Prognostic Value of Placental Growth Factor in Patients With Acute Chest Pain

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Context  Experimental data suggest that placental growth factor (PlGF), a member of the vascular endothelial growth factor family, acts as a primary inflammatory instigator of atherosclerotic plaque instability and thus may be useful as a risk-predicting biomarker in patients with acute coronary syndromes (ACS).

Objective  To determine whether blood levels of PlGF predict risk for death or nonfatal myocardial infarction in patients with acute chest pain.

Design, Setting, and Patients  Measurement of PlGF levels as well as levels of markers of myocardial necrosis (troponin T [TnT]), platelet activation (soluble CD40 ligand [sCD40L]), and inflammation (high-sensitivity C-reactive protein [hsCRP]) in an inception cohort of 547 patients with angiographically validated ACS participating in the CAPTURE (c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina) trial and in a heterogeneous cohort of 626 patients presenting with acute chest pain to an emergency department in Germany between December 1996 and March 1999.

Main Outcome Measure  Risk for death or nonfatal myocardial infarction after 30 days.

Results  In patients with ACS, elevated PlGF levels (>27.0 ng/L; 40.8% of patients) indicated a markedly increased risk of events at 30 days (14.8% vs 4.9%; unadjusted hazard ratio [HR], 3.34; 95% confidence interval [CI], 1.79-6.24; P < .001). In a multivariable model, elevated levels of TnT (HR, 1.83; 95% CI, 1.05-3.86; P = .03), sCD40L (HR, 2.65; 95% CI, 1.41-4.99; P = .002), and PlGF (HR, 3.03; 95% CI, 1.54-5.95; P = .001) were independent predictors, while elevated hsCRP level was not (HR, 0.98; 95% CI, 0.53-1.98; P = .94). In patients with acute chest pain, elevated levels of PlGF predicted risk (21.2% vs 5.3%) (unadjusted: HR, 4.80; 95% CI, 2.81-8.21; P < .001; adjusted: HR, 3.00; 95% CI, 1.68-5.38; P < .001). Patients negative for all 3 markers (TnT, sCD40L, and PlGF) were at very low cardiac risk (7 days: no event; 30 days: 2.1% event rate).

Conclusions  Plasma PlGF levels may be an independent biomarker of adverse outcome in patients with suspected ACS. A single initial measurement of plasma PlGF appears to extend the predictive and prognostic information gained from traditional inflammatory markers.
sclerotic plaque instability. Accordingly, we aimed to characterize the potential prognostic value of PlGF in addition to the well-established biomarkers troponin T (TnT), soluble CD40 ligand (sCD40L), and hsCRP in patients with unstable coronary heart disease. We investigated patients with angiographically documented ACS enrolled in the CAPTURE (c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina) trial, as well as a prospective cohort of patients presenting with acute chest pain to an emergency department.

METHODS

Clinical Trial Cohort

The European multicenter CAPTURE trial included patients with recurrent resting chest pain associated with electrocardiographic changes during treatment with intravenous heparin and nitroglycerin. All patients underwent prerandomization coronary angiography and had a culprit lesion measuring 70% or larger suitable for percutaneous transluminal coronary angioplasty (PTCA). Heparin was administered from before randomization until at least 1 hour after the PTCA procedure, which was scheduled between 18 and 24 hours after beginning study treatment. Patients were randomly assigned to receive either the glycoprotein IIb/IIIa receptor antagonist abciximab or placebo. Because other markers such as TnT and sCD40L have been shown to interact with the treatment effect of abciximab, the present analysis was restricted to patients receiving placebo and having available blood samples (n = 547; 86% of patients receiving placebo). Blood samples were collected a mean of 8.7 (SD, 4.9) hours after onset of symptoms but prior to PTCA. Each center in the CAPTURE trial received institutional review board approval for the blood drawings, and all patients provided written informed consent.

