. Recent cellular biomarkers have been found to be involved in endothelial dysfunction and the presence of CAD. As the role of these biomarkers becomes more defined, can the measurement of endothelial progenitor cells now predict the risk of experiencing future cardiovascular events?

Methods: Exhaustive search of available medical literature was performed on the databases CINAHL, Web of Science, and Medline-OVID. These were searched using the keywords “cardiovascular events,” “endothelial cells,” and “risk assessment”. All included studies were assessed for quality using the GRADE scale.

Results: Three observational trials met inclusion requirements. One of these studies evaluated the correlation between endothelial progenitor cells (EPCs) and cardiovascular (CV) risk along with endothelial function. This study was able to establish a significant correlation between the number of EPCs and their functional ability and endothelial dysfunction in patients with cardiovascular disease. The other two studies utilized a prospective approach and measured EPC numbers at baseline and then followed the sample to record the number of CV events. The first of these two studies was able to demonstrate that reduced numbers of EPC independently predicts atherosclerotic disease progression, while the second showed that EPC levels can predict the occurrence of CV events and aide in risk stratification of people with increased CV risk.

Conclusion: Endothelial progenitor cells have been proven to have a role in endothelial dysfunction, cardiovascular disease progression, and now risk assessment. While many factors play into the overall mechanism in which EPC levels affect cardiovascular risk and endothelial repair, monitoring blood levels of EPCs has become a feasible biomarker that can be added to further stratify ones CV risk profile.

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Can the Number of Endothelial Progenitor Cells Help Predict Future Cardiovascular Events?

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A Clinical Graduate Project Submitted to the Faculty of the
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Biography

[Redacted for privacy]
Abstract

Background: Despite advances in treatment and risk factor management, coronary artery disease (CAD) remains the largest cause of mortality worldwide. Much has been made of the role of endothelial dysfunction in CAD, but tools to measure and augment its progression are lacking. Recent cellular biomarkers have been found to be involved in endothelial dysfunction and the presence of CAD. As the role of these biomarkers becomes more defined, can the measurement of endothelial progenitor cells now predict the risk of experiencing future cardiovascular events?

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Acknowledgements

[Redacted for privacy]
# Table of Contents

Biography ............................................................................................................................ 2  
Abstract ............................................................................................................................... 3  
Acknowledgements ............................................................................................................. 4  
Table of Contents ................................................................................................................ 5  
List of Tables ...................................................................................................................... 6  
List of Abbreviations .......................................................................................................... 6  
BACKGROUND ................................................................................................................ 7  
METHODS ....................................................................................................................... 10  
RESULTS ......................................................................................................................... 10  
WERNER (2005) ET AL ....................................................................................................... 11  
SCHMIDT-LUCKE ET AL ...................................................................................................... 14  
WERNER (2007) ET AL ....................................................................................................... 17  
DISCUSSION ................................................................................................................... 20  
CONCLUSION ................................................................................................................. 25  
References ......................................................................................................................... 27  
Tables ................................................................................................................................ 33
List of Tables

Table 1: Characteristics of Reviewed Studies
Table 2: Summary of Findings

List of Abbreviations

ACE……………………………………………………..Angiotensin-Converting Enzyme
ACS……………………………………………………..Acute Coronary Syndrome
AMI……………………………………………………..Acute Myocardial Infarction
CAD……………………………………………………..Coronary Artery Disease
CABG……………………………………………….Coronary Artery Bypass Graft
CEC……………………………………………………..Circulating Endothelial Cell
CFU-EC………………………………………………Colony Forming Unit-Endothelial Cell
CV……………………………………………………..Cardiovascular
CVD…………………………………………………….Cardiovascular Disease
EMP………………………………………………….Endothelial Microparticles
EPC……………………………………………………Endothelial Progenitor Cell
HR……………………………………………………….Hazard Ratio
IGF-1………………………………………………….Insulin-like Growth Factor-1
PCI………………………………………………….Percutaneous Coronary Intervention
SA……………………………………………………..South Asian
STEMI………………………………………………ST-segment Elevation Myocardial Infarction
UK……………………………………………………..United Kingdom
VEGF………………………………………………..Vascular Endothelial Growth Factor
Can the Number of Endothelial Progenitor Cells help Predict Future Cardiovascular Events?

