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ACUTE CORONARY SYNDROME

Circulating Endothelial Cells and Endothelial Function Predict Major Adverse Cardiac Events and Early Adverse Left Ventricular Remodeling in Patients With ST-Segment Elevation Myocardial Infarction

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Endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) are mobilized from the bone marrow and increase in the early phase after ST-elevation myocardial infarction (STEMI). The aim of this study was to assess the prognostic significance of CECs and indices of endothelial dysfunction in patients with STEMI. In 78 patients with acute STEMI, characterization of CD34+/VEGFR2+CECs, and indices of endothelial damage/dysfunction such as brachial artery flow mediated dilatation (FMD) were determined. Blood samples for CECs assessment and quantification were obtained within 24 hours of admission and FMD was assessed during the index hospitalization. At 30 days follow up, the primary composite end point of major adverse cardiac events (MACE) consisting of all-cause mortality, recurrent nonfatal MI, or heart failure and the secondary endpoint of early adverse left ventricular (LV) remodeling were analyzed. The 17 patients (22%) who developed MACE had significantly higher CEC level ($P = 0.004$), von Willebrand factor (vWF) level ($P = 0.028$), and significantly lower FMD ($P = 0.006$) compared to the remaining patients. Logistic regression analysis showed that CECs level and LV ejection fraction were independent predictors of MACE. The areas under the receiver operating characteristic curves (ROC) for CEC level, FMD, and the logistic model with both markers were 0.73, 0.75, and 0.82, respectively, for prediction of the MACE. The 16 patients who developed the secondary endpoint had significantly higher CEC level compared to remaining patients ($P = 0.038$). In conclusion, increased circulating endothelial cells and endothelial dysfunction predicted the occurrence of major adverse cardiac events and adverse cardiac remodeling in patients with STEMI. (J Interven Cardiol 2016;29:89–98)

Introduction

Acute myocardial infarction (AMI) leads to tissue damage and deterioration of ventricular function over time. AMI induces a generalized inflammatory response reflected by increased plasma levels of chemoattractants leading to increased circulating CD34-positive (CD34+) hematopoietic stem cells (HSCs) as well as endothelial progenitor cells (EPCs). This mobilization occurs within few hours to days after the onset of AMI along with a significant increase in plasma levels of vascular endothelial growth factor.[1,2] A study by Wojakowski et al.[3] demonstrated that the mobilized peripheral blood mononuclear cells (MNCs) in the setting of AMI express specific cardiac, muscle, and endothelial markers. The capacity of EPCs isolated from the peripheral blood of healthy adult volunteers and patients with coronary artery disease (CAD) to transdifferentiate into cardiac myocytes when co-cultured with rat cardiomyocytes have also been reported.[4] Several studies have shown that intracoronary infusion of either circulating progenitor cells or bone marrow-derived progenitor cells was associated with significant improvement in global left ventricular (LV) function, and significant reductions in LV end-systolic volumes, suggesting a favorable

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LV remodeling process following AMI.[5–7] Levels of circulating EPCs were also found useful as a prognostic marker of cardiovascular risk and outcome. A strong correlation was detected between the number of circulating EPCs in peripheral blood samples from healthy men and their combined Framingham risk factor score.[8] In a prospective study of patients with CAD, a significantly higher incidence of death from cardiovascular (CV) causes was observed in patients with low baseline levels of EPCs at the end of 1 year observational period.[9] The aim of this study is to examine the prognostic role of circulating endothelial cell (CECs)/colony forming units-endothelial cells (CFU-ECs) level and indices of endothelial dysfunction in predicting major adverse cardiac events (MACE) and early adverse left ventricular remodeling (ALVR) in patients with STEMI.