Emergency Department Cohort

We separately analyzed a heterogeneous group of 626 consecutive patients with acute chest pain (161 women and 465 men; mean age, 61 [range, 38-82] years) presenting between December 1996 and March 1999 to the emergency department at the University of Hamburg, Hamburg, Germany, with acute chest pain lasting less than 12 hours. Patients with characteristic ST-segment elevations were excluded. The presence of coronary artery disease was documented by 1 of the following criteria: electrocardiographic evidence of myocardial ischemia (new ST-segment depression or T-wave inversion), a history of coronary heart disease (myocardial infarction or coronary revascularization, a positive exercise stress test, or narrowing of at least 50% of the luminal diameter of a major coronary artery on a current angiogram). Patients without coronary heart disease had to have normal coronary angiogram results. Blood samples were collected at the time of arrival in the emergency department (a mean of 3.1 [SD, 3.4] hours after onset of symptoms but prior to initiation of anticoagulation and antiplatelet treatment) and a second blood sample was drawn 4 hours later. The chest pain study was approved by the ethics committee of the General Medical Council, Hamburg. All patients provided written informed consent.

Biochemical Analyses

Measurement of cardiac marker levels was performed blinded to the patients’ histories. Levels of PlGF, VEGF, and sCD40L were measured by enzyme-linked immunosorbent assay (R&D Systems, Wiesbaden, Germany). Total imprecision (expressed as coefficient of variation) was less than 10% for all analytes.

Table 1. Baseline Characteristics for Patients in CAPTURE Trial Receiving Placebo, by PlGF Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI GF &lt; 27.0 ng/L</td>
<td>PI GF &gt; 27.0 ng/L</td>
<td></td>
</tr>
<tr>
<td>(n = 324)</td>
<td>(n = 223)</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>231 (71.3)</td>
<td>154 (69.1)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>61.4 (10.5)</td>
<td>62.5 (10.4)</td>
</tr>
<tr>
<td>Troponin T level &gt; 0.01 µg/L</td>
<td>203 (62.6)</td>
<td>146 (65.5)</td>
</tr>
<tr>
<td>hsCRP level &gt; 10 mg/L</td>
<td>94 (29.0)</td>
<td>151 (67.7)</td>
</tr>
<tr>
<td>Soluble CD40 ligand level &gt; 5 µg/L</td>
<td>123 (38.0)</td>
<td>99 (44.4)</td>
</tr>
<tr>
<td>Creatinine clearance, mean (SD), mL/min†</td>
<td>72 (16)</td>
<td>75 (21)</td>
</tr>
<tr>
<td>ST-segment depression</td>
<td>149 (46.0)</td>
<td>116 (52.0)</td>
</tr>
<tr>
<td>T-wave inversion</td>
<td>167 (51.5)</td>
<td>116 (52.0)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina prior to previous 7 d</td>
<td>179 (55.2)</td>
<td>128 (57.4)</td>
</tr>
<tr>
<td>MI within previous 30 d</td>
<td>41 (12.7)</td>
<td>30 (13.5)</td>
</tr>
<tr>
<td>MI prior to previous 30 d</td>
<td>66 (20.4)</td>
<td>45 (20.2)</td>
</tr>
<tr>
<td>PTCA</td>
<td>53 (16.4)</td>
<td>41 (18.4)</td>
</tr>
<tr>
<td>CABG surgery</td>
<td>10 (3.1)</td>
<td>8 (3.6)</td>
</tr>
<tr>
<td>Risk factors‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>27 (8.3)</td>
<td>28 (12.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>108 (33.3)</td>
<td>89 (39.9)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>128 (39.5)</td>
<td>93 (41.7)</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>317 (97.9)</td>
<td>219 (98.2)</td>
</tr>
<tr>
<td>Intravenous heparin</td>
<td>321 (99.1)</td>
<td>221 (99.1)</td>
</tr>
<tr>
<td>Intravenous nitrates</td>
<td>322 (99.4)</td>
<td>221 (99.1)</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>206 (63.6)</td>
<td>140 (62.8)</td>
</tr>
</tbody>
</table>

Abbreviations: CABG, coronary artery bypass graft; CAPTURE, c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina; hsCRP, high-sensitivity C-reactive protein; MI, myocardial infarction; PlGF, placental growth factor; PTCA, percutaneous transluminal coronary angioplasty.