BACKGROUND
Cardiovascular disease (CVD) is the number one killer of people in the United States and worldwide. Every minute, an American dies from coronary artery disease (CAD) and 37% of people who suffer from an acute cardiovascular event will die within the year. The treatment and recognition of CAD has been improving and a dramatic decline has been experienced in death rates over the last 35 years. This is in part from new targeted treatments of CAD and management of cardiovascular risk factors. Despite this improvement, CAD still affects about 16 million adults in the US and is still responsible for approximately one in five deaths in the US. This accounts for around 600,000 deaths per year in the United States. Coronary artery disease alone is responsible for $108.9 billion cost to the United States including health care costs, medication, and lost productivity. Since the incidence of CAD increases with increasing age, the US population will observe an increase in the burden of CAD on our medical system.

It is well known that presence of traditional cardiovascular risk factors: age, family history of premature heart disease, diabetes, male gender, smoking, hypertension, hyperlipidemia, obesity, and physical inactivity put one at a higher risk of developing CAD and contribute to endothelial dysfunction; less understood is how these factors interplay with the pathogenesis of CAD. CAD has now has been established as an endovascular inflammatory process that triggers a cascade of destructive events, but what
causes this process to start? More importantly, how can clinicians measure this dysfunction and attempt to predict its course?

Endothelial dysfunction is a term that describes the shift from healthy endothelium to a damaged, pro-inflammatory, pro-coagulative, pro-vasoconstrictive endothelium which lends itself to cardiovascular complications. The recognition of this process as a key part of the pathogenesis of CAD has led to the investigation of several biomarkers that play roles in this dysfunction: circulating endothelial cells (CECs), endothelial microparticles (EMPs), and endothelial progenitor cells (EPCs). Current consensus is that CECs are mature endothelial cells that shed from the vascular lining in response to injury. EMPs are similar in the fact that their presence is often attributed with injury to the endothelial lining. EPCs are immature endothelial cells that originate from bone marrow; these cells are currently associated with vascular regeneration and endothelial repair. While lower levels of this cell type would indicate inability to repair, there also has been an association of increased number of EPCs and acute ischemic events. It has also been shown that differentiation of these cells has led to the new growth of vascular structures. This has led to investigating these cells as different therapeutic options where new vessel growth is indicated.

Recording endothelial dysfunction in subjects is not a simple variable to measure. Most often this is done non-invasively through flow mediated dilation of the brachial artery which takes advantage of the fact that shear stress applied to a vessel will cause nitric oxide release and subsequent vasodilation. Other methods include invasively measuring vascular response to acetylcholine infusion. Measuring the levels of these cellular biomarkers is best accomplished through flow cytometry. This process involves
blood being drawn from a subject and then staining of cells to allow detection of specific receptors that are associated with each type of cell. EPCs are mainly categorized into those which express CD34+/KDR+ receptors or CD133+ receptors. KDR is another name for vascular endothelial growth factor (VEGF) receptor. Occasionally, these CD133+ cells are referred to as “immature” EPCs while CD34+ are thought to be “mature” EPCs, but more commonly these cells are referred to in terms of “early-growth” and “late-growth” EPCs which refers to length of time to grow on a cultured medium and the expression of other hematopoietic markers that tell their lineage from either hematopoietic stem cells or vascular stem cells. Both lines are thought to have roles in endothelial modification.

Other measurable characteristics of these cells are the colony-forming and migratory ability; these are measured on cultured growth assays. After a period of growth, the amounts of colony forming units (CFU-ECs) are counted and migration measured using fluoroscopy. These characteristics are thought to be tied to these cells functional capabilities. Speculation also lies in the process in which these cells are called to the area of injury as an area where disruption can occur. It has been theorized that many inflammatory cytokines and communication through the Akt-nitric oxide synthase pathway control the mobilization of these cells to areas of injury.

It has been seen that different disease processes as well as possessing certain cardiovascular risk factors can affect endothelial progenitor cells. Number of cells, mobilization to injury, and colony forming ability are all shown to be affected. With the observation that these factors have been associated with decreases in EPCs and EPC functionality, and that low numbers of EPCs are associated with endothelial dysfunction,
the question becomes what role do EPCs play in cardiovascular disease. Much has been
done in determining the function and purpose of these cells. What if they can help with
predicting future cardiovascular events and stratifying risk in an already diseased
population?