Methods

Study Population. A total of 85 patients with an acute STEMI (18–85 years) were prospectively screened for inclusion in the study between July 2013 and September 2013. Exclusion criteria were patients with prior STEMI within 3 months, renal failure, chronic inflammatory disease, malignancy and surgery or trauma during the preceding 2 months. The study was approved by the ethics committee of Cairo University hospital and conducted in accordance with the Declaration of Helsinki. A written, informed consent was obtained from each patient. All the patients underwent either primary percutaneous coronary intervention (PCI) or IV thrombolytic therapy followed by coronary angiography within 24 hours according to the timing of presentation and at the discretion of the treating physician. Patients received standard treatment for STEMI according to the recommendations of the American College of Cardiology Foundation guidelines.[10]

Laboratory and Echocardiography Assays. A peripheral blood (PB) sample was collected within 24 hours of admission from all patients for CECs in vitro culture and identification using flow cytometry and functional assays as well as vonWillebrand factor estimation by enzyme-linked immunosorbent assay (ELISA). Within 48 hours of admission, a baseline trans-thoracic echocardiography (TTE) was performed to calculate LV end diastolic dimension (LVEDD) and volume (LVEDV), LV end systolic dimension (LVESD) and volume (LVESV), LV wall motion score index (LV WMSI), LV myocardial performance index (LV MPI) and LV ejection fraction (EF) using biplane Simpson's method. At 30-day follow up, a repeat TTE could be obtained in 62 patients.

Brachial arterial flow-mediated dilation (FMD) was performed according to the previously described protocol by Donald et al.[11]

Identification and Quantification of Circulating Endothelial Cells. Peripheral blood mononuclear cells (PB-MNCs) fraction were isolated using density-gradient centrifugation with Ficoll-Paque (Gibco-Invitrogen, Grand Island, NY). PB-MNCs were cultured in endothelial culture medium (EC basal medium-2; Lonza-Walkersville, MD) and incubated at 37°C with 5% CO2 for 14 days. By day 14, cells were further characterized using capillary formation assays and flow cytometry.

Circulating Endothelial Cell Functional Assessment. Total RNA was extracted from identified human CECs and Real-time qPCR was performed to asses the expression of vascular endothelial growth factor receptor-2 (VEGFR2), endothelial nitric oxide synthase (eNOS), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes according to previously published methods.[12–14]

Calculation of the Thrombolysis In Myocardial Infarction Risk Score. Clinical Thrombolysis In Myocardial Infarction (TIMI) risk score was calculated for all enrolled patients. Full details of the design and methods of the TIMI risk score has been previously published.[15] The TIMI risk score consists of age, prior angina, diabetes, hypertension, systolic blood pressure <100 mmHg, heart rate >100 beats/min, Killip class II–IV, weight <67 kg, anterior
ST-segment elevation or left bundle branch block on electrocardiogram, and time to treatment >4 hours.

Study Endpoints. At 30 days from admission, the primary composite end point of the following MACE: heart failure, recurrent nonfatal MI, or all-cause mortality and the secondary endpoint of early adverse LV remodeling (ALVR) defined as $\geq 20\%$ increase in the echocardiographic LVEDV compared to baseline were analyzed.

Statistical Analysis. Differences between groups were assessed by unpaired 2-tailed t-test and the Mann–Whitney U test for continuous variables, as appropriate. Categorical data and proportions were analyzed by the use of chi-square or Fisher's exact test

when required. We assessed predictors of the MACE and the secondary endpoint at 30-day follow-up using a binary logistic regression. All significant variables in the univariate analysis were entered into a multivariable logistic regression model, and variables were backwards eliminated. Optimal cut-off points of CECs level and FMD percent to predict MACE were calculated using receiver-operator characteristics (ROC) analysis. Binary logistic regression was also employed to measure the predictive probabilities of the combination of CECs level, FMD and TIMI risk score model for 30-day MACE. Comparison of the predictive accuracy with the c-statistics, a measure of the area under the receiver-operator characteristic curve, was carried out using the procedure proposed by DeLong and colleagues.[16] P value was considered significant if less than 0.05.