*Except where otherwise noted.
†Estimated by Cockcroft-Gault formula.
‡Self-reported, or based on medical records when available.

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Figure 1. Association Between PlGF Levels and Composite Event Rate (Death or Nonfatal Myocardial Infarction) at 24 Hours, 72 Hours, 30 Days, and 6 Months, by PlGF Quintile in CAPTURE Patients Receiving Placebo (n=547)

The single end-point mortality according to placental growth factor (PlGF) quintiles is presented for 6 months of follow-up only. The ranges of PlGF levels across quintiles were ≤13.3 ng/L, 13.4-19.2 ng/L, 19.3-27.3 ng/L, 27.4-40.0 ng/L, and >40.0 ng/L. Differences in the composite end point between the quintiles were significant at 72 hours (P=.02), 30 days (P=.001), and 6 months (P<.001) of follow-up. The difference in mortality was significant at 6 months of follow-up (P=.01). CAPTURE indicates c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina.

Statistical Methods

All results for continuous variables are expressed as mean (SD). Comparisons between groups were analyzed by Mann-Whitney U test. Comparison of categorical variables was generated by the Pearson χ² test. The primary end points were mortality or nonfatal myocardial infarction during 30 days of follow-up. Secondary end points for the CAPTURE trial were mortality or nonfatal myocardial infarction up to 6 months. The distribution of hsCRP and PlGF blood levels was nonnormal, but logarithmic transformation resulted in normal distribution of the values. The Cox proportional hazards regression model was used to estimate the relative risk for cardiovascular events in relation to the logarithmically transformed variables. The assumption that the hazards are proportional over time was validated by defining a time-dependent covariate as a function of time and PlGF level. Analysis of quintiles of PlGF levels based on nontransformed values was performed using the Cox proportional hazards regression model with the lowest quintile serving as the reference group. Analysis of the receiver operating characteristics (ROC) curve over the dynamic range of the PlGF assay was used to identify the threshold level for PlGF providing highest predictive value. The effect of baseline characteristics (with P = .10 necessary to enter a variable into the model) and other biochemical markers on any observed associations between PlGF levels and cardiovascular events was analyzed using stepwise multivariable Cox proportional hazards models adjusted for age, sex, history of diabetes, ST-segment changes, and the measured biochemical markers. All included variables were significant predictors in a univariate model. Reported P values are 2-sided; P < .05 was used to determine statistical significance. All statistical significance. All statistical
Diagnosing myocardial infarction (MI) remains a major challenge, even in well-equipped centers. Several biomarkers, such as troponin T (TnT) and high-sensitivity C-reactive protein (hsCRP), have been used successfully to identify patients who may have had MI, and their combined use with other biomarkers is often superior to single-marker analysis. However, the predictive value of these biomarkers is not always consistent, and some patients may remain at risk, particularly those with undetectable TnT levels. Placental growth factor (PlGF) is a novel biomarker that has been found to be detectable in 95.6% of patients with acute coronary syndrome (ACS) and is associated with increased risk for death or nonfatal MI, independent of TnT and sCD40L levels, in patients with ACS. In a multivariable analysis, PlGF level was the more powerful predictor of the patients' outcome (HR, 3.74; 95% CI, 1.79-6.54; P = .001). The single end point mortality at 6-month follow-up also significantly differed between both groups (4.0% vs 0.6%; HR, 6.75; 95% CI, 1.44-31.55; P = .009). A total of 11 patients died during 6 months of follow-up.