METHODS

A comprehensive search of the medical literature was done by searching the
databases CINAHL, WEB OF SCIENCE, and MEDLINE-OVID using the keywords
“cardiovascular events,” “endothelial cells,” and “risk assessment.” The returned search
results were then screened for relevance by including only English articles and study that
utilized human subjects. Further exclusion criteria were applied that include: studies
where epithelial progenitor cells (EPC) were not a primary area of focus, studies that
focused on EPC’s as a therapeutic option, those not related to cardiovascular risk
assessment, and those that looked at EPC’s in acute events. The bibliographies of
relevant studies were then searched for further relevant studies. All included studies were
then assessed for quality using the Grading of Recommendations, Assessment,
Development and Evaluation (GRADE) scale\(^{15}\); however, no exclusions were made
based on these results.

RESULTS

An initial search yielded 126 results. These were screened and narrowed to two
relevant articles\(^{10,13}\) after application of inclusion criteria, all being observational trials. A
search of the bibliographies yielded one additional relevant study\(^{16}\) that met inclusion
criteria, which is also observational in design. See Table 1.
WERNER (2005) ET AL

This prospective observational trial\textsuperscript{16} looked to answer the question if the number of circulating endothelial progenitor cells would correlate with cardiovascular outcomes. In order to answer this question the authors assessed the number of endothelial progenitor cells in 587 patients who underwent coronary angiography between March 2003 and January 2004. They then followed these patients for a follow-up period of 12 months to record the amount of cardiovascular outcomes or events that occurred. Cardiovascular events were defined as death from cardiovascular cause or occurrence of first cardiovascular event, which included acute myocardial infarction, hospitalization from cardiovascular event, need for revascularization, and death from CV cause. Death from all causes was also monitored.\textsuperscript{16}

Subjects who underwent angiography were scored for disease presence by two independent interventional cardiologists. Of the original 587 patients evaluated 49 patients without evidence of CAD on angiography and 19 patients with malignant or inflammatory disease or severe acute ischemia were excluded. Informed consent was obtained on all patients and the study protocol was approved by the ethics committee of the University of Saarland. The 12 patients who did not complete the follow-up were also excluded from the final results leaving a total of 507 subjects.\textsuperscript{16}

Subjects were assessed for previous cardiovascular events and follow-up data based on previous medical records and personal interviews. Individual risk factors for CVD and current treatment regimens were taken into account for risk stratification and included in statistical analysis. Causes of death were determined by examination of hospital records, autopsy reports, and medical files of the subject’s general practitioners.
Flow cytometry was done on arterial blood samples and samples were stained looking for CD34+ cells, KDR+ cells, and CD133+ cells. Samples were also assessed for colony forming ability.16

To better perform statistical analysis on results, values of EPCs were analyzed as categorical variables after log transformation to normalize distribution. Specific thresholds were then determined to categorize subjects into cohorts based on their EPC levels at the time of enrollment, levels being either “low,” “medium,” or “high.” Continuous variables were assessed for normal distribution using the Kolmogorov-Smirnov test and one-way analysis-of-variance test was used for comparisons of categorical variables. Multivariate proportional-hazards regression analysis was performed to determine the association between EPC counts and each outcome measured. Analyses were adjusted for age, sex, smoking status, hypertension, diabetes, hyperlipidemia, left ventricular ejection fraction, percutaneous coronary intervention, diagnosis of ACS at time of enrollment, severity of CAD, and treatment with ACE-I, beta-blockers, statins, or platelet inhibitors. Survival was determined with the Kaplan-Meier method and Cox regression analysis, and statistical significance was found with a p-value < 0.05. All data analyses and event classifications were performed by investigators blinded to EPC status of patients.16

Investigators found that cumulative event-free survival increased in a step-wise fashion across increasing levels of baseline endothelial progenitor cells in regards to deaths from cardiovascular causes (p = 0.01) and occurrence of one’s first cardiovascular event (p < 0.001). Hospitalization and revascularization were also found to be more frequent occurrences in the subgroup with lower EPC counts. It was found that increasing
numbers of CD34+/KDR+ endothelial cells were associated with a decreased risk of death from cardiovascular causes. The risk of death from CV causes was found to be increased by greater than 3 times when comparing subjects in the low EPC group to those in the highest EPC group, and resulting in a hazard ratio of 0.31 (95% CI 0.16 to 0.63). After adjustment for variable listed above, the association between increasing levels of EPCs and decreased risk of CV death remained significant (p = 0.001).16