Results

A total of 78 patients with acute STEMI were included in the analysis of the baseline characteristics. The mean age ($\pm$ SD) of the patients was 53.8 ± 10.6 years (range 28–83). More than half of the patient population had anterior STEMI and 57.7% of them were treated with successful PPCI. The majority of patients were clinically stable after their STEMI (85.9% Killip class I) (Table 1). The differences between patients who developed MACE and those who did not are summarized in Table 2. Overall, there were no significant differences between the 2 groups in the baseline characteristics and comorbidities. However, patients who developed MACE were more likely to have anterior STEMI (82% vs 48%, $P = 0.011$) and had a significantly higher TIMI risk score [6 (3–7) vs. 2 (1–3), $P < 0.001$] compared to those who did not develop MACE. There was a trend towards higher peak troponin I levels in patients who developed MACE.

Clinical Study Endpoints. Seventeen patients (21.8%) out of 78 patients developed the primary clinical endpoint of the following MACE: CHF (12 patients), recurrent nonfatal MI (1 patient), or all-cause mortality (5 patients). One patient had evidence of congestive heart failure (CHF) during hospital stay and died before the 30-day follow-up visit. Patients of the MACE group had a significantly higher EPCs level ($P = 0.004$), VEGFR2 gene expression ($P = 0.023$), vWF level ($P = 0.028$), and significantly lower FMD ($P = 0.006$) compared to the non-MACE group (Table 2).

Sixteen patients (25.8%) out of 62 patients (who underwent 30-day TTE) developed ALVR. These patients had significantly higher CECs level ($P = 0.038$) compared to the rest of the patients without evidence of early ALVR (Table 3).

CD34+/VEGFR2+ Circulating Endothelial Cell Level and Function. Previous studies have demonstrated that MI can lead to the mobilization of bone marrow-derived stem cells and that this mobilization correlates with the extent of injury. Patients who developed MACE had higher percentage of anterior MI and trends toward higher troponin levels. This correlated with significantly higher numbers of CECs in this population (1.74%; IQR: 1.62–1.89% vs 1.5%; 0.52–1.76%, $P = 0.004$). The increased number of CECs was also associated with significantly higher gene expression of VEGFR2 (1.02 ± 0.31 vs 0.8 ± 0.36, $P = 0.023$). Similarly, the level of vWF was significantly higher among patients with MACE (835 ± 349 vs 623 ± 343 ng/ml, $P = 0.028$). However, we did not observe significant difference in eNOS gene expression between the two groups. This higher CECs numbers and the elevated endothelial gene expression could be related to larger myocardial injury among those who developed MACE. ROC analysis revealed that a cut-off point CEC level of $>1.64\%$ best predicted 30-day MACE with a sensitivity of 76.5% and a specificity of 63.9%.

Indices of Endothelial Function. The level of vascular reactivity as measured by FMD of the brachial artery has been shown in multiple human studies to reflect the prognosis of patients with CAD. In our study population, FMD was significantly lower in patients who developed MACE compared to those who did not suffer MACE (3.5 ± 1.4 vs $5.9 \pm 3.1\%$, $P = 0.006$). ROC analysis revealed that a cut-off point FMD of $\leq 4.7\%$ best predicted 30-day MACE with a sensitivity of 93.3% and specificity of 63.9%.

Predictors of Outcomes. The predictors of MACE at 30-day follow-up after STEMI were assessed using a binary logistic regression model that included anterior MI location, LV EF, LV MPI, LV WMSI, WBCs count, CD34+/VEGFR2+ CEC level, VEGFR2 gene expression, plasma vWF level, and FMD. Multivariable analysis showed that only LVEF ($P = 0.002$) and CEC level ($P = 0.019$) were the independent predictors of MACE at 30-day follow-up (Table 4).

Table 1. Baseline Demographic, Clinical, Procedural, Laboratory, Indices of Endothelial Function, and Echocardiographic Data of 78 Patients With STEMI