**Multivariable Analyses**

The predictive value of PlGF level was independent of myocardial necrosis as evidenced by TnT level (Table 2). High PlGF levels indicated increased cardiac risk in patients with undetectable TnT levels (4.5% vs 0.3% for high and low PlGF levels, respectively; P = .01), in patients with low TnT levels (21.0% vs 3.6%; P < .001), and in patients with high TnT levels (31.4% vs 10.4%);
Discharge PlGF Levels and Long-term Outcome

A second blood sample, drawn before discharge (a mean of 7.2 [SD, 4.5] days after randomization), was available for 489 (89.4%) of the 547 patients receiving placebo. Levels of PlGF decreased only slightly from a mean (SD) of 27.95 (9.83) ng/L at baseline to 25.04 (11.29) ng/L at discharge (–10.4%; P = .01), whereas hsCRP levels had increased by 52.6% (P < .001). For patients with PlGF levels above 27.0 ng/L at discharge, the incidence of mortality and nonfatal myocardial infarction was significantly higher compared with patients with low PlGF levels, both at 30-day follow-up (4.6% vs 0.8%; P = .02) and at 6-month follow-up (7.4% vs 2.2%; P = .005).

Emergency Department Cohort

Despite similar clinical presentation at the time of arrival, patients in the emergency department cohort were classified as follows at the time of discharge: 308 patients with ACS (117 patients had non–ST-elevation myocardial infarction), 91 patients with stable angina, 10 patients with pulmonary embolism, 11 patients with congestive heart failure, 7 patients with myocarditis, and 199 patients with no evidence of heart disease.

Mean (SD) PlGF levels were significantly higher in patients with unstable angina or non–ST-elevation myocardial infarction (n = 308) (28.3 [7.3] ng/L) compared with patients with stable angina (16.2 [3.6] ng/L) (P < .001), and with patients having no evidence of heart disease (9.6 [4.2] ng/L) (P < .001), respectively (Figure 4). Mean PlGF levels in patients with non–ST-elevation myocardial infarction did not significantly differ from mean PlGF levels in patients with unstable angina (30.5 [15.2] vs 28.3 [9.8] ng/L; P = .42). The 97.5th percentile upper reference limit in patients without evidence for heart disease was 24.9 ng/L and the 99th percentile upper reference limit was 30.5 [15.2] vs 28.3 [9.8] ng/L; P = .42).

A total of 65 events (including 18 fatal events) were recorded during 30 days of follow-up. Using the threshold value for PlGF level of 27.0 ng/L derived from the CAPTURE cohort, patients with high PlGF levels were at significantly increased risk compared with patients with low PlGF levels (21.2% vs 5.3%; univariate analysis; HR, 4.80; 95% CI, 2.81-8.21; P < .001; multivariable analysis: adjusted HR, 4.52; 95% CI, 2.23-9.17; P < .001) (Figure 5). Levels of TnT (HR, 7.37; 95% CI, 4.10-13.26; P < .001), sCD40L (HR, 2.74; 95% CI, 1.54-4.86; P < .001), and PlGF (HR, 3.01; 95% CI, 1.68-5.38; P < .001) emerged as independent powerful predictors for cardiovascular events during 30 days of follow-up. Patients who were negative for all 3 markers were at very low cardiac risk (7 days: no event; 30 days: 2.1% event rate [95% CI, 1.5%-2.6%]) (Figure 6). A second blood sample collected 4 hours after arrival in the emergency department did not increase the predictive value of PlGF for...
30-day outcome (area under the ROC curve: 0.70 [95% CI, 0.62-0.78] vs 0.71 [95% CI, 0.65-0.77] for the second vs the baseline blood samples, respectively; P = .84).

COMMENT

We found that PlGF blood levels at presentation are of prognostic value of clinical outcome in patients with known or suspected ACS. The predictive value of PlGF levels is independent of evidence for myocardial necrosis as determined by TnT levels and platelet activation as evidenced by sCD40L levels. Moreover, elevated PlGF levels did not identify only those patients with acute chest pain who developed ACS, but also those patients with an increased risk of recurrent instability after hospital discharge.