It was also shown that decreasing levels of endothelial progenitor cells were associated with the development of a first major cardiovascular event. After multivariate analysis for covariates it was confirmed that there was statistically significant association between CD34+/KDR+ EPCs and the occurrence of one’s first major CV event with a hazard ratio of 0.74 (95% CI 0.62 to 0.89, p = 0.002). The results followed a similar trend for CD133+ endothelial progenitor cells. The cumulative event-free survival also increased in a step-wise fashion with increasing baseline levels for colony forming units of endothelial cells. This was shown to maintain significance after analysis for a person’s first major cardiovascular event (p = 0.03), revascularization (p = 0.01), and hospitalization (p = 0.01). A multivariate analysis confirmed these associations and also showed a hazard ratio of 0.68 (995% CI 0.49 to 0.96, p = 0.03) for one suffering their first major CV event.16

Through this data the authors were able to illustrate that a single measurement of CD34+/KDR+ endothelial progenitor cells can be a useful tool in predicting CV outcomes in patients with known CAD.16 The associations of these cells and the outcomes measured were found to be independent of disease severity, diagnosis of acute coronary syndrome, cardiovascular risk factors, or current drug therapy. The authors infer
that these finding suggest that EPCs contribute to the restoration of the endothelial monolayer. They admit that through their findings they were not able to establish a significant connection between EPC levels and death of all causes or acute myocardial infarction. They put forth that an excess of deaths from non-cardiovascular cause could have led to this finding. They also suggest that it is possible that there is an up regulation of EPCs during acute ischemic events. They call for more research to help understand the complex interplay between EPCs and acute myocardial infarctions. They also call for more research to further enlighten the connection between congestive heart failure and EPC dysfunction, as this can contribute to the dysfunction in patients with AMI. Regardless, they state that this biomarker has a place in the stratification of patients with CAD and a future lies in therapeutic targeting of these cells and vascular healing.16

**SCHMIDT-LUCKE ET AL**

In this prospective observational trial,13 the authors looked to determine whether circulating EPC levels correlated with atherosclerotic disease progression. In order to test this question, the authors recruited 120 subjects from a single center between the months of October 2000 and June 2004. These 120 subjects were stratified into three groups; those with stable CAD, those with unstable CAD which was termed the acute coronary syndrome (ACS) group and a control group. The stable CAD group (n=44) was defined as having angiographically documented CAD in the absence of ACS in the previous 3 months before blood samples were drawn. People with unstable CAD (n=33) were defined as having de novo angina or angina at rest. They were also stratified as for troponin positivity to account for myocardial necrosis on EPC levels. Moreover, extent of
CAD to number of coronary arteries affected was measured. Healthy subjects (n=43) were defined as having no CAD by history and physical exam.\textsuperscript{13}

Further inclusion and exclusion criteria were applied to the groups. Inclusion criteria was defined as persons aged 18 to 85 years, with signed informed consent, documented CAD for subjects with stable CAD or ACS or acute myocardial infarction (AMI). Exclusion criteria was defined as persons with clinical or biochemical evidence for the presence of concomitant inflammatory disease, chronic renal insufficiency (creatinine > 1.4 mmol/L), impaired left ventricular ejection fraction (EF < 45\%), autoimmune or malignant disease, thrombocytopenia (platelets <100 000/L), anemia (hemoglobin < 8.5 g/dL), inability to understand the consent form, participation in or consent to participate in another study, previous coronary artery bypass grafting (CABG), severe peripheral artery disease, or atrial fibrillation.\textsuperscript{13}

Levels of EPC were measured using flow cytometry. Clinical long-term follow-up of the subjects was performed using a questionnaire that was sent to the subjects and telephone contact. Through this form all information regarding potential CV events was validated by source data, including coronary angiogram analysis, discharge letters, or hospital charts. CV death was defined as death from myocardial infarction, or documented sudden death. Further events were recorded including unstable angina events, AMI, and progression of CVD by need of new revascularization either by percutaneous coronary intervention (PCI) or CABG due to documented ischemia.\textsuperscript{13}

Statistical analysis of results was applied toward all results. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test and non-normally distributed variables (age, CV risk factors, EPC numbers, extent of disease, high-
sensitivity C-reactive protein score) were compared by the Mann-Whitney \( U \) test. Comparisons between groups were done by the \( t \) test or ANOVA for normally distributed variables with > 2 subgroups and by the Kruskal-Wallis test for non-normally distributed variables. Comparison of categorical variable was done using the Pearson \( x^2 \) test. Multivariate linear regression analysis and nonparametric bivariate correlation were used to correlate circulating EPC levels with CV risk factors, and regression analysis was performed against risk factors to determine independent determinants of EPC counts. Cumulative event-free survival was univariately evaluated by Kaplan-Meier analysis, and Cox proportional-hazard ratio was used to estimate relative risk for major adverse cardiac events (MACE) association with identified variables. Statistical significance was assumed if the null hypothesis could be rejected at a \( p \leq 0.05 \).