Variable	Value
Demographics	
Age (years), mean (range)	53.8 ± 10.6 (28–83)
Male gender (%)	62 (79.5)
BMI (kg/m^2)	29 ± 3
Cardiovascular risk factors (%)	
Family history	10 (12.8)
Dyslipidemia	59 (75.6)
Hypertension	30 (38.5)
Diabetes mellitus	37 (47.4)
Smoking	41 (52.6)
Previous MI (%)	3 (3.8)
MI location (%)	
Anterior location	43 (55.1)
Inferior/lateral location	35 (44.9)
Physical exam	
Killip Class on admission (%)	
I, II, II, IV	67 (85.9), 6 (7.7), 2 (2.6), 3 (3.8)
Reperfusion therapy (%)	
Primary PCI	45 (57.7)
Thrombolytic therapy	33 (42.3)
Laboratory data	
WBCs (10^3 cells/ml)	12.5 ± 4.4
Creatinine (mg/dl)	0.99 ± 0.34
CECs level (%), median (IQR)	1.59 (0.64–1.78)
VEGFR2 gene expression	0.85 ± 0.36
eNOS gene expression	1.26 ± 0.61
Indices of endothelial function	
vWF level (ng/ml)	669 ± 353
FMD (%)	5.4 ± 3.0
Echocardiographic data	
LV EDD (cm)	5.4 ± 0.7
LV EDV (ml)	84 ± 29
LV ESD (cm)	3.9 ± 0.8
LV ESV, median (IQR) (ml)	35 (26–46)
LV EF (%)	50 ±13
LV MPI	0.69 ± 0.15
LV WMSI	1.73 ± 0.37
MACE (%)	17 (21.8)
Heart failure	12 (15.4)
Recurrent nonfatal MI	1 (1.3)
All-cause mortality	5 (6.4)
Early ALVR (%)	16 (20.5)

MI, myocardial infarction; BMI, body mass index; PCI, percutaneous coronary intervention; WBCs, white blood cells; CECs, circulating endothelial cells; IQR, interquartile range; VEGFR, vascular endothelial growth factor receptor; eNOS, endothelial nitric oxide synthase; vWF, vonWillebrand factor; FMD, flow mediated dilation; LV, left ventricular; EDD, end diastolic dimensions; EDV, end diastolic volume; ESD, end systolic dimensions; ESV, end systolic volume; EF, ejection fraction; MPI, myocardial performance index; WMSI, wall motion score index; MACE, major adverse cardiovascular events; ALVR, adverse left ventricular remodeling.

Additive Prognostic Value of Combined CD34+/VEGFR2+CECs Level and Brachial Artery FMD. The accuracy of CD34+/VEGFR2+ EPCs level and FMD test as well as the combination of both tests to predict MACE were determined by using ROC analysis. EPCs level yielded an AUC of 0.73 (95%CI: 0.61–0.82, $P = 0.004$) with 78.2% of the cases correctly classified. As regards FMD test, the AUC was 0.75 (95%CI: 0.64–0.84, $P = 0.003$) with 78.9% of the cases correctly classified. The predicted probability from the binary logistic model combining the 2 tests yielded an AUC of 0.82 (95%CI 0.72–0.90, $P < 0.001$) with 80.3% of the cases correctly classified, thus exceeding that of either test alone (Table 5 and Fig. 1).

Additive Prognostic Value of Combined CD34+/VEGFR2+ CEC Level and Brachial Artery FMD to TIMI Score. By further implementation of the ROC analysis, the AUC for TIMI risk score was: 0.77 (95%CI 0.61–0.92, $P < 0.001$), for combined TIMI risk score and CEC level: 0.89 (95%CI 0.81–0.97, $P = 0.115$ for comparison) and for combined TIMI risk score, EPC level and FMD: the highest value of 0.92 (95%CI 0.86–0.98, $P = 0.025$ for comparison) (Table 5 and Fig. 2).

Discussion

Various clinical, electrocardiographic, laboratory, and echocardiographic prognostic indices have been suggested to classify patients after AMI.[17–20] However, the use of a biological prognostic marker is still very appealing. The current study is the first, to investigate the prognostic potential of the combined measurement of CEC level and function together with indices of endothelial dysfunction (FMD) in patients with acute STEMI. Our study demonstrated significantly higher CEC level ($P = 0.004$), and VEGFR-2 gene expression ($P = 0.023$) in patients who developed MACE at 30-day follow-up compared to the non-MACE group. On the other hand, patients who developed MACE and secondary endpoints had lower endothelial function as assessed by FMD measurements. In animal models of MI, studies have suggested that EPCs stimulated by endogenous or exogenous stimuli can lead to a remarkable recovery in ventricular performance and reduction in post-MI mortality.[21–23] In a study by Leone et al.,[24] the concentration of CD34+ bone marrow derived stem cells was not only