The role of PlGF as a primary inflammatory instigator of atherosclerotic lesion instability is supported by its proinflammatory effects in animal models of atherosclerosis or arthritis. Although PlGF belongs to the VEGF family of growth factors, its pathophysiologic role appears to be more related to vascular inflammation than to angiogenesis. Whereas VEGF is activated by hypoxia and elevation of VEGF levels is regarded as an early adaptation of the myocardium to deprivation of blood flow, PlGF is not affected or even downregulated by hypoxia. We did not find any correlation between PlGF levels and VEGF levels (data not shown) as a marker of myocardial ischemia or between PlGF levels and TnT levels as a marker of myocardial necrosis. Thus, PlGF levels do not appear to be confounded by myocardial necrosis, whereas VEGF levels are linked to elevated levels of TnT, impaired TIMI flow, and clinical evidence of myocardial ischemia. The lack of PlGF levels being sensitive to minor myocardial injury might be specifically important in patients with ACS, of whom approximately one third are positive for TnT and troponin I at the time of arrival in the hospital. In contrast, myocardial injury appears to compromise the value of hsCRP levels for predicting outcome in patients with ACS. As a classic unspecific downstream acute-phase marker, hsCRP levels can rise substantially after acute myocardial ischemia (TnT ≥0.01 µg/L: 14.1 [SD, 19.6] mg/L; TnT >0.01 µg/L: 23.6 [SD, 28.9] mg/L; P < .001) such that determining an individual’s underlying basal level is difficult and may result in misclassification.

By multivariable proportional hazards analysis, levels of PlGF, sCD40L, and TnT all emerged as independent predictors of adverse outcome. Combining PlGF and sCD40L was especially revealing in patients negative for TnT, suggesting that both markers reflect distinct pathways that eventually contribute to a proinflammatory and procoagulant milieu in the coronary circulation. Given the superiority of PlGF level over hsCRP level for predicting cardiovascular events in patients with ACS, the identification of PlGF as a primary inflammatory instigator of coronary lesion instability will substantially enhance our diagnostic armamentarium for the diagnosis and risk stratification of patients with ACS.

Potential limitations of the current study merit consideration. First, because our blood samples were stored at −80°C until analysis, we cannot exclude the possibility of protein degradation. However, the samples for this study were only thawed once and we have noted measured PlGF values to be similar in fresh and frozen samples (data available from authors upon request). Second, because of the selected nature of the CAPTURE cohort and the relatively small number of 30-day events, we were concerned that our results might not be generalizable. We therefore prospectively tested the prognostic value of PlGF and other markers in a “real-world” cohort of patients presenting with chest pain. Third, we could not determine from this cohort whether PlGF can reliably identify patients who will benefit from aggressive management, as has been shown for example with troponin T and interleukin 6.

In summary, PlGF plasma levels represent a potentially powerful clinical biomarker of vascular inflammation and adverse outcome in patients with ACS.
Measuring PLGF levels may extend the predictive and prognostic information gained from traditional inflammatory markers in patients with ACS. Since the proinflammatory effects of PLGF can be specifically inhibited by blocking its receptor, Fms-like tyrosine kinase, these findings may also provide a rationale for a novel anti-inflammatory therapeutic target in patients with coronary artery disease.29

Author Contributions: Dr Heeschen, as principal investigator of the study, took responsibility for the integrity of the data and the accuracy of the data analyses. Study concept and design: Heeschen, Dimmeler, Fichtlscherer, Hamm, Zeiher. Acquisition of data: Heeschen, Simonos, Zeiher. Analysis and interpretation of data: Heeschen, Fichtlscherer, Hamm, Berger, Zeiher. Drafting of the manuscript: Heeschen, Zeiher. Critical revision of the manuscript for important intellectual content: Heeschen, Dimmeler, Fichtlscherer, Hamm, Berger, Simonos, Zeiher. Statistical expertise: Heeschen, Fichtlscherer, Berger.

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REFERENCES