Through this analysis the authors were able to show that the control group had significantly higher levels of EPCs when compared to subjects with documented CAD. By univariate analysis of the entire cohort, the classic risk factors of CAD: age, hypertension, smoking, family history, as well as disease progression, and atherosclerotic extent, were all found to be inversely related to the number of circulating endothelial progenitor cells. Multivariate analysis showed that age and positive family history of CAD remained as the only independent predictors of EPC levels.

During the follow-up period for this study it was found that 11 subjects suffered from a cardiovascular event. Subjects that suffered an event were found to have significantly lower EPC levels when drawn at the beginning of the study at 0.0067 +/- 0.0097 vs. 0.02 +/- 0.02 in patients without a CV event. Subjects were also arranged into quintiles according to EPC levels and those subjects in the lowest quintile were found to
have significantly (p < 0.05) more cardiovascular events. Kaplan-Meier analysis found that across subjects, those that had EPC levels below the threshold of a CD34+/KDR+ count of 0.0038 had a significantly higher incidence of CV events. It was found, that the crude hazard ratio (HR) for suffering from CV event during follow-up with an EPC count below this threshold was 6.3, p = 0.003. When this number was adjusted for disease activity and risk factors of CVD, being below this threshold for EPCs was still associated with a significant, nearly 4-fold increased risk of suffering from a CV event (HR 3.9, p = 0.036).13

The authors were able to conclude through this study13 that reduced EPC levels independently predict atherosclerotic disease progression and future cardiovascular events. This supports that endogenous repair of vasculature has an important role in modulating the clinical course of CAD. They recognize that by measuring only CD34+/KDR+ EPC and leaving out immature CD133+ cells, they are possibly missing mechanistic insights to colony forming capacity and migratory capacity of EPCs. While they declare that a link between CV risk factor presence and reduced numbers of EPC exists, they call for further research that identifies more clear correlations between EPC functional capacity and endogenous vascular repair.13

**Werner (2007) et al**

This particular study10 was designed by the authors to correlate endothelial function with endothelial progenitor cells in patients with coronary artery disease (CAD). Observational in design, the authors examined EPC levels and functional ability as well as endothelial function in patients with known CAD. Subjects were enrolled in the study and all received a coronary angiogram due to suspected myocardial ischemia. Only
subjects with <= 50% luminal reduction and no flow limiting stenosis in at least one coronary artery were included. Subjects were excluded if there was a malignant process or if there was inflammatory disease present. Also if there was evidence of acute myocardial ischemia subjects were excluded. All patients were fasting for 12 prior to catheterization, and all blood samples were drawn prior to catheterization. Ninety subjects were eligible and one was excluded based on difficulty of their coronary anatomy. Medical histories including CV risk factors, previous and present CV events, current medication regiment, and vital signs were obtained from medical files and personal interview for stratification and statistical analysis of subjects.\textsuperscript{10}

During catheterization, acetylcholine was infused into one coronary artery in an attempt to induce vasospasm, and angiography was performed to record luminal diameter and differences were recorded in comparison to angiography without infusion of any drug. Flow cytometry was also done in attempt to measure each individual’s amount of endothelial progenitor cells. After samples were drawn, an attempt to culture these cells was made to determine the amount of colony forming units and ascertain functional ability. All data of EPC and colony forming unit endothelial cell (CFU-EC) levels were converted using natural-log methods to express this data as continuous variables. Univariate and non-parametric bivariate correlations were performed using the Pearson correlation coefficient when data was normally distributed. Stepwise linear regression analysis was done where indicated to determine independent variables that could influence the prediction of EPC and CFU-EC changes in peripheral blood and statistical significance was assumed with a p-value < 0.05.\textsuperscript{10}
Of the eighty-nine patients remaining, it was shown that endothelial function significantly correlated with the number of either CD133+ or CD34+/KDR+ EPC’s with a regression value of $r = 0.239$, $p = 0.024$ and $r = 0.427$, $p < 0.001$ respectively. This showed that subjects that had lower total number of EPCs suffered from severe endothelial dysfunction. Stepwise linear regression analysis demonstrated that this was finding was independent of age, gender, diabetes, hypertension, hyperlipidemia, family history of premature CAD, smoking, and statin treatment ($p < 0.001$ for CD34+/KDR+ and $p = 0.017$ for CD133+). Function of EPC was quantified by the ability of isolated cells to form colony forming units. Initial analysis showed that EPC function correlated with endothelial function ($r = 0.271$, $p = 0.038$), thus showing that poor EPC function would associate with greater endothelial dysfunction. However, after linear regression analysis, accounting for the same variables, this was no longer found to be significant.$^{10}$