Table 2. Baseline Demographic, Clinical, Procedural, Laboratory, Indices of Endothelial Function, and Echocardiographic Data of Patients Who Developed the Primary Composite End Point (MACE Group) in Comparison With the Non-MACE Group

Variable	MACE Group (N 17)	Non-MACE Group (N 61)	P value
Demographics			
Age (years)	57 ± 14	53 ± 9	0.150
Male gender (%)	15 (88.2)	47 (77.0)	0.312
BMI (kg/m^2)	29.5 ± 2.4	28.8 ± 2.5	0.342
Cardiovascular risk factors (%)			
Family history	0	10 (16.4)	0.074
Dyslipidemia	10 (58.8)	49 (80.3)	0.068
Hypertension	8 (47.1)	22 (36.1)	0.410
Diabetes mellitus	10 (58.8)	27 (44.3)	0.288
Smoking	8 (47.1)	33 (54.1)	0.607
Previous MI (%)	2 (11.8)	1 (1.6)	0.055
Anterior MI Location (%)	14 (82.4)	29 (47.5)	0.011*
ACEI	14 (82.4)	57 (93.4)	0.157
Statins	16 (94.1)	60 (98.4)	0.328
TIMI risk score, median (IQR)	6 (3–7)	2 (1–3)	<0.001*
Laboratory data			
WBCs (10^3 cells/ml)	15.5 ± 4.1	11.7 ± 4.1	0.001*
Troponin I (ng/ml) median (IQR)	44 (9–101)	19 (7–65)	0.202
Creatinine (mg/dl)	1.09 ±0.39	0.97 ± 0.33	0.196
CECs level(%), median (IQR)	1.74 (1.62–1.89)	1.5 (0.52–1.76)	0.004*
VEGFR2 gene expression	1.02 ± 0.31	0.8 ± 0.36	0.023*
vWF level (ng/ml)	835 ±349	623 ± 343	0.028*
eNOS gene expression	1.5 ± 0.6	1.19 ± 0.6	0.056
Indices of endothelial function			
FMD (%)	3.5 ± 1.4	5.9 ± 3.1	0.006*
Echocardiographic data			
LV EDD (cm)	5.9 ± 0.5	5.2 ± 0.7	<0.001*
LV ESD (cm)	4.5 ± 0.6	3.7 ± 0.8	<0.001*
LV EDV (ml)	93 ± 32	78 ± 26	0.042*
LV ESV (ml)	60 (38–78)	33 (25–44)	0.001*
LV EF (%)	40 ± 8	53 ± 12	<0.001*
LV WMSI	1.98 ± 0.29	1.66 ± 0.36	0.001*
LV MPI	0.79 ± 0.16	0.66 ± 0.14	0.002*

MI, myocardial infarction; BMI, body mass index; ACE I, angiotensin converting enzyme inhibitors; TIMI, thrombolysis in myocardial infarction; WBCs, white blood cells; CECs, circulating endothelial cells; IQR, interquartile range; VEGFR, vascular endothelial growth factor receptor; eNOS, endothelial nitric oxide synthase; vWF, vonWillebrand factor; FMD, flow mediated dilation; LV, left ventricular; EDD, end diastolic dimensions; EDV, end diastolic volume; ESD, end systolic dimensions; ESV, end systolic volume; EF, ejection fraction; MPI, myocardial performance index; WMSI, wall motion score index. *$P < 0.05$.

higher in AMI patients compared to patients with stable CAD but was also an independent predictor of global and regional improvement of LV function at the end of 1 year follow-up. We suspect that the elevated levels of CECs, vWF and VEGFR2 may be a reflection of larger myocardial infarction in patients who went on to develop MACE.