These results show that the number of circulating endothelial progenitor cells closely correlate with endothelial function, independent of other risk factors associated with cardiovascular disease. By this, the authors infer that the integrative regenerative capacity of EPCs may be relevant toward the state of vascular dysfunction. They also conclude that these cells are involved in the pathogenesis of not only endothelial dysfunction, but also atherosclerosis and CAD. The precise factors that regulate these cells are not as well understood. The authors speculate that it is possible that certain cytokines take on this responsibility. They call for more research into the factors that regulate these cells numbers in the human body so that new treatment options for CAD can be developed.$^{10}$
DISCUSSION

It has been shown across these studies\textsuperscript{10,13,16} that not only do endothelial progenitor cells have a link with endothelial dysfunction, but that levels can be used to predict future cardiovascular events. This measurement has now been qualified as an independent marker of risk. While the results of these studies do excite the topic for future research, the studies above were not without their own limitations. Werner et al\textsuperscript{10} (2007) study was limited by its population size and additionally had no follow-up as a part of their study design. While this was not inherent to their design, this would have allowed for more credibility to be given to their results, as these correlations could have been tested over time. Due to the fact that this study started with such a small sample size, it is difficult to extrapolate these results confidently across the general population.

Schmidt-Lucke et al\textsuperscript{13} was another underpowered study with only having a population size of 120 total subjects with only 11 CV events. This is an unfortunate limitation because this study was able to demonstrate a dose-response sort of relationship with EPC levels and CV outcomes as well as a large treatment effect and due to its earlier limitation no more weight can be given this study’s drawn conclusions. One additional considered limitation was neglecting to mention the discussion of blinding between the ones manipulating the data and patients results, however, with the objectivity of the resultant data this was not viewed as a serious limitation.

One important aspect was that both Schmidt-Lucke et al\textsuperscript{13} and Werner et al\textsuperscript{16} (2005) did have the additional element of following its subjects over time and plotting events on a survival curve. Werner et al\textsuperscript{16} (2005), was a well done observational trial, that with a large treatment effect and dose-response relationship with EPC levels was able to
give high quality results as this study was not limited the was the others were with small sample sizes. As stated above, all these studies\textsuperscript{10,13,16} were able to show the connection between EPCs and endothelial dysfunction, also as a prediction tool for future cardiovascular events. These authors recognize that in practical laboratory purposes, the number of cells would be much more feasible number to ascertain as opposed to CFU-EC numbers which can take weeks to retrieve.\textsuperscript{10} It has also been shown that age and positive family history of premature CAD were shown to independently predict reduced EPC levels showing that these risk factors have a strong influence on EPC levels.\textsuperscript{13} Other factors that influence individuals EPC numbers, functional ability, and response to endothelial injury need to be further explored.\textsuperscript{10,13,16}

It has been shown that there is a clear association between the numbers of cardiac risk factors an individual has and EPC senescence in healthy individuals, this indicates that CVD risk factors not only lead to endothelial dysfunction but also EPC dysfunction.\textsuperscript{14} Studies have looked at this specific issue and found that persons at risk for or with established CAD have a 40% reduction in EPC count, reduced migratory capacity of EPCs.\textsuperscript{14,18} Smoking has the strongest negative correlation to EPC number and it has been seen that smokers can have a two to three fold decrease in EPC count.\textsuperscript{19} Men who are more advanced in age (56-70 years old) showed a 70% reduction in EPC numbers and 50% reduction in migratory capacity when compared to those in a 22-35 year old age group.\textsuperscript{20} It has also been established that those with either type I or II diabetes show up to 50% decreases in EPC count along with a decrease in angiogenic capacity and vascular incorporation.\textsuperscript{21,22} The trend continues in persons with concomitant disease, those with chronic kidney disease can expect EPC numbers to be decreased as much as 75% with
subsequent decreases in migratory capacity and the cells ability to incorporate into developing vascular networks.\textsuperscript{23} However, this still does not leave us at the conclusion that presence will mitigate response.

It is perhaps equally important to distinguish if in fact a defective phenotype of EPC is as much to blame for increased endothelial dysfunction as the complete lack of cell numbers.\textsuperscript{8} In a study that aimed to evaluate if the administration of EPCs would improve CV outcomes in AMI patients,\textsuperscript{24,25} the TOPCARE-AMI trial gave subjects with MI 3-day old EPCs in the infarct related artery. It was shown that LVEF improved and infarct area decreased post EPC infusion when compared to control. This was shown without evidence of arrhythmias, inflammatory reactions, or obstruction of blood vessels. These results are complicated by the results of a similar study that used immature EPCs.\textsuperscript{26} When CD133\textsuperscript{+} cells when used in a similar fashion, the result was the CD133\textsuperscript{+} administration was associated with enhanced current ischemia and need for repeat vascular intervention.

A similar finding was demonstrated by a group of Taiwanese researchers through research done in 2010 looking at EPC levels in patients undergoing PCI for acute MI.\textsuperscript{6} They were first able to show that patients with ST-segment elevation myocardial infarctions (STEMI) were a more likely population to have lower number of EPCs. Then they also found a high EPC count was more likely in subjects with concomitant congestive heart failure, lower EF, and more advanced Killip score. These patients also had lower levels of angiogenesis, and it was found through multivariate regression that a high EPC level was the most important independent marker that predicted a major adverse cardiovascular outcome in the next thirty days.\textsuperscript{6} There may be some question to
these findings as these researchers measured CD31+ levels, a phenotype which has also been associated with endothelial microparticles instead of endothelial progenitor cells.\textsuperscript{5} The existence of these specific microparticles has been associated with the presence of high risk cardiovascular lesions.\textsuperscript{5} This goes along with previous research that shows that there is a release or generation of these cells with an acute ischemic event,\textsuperscript{4,5} but also that the specific phenotype of cell is of significant importance when evaluating cellular function.\textsuperscript{8} This also lends credibility to the theory that persons with congestive heart failure\textsuperscript{27} or multiple risk factors for CAD have some degree of endothelial progenitor cell dysfunction and perhaps do not have the same degree of endothelial repair ability as someone without these comorbidities.\textsuperscript{14}

This along with Werner et al\textsuperscript{16} (2005) showing that EPCs were not independent predictors of occurrence of acute myocardial infarction, prove that there is a relationship between these cells and their presence in acute events that we have yet to completely uncover. It can be inferred that in people with endothelial dysfunction, they will have an up-regulation of and higher amount of EPCs, functional or not. If this response causes an increase in dysfunctional EPCs to attempt to repair endothelial damage there can be no effect or perhaps even a process which worsens ones outcome. People without endothelial dysfunction would presumably not have this need for endothelial remodeling and therefore would not have an increased number endothelial progenitor cells. This theory would say that context is paramount in measuring this value and a one-time measurement of a patient’s EPC level may not be the most helpful without first quantifying this persons own degree of endothelial damage.
It can be described that endothelial damage represents a balance between the magnitude of injury and a capacity for repair.\textsuperscript{13} Since circulating endothelial cells are cells that are also found in the face of acute injury, a ratio of CEC to EPC may be a more helpful tool when trying to decide if there is indeed structural damage, and then assess the patient’s ability to repair that damage.\textsuperscript{5} Again, characteristics that influence these cells functional ability should be identified so that if possible this can be determined in some manner other than growing CFU-ECs. This may be the more important piece than just sole number count when determining a patient’s ability to repair endovascular damage.

The other factor at play is the degree regulation of these cells that falls to regulatory cytokines, if the signaling pathway has some defect, there will be a problem in cells mobilizing to the area of injury.