Our results were, however, in agreement with a recent study by Chang et al.[25] investigating the level of EPCs in STEMI patients undergoing primary coronary angioplasty. Although the circulating level of EPCs was considerably lower in STEMI patients compared to normal subjects in their study, the patients with high EPCs (≥1.2%) had lower LVEF, elevated WBCs

Table 3. Baseline Demographic, Clinical, Procedural, Laboratory, Indices of Endothelial Function, and Echocardiographic Data of Patients Who Developed the Secondary End Point of Early Adverse LV Remodeling (ALVR Group) in Comparison With the Non-ALVR Group

Variable	ALVR Group (N = 16)	Non-ALVR Group (N = 46)	P value
Demographics			
Age (years)	49 ± 10	52 ± 9	0.204
Male gender (%)	15 (93.8)	35 (76.1)	0.123
BMI (kg/m^2)	29.4 ± 2	28.6 ± 2.4	0.221
Cardiovascular risk factors (%)			
Family history	4 (25)	5 (10.9)	0.167
Dyslipidemia	9 (56.2)	37 (80.4)	0.057
Hypertension	6 (37.5)	16 (34.8)	0.845
Diabetes mellitus	7 (43.8)	25 (54.3)	0.465
Smoking	11 (68.8)	23 (50)	0.194
Anterior MI Location (%)	10 (62.5)	23 (50)	0.388
ACE I	13 (81.2)	43 (93.5)	0.154
Statins	16 (100)	45 (97.8)	0.552
Laboratory data			
WBCs (10^3 cells/ml)	14.0 ± 3.8	11.6 ± 4.4	0.065
Troponin I (ng/ml) median (IQR)	20 (8–54)	15 (7–44)	0.605
Creatinine (mg/dl)	1.09 ± 0.40	0.94 ± 0.32	0.145
CECs level(%), median (IQR)	1.76 (1.13–1.89)	1.5 (0.52–1.74)	0.038*
VEGFR2 gene expression	0.86 ± 0.30	0.88 ± 0.39	0.892
vWF level (ng/ml)	820 ± 402	675 ± 338	0.165
eNOS gene expression	1.40 ± 0.66	1.29 ± 0.60	0.538
Indices of endothelial function			
FMD (%)	4.6 ± 2.9	5.7 ± 3.2	0.230
Echocardiographic data			
LV EDD (cm)	5.5 ± 0.6	5.3 ± 0.7	0.315
LV ESD (cm)	3.9 ± 0.7	3.8 ± 0.9	0.445
LV EDV (ml)	68.7 ± 20.5	79.6 ± 25	0.129
LV ESV (ml)	33 (27–39)	33 (25–44)	0.822
LV EF (%)	50 ± 9	53 ± 13	0.490
LV WMSI	1.66 ± 0.32	1.66 ± 0.35	0.953
LV MPI	0.68 ± 0.19	0.66 ± 0.13	0.637

ALVR, early adverse LV remodeling; MI, myocardial infarction; BMI, body mass index; ACE I, angiotensin converting enzyme inhibitors; WBCs, white blood cells; CECs, circulating endothelial cells; IQR, interquartile range; VEGFR, vascular endothelial growth factor receptor; eNOS, endothelial nitric oxide synthase; vWF, vonWillebrand factor; FMD, flow mediated dilation; LV, left ventricular; EDD, end diastolic dimensions; EDV, end diastolic volume; ESD, end systolic dimensions; ESV, end systolic volume; EF, ejection fraction; MPI, myocardial performance index; WMSI, wall motion score index. *$P < 0.05$.

count, more advanced CHF (≥class III), and increased 30-day mortality than those with a low EPC level (<1.2%). Similarly, patients who developed MACE in our study had a significantly lower LVEF compared to the non-MACE group (<0.001) and 70% of them had evidence of CHF. Thus, the elevated levels of CECs in our study and the study by Chang et al. could be a reflection of the higher myocardial damage. The peripheral recruitment of CECs and CD34+ MNCs has been reported to occur in early stages following STEMI and in CHF[26] and hence the higher CEC level in the MACE group.