While questions still exist of the precise mechanism of action and all influencing factors that act on these cells, researchers feel that monitoring levels of EPC as surrogate biological markers may be useful for identifying novel therapeutic approaches targeted to enhance endogenous vascular repair capacity, and may be able to modify the progression of cardiovascular disease.\textsuperscript{13} Due to the angiogenic and reparative capabilities attributed to EPCs, therapies that look to capitalize on this are being investigated in the fields of pulmonary hypertension, AMI, post- PCI, cerebral vascular events, and peripheral artery disease\textsuperscript{8}. On top of using cell therapies in these areas, methods to increase cell numbers and mobilization are being investigated. It has been shown that certain drugs and behavior can increase these variables associated with EPCs. EPCs have been shown to mobilize into the bloodstream with exercise.\textsuperscript{28} This is thought to be in part secondary to the release of vascular endothelial growth factor (VGEF) and other cytokines.\textsuperscript{7,28} Drugs
that have been associated with a similar increase include statins, angiotensin-converting enzyme (ACE) inhibitors, erythropoietin, and insulin-like growth factor-1 (IGF-1).\textsuperscript{29-32}

One issue that needs to be addressed in any therapy that looks to increase or supplement the number of EPCs in individuals for any treatment purpose would be patients' risk for or history of cancer. EPCs have been shown to be sufficient for the development of tumor vasculature and risk exacerbating or furthering the growth of existing malignancies.\textsuperscript{33}

**CONCLUSION**

Endothelial progenitor cells have been proven to have a role in endothelial dysfunction, cardiovascular disease progression, and now risk assessment. While many factors play into the overall mechanism in which EPC affect cardiovascular risk and endothelial repair, monitoring blood levels of EPCs has become a feasible biomarker that can be added to further stratify a patient's CV risk profile. While the overall evidence from this review falls in a moderate category based on GRADE results, a strong clinical foundation for this area of research currently exists in previous animal trials and blossoming studies. To better understand some of these complex relationships in humans, further in vivo trials need to be done. Additionally, more credibility could be lent to this topic with more well-designed, larger observational trials, double-blinded randomized control trials; and this can give providers set values that can be applied to the general public. However, the future in this area lies in the discovery of new therapeutic modalities that can modify cardiovascular disease progression. To do this, more has to be understood about factors that hold influence over EPC count, mobility, and colony forming ability, and how these functional performance issues specifically tie into disease
modification. Once this is better understood, this cell number can become more than just a count to determine risk, but a tool to help improve people’s lives.
References


### Table 1. Characteristics of Reviewed Studies

<table>
<thead>
<tr>
<th>Quality Assessment</th>
<th>Importance</th>
</tr>
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<tbody>
<tr>
<td>Outcomes</td>
<td>Design</td>
</tr>
<tr>
<td>Werner et al (2005): EPC and CV outcomes$^{16}$</td>
<td>First Major CV event</td>
</tr>
<tr>
<td></td>
<td>Death from CV cause</td>
</tr>
<tr>
<td>Schmidt-Lucke et al: Reduced EPC predicts future CV events$^{13}$</td>
<td>First major CV event</td>
</tr>
<tr>
<td>Werner et al (2007): EPC correlate w/ endothelial function in patients w/ CAD$^{10}$</td>
<td>Low EPC correlate to severe endothelial dysfunction</td>
</tr>
<tr>
<td></td>
<td>EPC function correlate with endothelial fn</td>
</tr>
</tbody>
</table>

$^a$ Study upgraded for RR of 4.6 and dose-gradient response to higher number of EPC correlating with decreased risk of CV deaths and 1st major CV event.

$^b$ Study downgraded for lack of precision, study population only consisted of 120 subjects and only had a total of 11 adverse events throughout follow-up. Despite large treatment effect and dose-response correlation of results, study is unable to be upgraded.

$^c$ The study shows limitations in lack of follow-up and therefore cannot be upgraded for any reason. Also, the study had a total population of 90 subjects recruited from one center. Although a correlation was shown, there is no documented treatment effect or dose response gradient to attempt upgrade.
**Table 2. Summary of Findings**

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low EPC levels</td>
<td>High(normal) EPC</td>
</tr>
<tr>
<td>Werner et al(2005)</td>
<td>(n = 168)</td>
<td>(n = 167)</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>First major CV event (AMI, hospitalization, revascularization, death from CV cause)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death from CV cause</td>
<td>14 (8.3%)</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>First major CV event (HR for MACE with 95% CI)</td>
<td></td>
</tr>
<tr>
<td>Werner et al(2007)</td>
<td>CD34+/KDR+</td>
<td>CD133+</td>
</tr>
<tr>
<td></td>
<td>RF adjusted P-</td>
<td>value</td>
</tr>
<tr>
<td></td>
<td>Colony forming Fxn</td>
<td>RF Adjusted</td>
</tr>
</tbody>
</table>