The angiogenic cytokine VEGF expression was also found to be enhanced as a result of an increase in the level of hypoxia-inducible factor 1 (HIF-1) as an early response to AMI.[27] A study by Shintani et al.[2] revealed

Table 4. Factors Associated With 30-Day MACE by Univariate and Multivariable Logistic Regression Analysis (N = 78)

	Univariate Analysis			Multivariable Analysis		
Variable	Odds Ratio	95%CI	P value	Adjusted Odds Ratio	95%CI	P value
Anterior MI location	5.15	1.34–19.75	0.017	–	–	–
CECs $CD34^+/VEGFR2^+$%	4.40	1.24–15.61	0.022	5.43	4.14–7.11	0.019
VEGFR2 gene (fold change)	6.56	1.22–35.12	0.028	–	–	–
vWF level	1.002	1.0001–1.004	0.033	–	–	–
FMD	0.66	0.49–0.9	0.007	–	–	–
LV EF	0.9	0.84–0.96	0.001	0.82	0.72–0.93	0.002
LV MPI $\geq 0.68^*$	3.778	1.09–13.04	0.036	–	–	–
LV WMSI	15.61	2.50–97.48	0.003	–	–	–
WBCs count	1.23	1.07–1.41	0.003	–	–	–

CI, confidence interval; MI, myocardial infarction, CECs, circulating endothelial cells; VEGF, vascular endothelial growth factor; vWF, vonWillebrand factor; FMD, flow mediated dilation; LV, left ventricular; EF, ejection fraction; MPI, myocardial performance index; WMSI, wall motion score index; WBCs, white blood cells. *Median LVMPI

significant increase in the circulating EPCs and CD34+ MNCs in patients with AMI with a significant positive correlation between CD34+ MNC count and the plasma levels of VEGF2. In our study, a significantly higher VEGFR2 gene expression was identified in the MACE group who suffered larger infarcts. By multivariable analysis, the 2 independent predictors of 30-day MACE in the current study were the CEC level and the LVEF. A greater ischemic insult in the patients who will eventually develop MACE seems to be the triggering factor for a greater recruitment of CECs. On the other hand, an impaired LV systolic function is an established marker of short and long-term adverse clinical outcome after acute MI.[28–30] In the recent HORIZON-AMI trial, STEMI patients with impaired LVEF had significantly higher 1-year MACE compared to patients with normal LV function even after adjustment for baseline characteristics.[31] A high level of CEC does not necessarily indicate increased functional regenerative activity of these cells. In vitro assays demonstrate that EPCs isolated from STEMI patients with high Killip score have lower angiogenic potential compared to patients with a low Killip score and normal control subjects.[25] Therefore, a high level of CEC in patients with evidence of early ALVR may be once again a marker of a worse clinical profile and larger infarcts in these patients rather than a marker of greater regenerative capacity of these cells. Other findings of a significantly lower FMD in the MACE compared to the non-MACE group confirm the major link between endothelial injury or dysfunction and CV outcome described in many studies.[32–36] vWF is a critical factor for platelet aggregation and adhesion.[37,38] In patients with non-

Table 5. Predictive Accuracy of EPCs Level and Brachial Artery Flow Mediated Dilation Test in Detection of 30-Day Major Adverse Cardiovascular Events

Parameter	AUC	CI 95%	P value	Correctly Classified Cases (%)
CECs level	0.73	0.61–0.82	0.004	78.2
FMD test	0.75	0.64–0.84	0.003	78.9
CECs level + FMD test	0.82	0.72–0.90	<0.001	80.3
TIMI score	0.77	0.61–0.92	<0.001	87.2
TIMI score + CECs level	0.89	0.81–0.97	<0.001	85.9
TIMI score + CECs level + FMD	0.92	0.86–0.98	<0.001	86.8

AUC, area under curve; CI, confidence interval; CECs, circulating endothelial cells; FMD, flow mediated dilation.

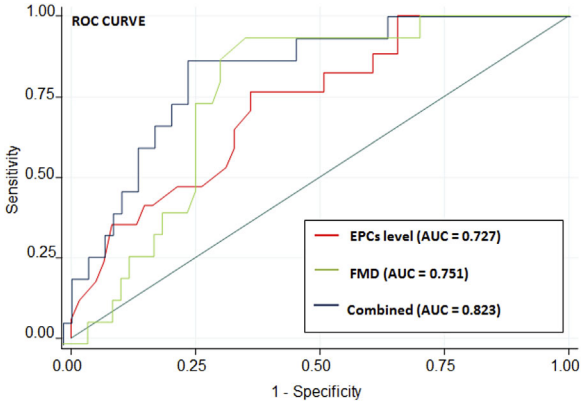

Figure 1. The receiver-operator characteristic curve shows the performance of circulating endothelial cells (CEC) level, brachial artery flow mediated dilation (FMD) test and combination of both tests to detect 30-day major adverse cardiovascular events (MACE).

STEMI or unstable angina pectoris, increasing plasma vWF level was found to be an independent predictor of adverse CV outcomes at 14-day, 30-day and 1-year follow-up.[39,40] In STEMI patients, the acute release of vWF was significantly higher in patients developing heart failure and in those dying within the first month after MI.[41] The predictive accuracy of either CEC level or FMD test for detection of 30-day MACE were both good (AUC: 0.73 and 0.75, respectively) and could correctly classify 78.2% and 78.9% of patients, respectively. Combining the results of both tests increased the accuracy to predict 30-day MACE with an AUC of 0.82% and 80.3% of patients were correctly classified. Additionally, combination of the admission CEC level and FMD to the widely accepted TIMI risk score improved its value in predicting 30-day MACE. It is important to note that the study population was small and larger studies are needed to examine the clinical prognostic value of CECs and FMD in STEMI patients. Assessment of the cost of using the combination of these two tests over conventional prognostic markers to prevent MACE should be further studied in randomized studies.

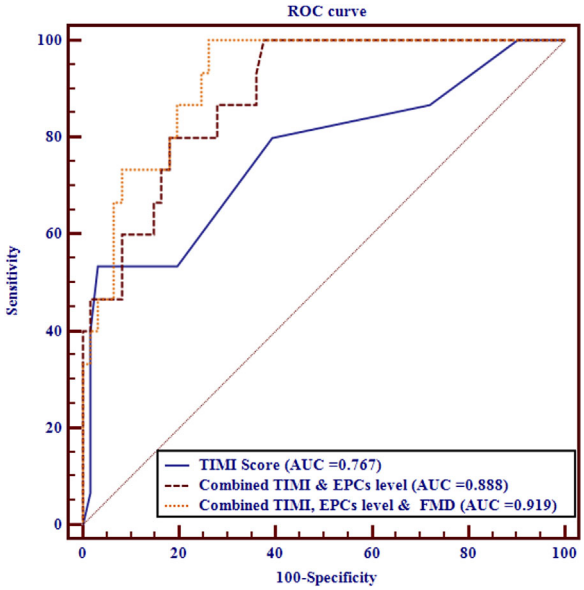

Figure 2. The receiver-operator characteristic curve shows the performance of TIMI risk score and the combination of TIMI risk score and endothelial progenitor cells (CEC) level to detect 30-day major adverse cardiovascular events (MACE).

Study Limitations. The main limitation of this study is the short 30-day follow-up and longer-term studies may be needed. Another limitation is the relatively small number of patients included in this single center study. The results need to be replicated in a larger that examines the prognostic value of CEC and FMD on the individual endpoints, namely all-cause mortality, recurrent nonfatal MI, or heart failure. Our analysis of CECs was performed on cells cultured for 14 days. We believe this method allows us to enrich the PB-MNCs and thus allows for better assessment of CECs. The approach may explain some of the difference between our findings and other published reports.

Conclusions

This study suggests that higher CEC levels and poor endothelial dysfunction could be markers of large myocardial infarction in patients at risk of developing adverse clinical events. They could serve as prognostic markers of clinical outcomes in patients with acute coronary syndrome independent of the established conventional risk factors. Our data suggest a multi-marker approach, inclusive of CECs, is warranted to evaluate the prognosis in patients presenting with ST elevation myocardial infarction.

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