Placental Growth Factor in Acute Coronary Syndrome Patients with Non ST-Elevation

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Abstract

Background: Markers of plaque instability are considered as predictors of future coronary events in patients with non ST-elevation myocardial infarction (NSTEMI) in acute coronary syndrome (ACS). The aim of this study was to determine the role of placental growth factor (PlGF) as a possible predictor of future coronary events such as non-fatal myocardial infarction or cardiac death measured at the admission and 30 days after discharge.

Methods: We prospectively collected data from 102 patients admitted to the emergency department (ED) for ACS with chest pain at rest within the preceding 12 hours who were evaluated by risk factors and electrocardiogram changes (ECG).

Results: The time course of PlGF levels determined on 102 patients showed a mean value 13.21 ng/L and standard deviation 8.76 ng/L but in the sample of the 53 patients who survived after 30 days, these values decreased to 10.48 ng/L and 5.45 ng/L, respectively.

Conclusions: Results of this study indicate a clinically useful role of PlGF in the detection of ACS and NSTEMI patients who are at a higher risk for different cardiovascular disorders within 30 days of admission.

Introduction

An acute coronary syndrome (ACS) without ST-segment elevation (NSTEMI)[1] or T-wave inversion requires special treatment in those patients admitted to the emergency department (ED) with strong chest pain.[2,3] A history of previous cardiovascular diseases, electrocardiogram (ECG) changes, on admission as well as potential risk factors such as sex, age,[4] smoking, hypertension, and diabetes mellitus could provide helpful information about the health conditions of patients. In making adequate triage diagnostic and early therapeutic decisions different biochemical blood biomarkers[5-10] as risk indicators could be important in predicting the development of future possible cardiovascular disorders.

As a risk-predicting biomarker in patients with ACS, placental growth factor (PlGF)[11] was recently shown to be important in early and advanced atherosclerotic lesions and coronary plaque rupture. This biomarker could be up-regulated in the crucial mechanism of atherosclerosis in ACS patients[12] based on subsequent platelet aggregation and systematic thrombosis, which may lead to the acute myocardial infarction (AMI) or sudden cardiac death. Hence, as a member of the vascular endothelial growth factor family (VEGF)[13] PlGF may be a primary inflammatory instigator of atherosclerotic plaque instability during the acute phase of ACS.

Accordingly, we attempted to establish the potential prognostic value of PlGF in ACS patients with non ST-segment elevation for the recurrence of a new vascular event, including death and non-fatal MI after 30 days of follow up.

Materials and Methods

Subjects

The study protocol No. 01/33 was approved by the Clinical Center of Kragujevac Institutional Ethics Review Comittee, and written informed consent was obtained from all participating patients. We enrolled 102 patients presenting acutely with ACS who had chest pain at rest within the preceding 12 hours. Variables associated with coronary events were sex, previous history of coronary artery disease (CAD), diabetes mellitus (DM), chronic infections, and smoking. Obesity and body mass index (BMI) were the risk factors observed at presentation, and we paid attention to shortness of breath as a symptom of current physical condition of patients.

Study patients had peripheral venous blood samples drawn at presentation because PlGF is detectable in the peripheral circulation. These samples were centrifuged and serum was used for analysis at the Institute of Biochemistry. After 30 days, 53 patients survived, and 49 of them died. There were no patients lost due to follow up.

PlGF Biomarker Method

PlGF was determined in 102 patients within the 12 hours after pain onset. These patients were followed up for 30 days when the second determination of this marker was obtained. Quantitative detection of PlGF serum concentrations was performed using an enzyme linked immunosorbent assay (ELISA) technique based on a commercially available assay kit (R&D Systems, Minneapolis, MN).[14]

Statistical Analysis

Statistical analysis was performed using an SPSS software package (SPSS, Chicago, IL). Descriptive statistics were given as mean ± SD. The primary end point was mortality or non-fatal myocardial infarction during 30 days of follow-up. Risk factors (sex, previous history, diabetes mellitus, chronic infections, smoking, obesity, and BMI) as well as ECG data (ST-segment depression, T-wave inversion) were taken as independent variables. Namely, the effect of baseline characteristics between PlGF levels and cardiovascular events was analyzed using a stepwise multivariable adjusted for sex, previous history, diabetes mellitus, chronic infections, smoking, obesity, BMI, and ST-segment changes. Multiple regression analysis was applied in order to evaluate the relationship between PlGF and short-term outcome and a probability value of $P<0.05$ was considered significant. Box and Whisker plots were used to display analyzed

data. Additional analyses were performed in the group of patients who survived the first 30 days, using Kaplan-Meier, with fatal events counted after this period.

Table 1_The Values of Serum PlGF Concentrations of ACS Patients and Risk Factors Observed at Admission

Risk Factors	Status of Patients	Number of Observed Patients (%)	Mean
ECG	ST	66 (64.70%)	12.46 ± 8.85
ECG	T	36 (35.29%)	14.56 ± 8.55
Sex	Male	70 (68.62%)	13.56 ± 8.21
Sex	Female	32 (31.37%)	12.44 ± 9.95
Previous history	Yes	56 (54.90%)	13.76 ± 8.46
DM	Yes	47 (46.07%)	12.39 ± 8.06
Chronic infection	Yes	68 (66.66%)	12.22 ± 8.63
Smoker	Yes	59 (57.84%)	13.31 ± 8.54
Obesity	Yes	31 (30.39%)	15.37 ± 7.97
BMI	Normal	62 (60.78%)	12.80 ± 9.25

Statistic data were given as mean ± SD, and a probability value of p<0.05 was considered significant.

Results

At the beginning of our experiment we included 102 ACS patients admitted to the ED. Based on ECG changes recorded at the admission we separated 66 (64.70%) patients with ST-segment depression and 36 (35.29%) patients with T-wave inversion (**Table 1**). The rationale for separating ST-segment depression and T-wave inversion groups was the difference between the duration of ST-segment depression (80-120ms) and the appearance of T-wave. Putting the patients into these 2 groups was the consequence of recorded changes of their ECG. Neither of them had a worse outcome than the other. Of 102 patients who were eligible, 70 (68.62%) were men and 32 (31.4%) were women mean age 56 (50%). Of them, 56 (54.90%) had a statistically significant (P<0.05) history of cardiovascular disease in the ST-group. Forty-seven (46.07%) had diabetes mellitus, 68 (66.66%) had chronic infections, and 59 (57.84%) reported being current smokers. At the time of presentation, 31 (30.39%) of patients were obese, and the value of BMI was a total of 62 (60.78%). As illustrated in **Table 1**, the concentrations of PlGF dependents mostly with a previous history of cardiovascular disease, obesity, and body mass index (P<0.05). The time course of PlGF levels based on 102 observed patients (**Figure 1**) showed mean of 13.21 ng/L and standard deviation of 8.76 ng/L. After 30 days 49 (48.00%) patients died (P<0.05) and 53 (51.96%) survived. Of the 53 who survived, the PlGF concentration decreased to a mean of 10.48 ng/L and a standard deviation of 5.45 ng/L (**Figure 2**). A line graph linking before and after would clearly provide the PlGF levels of the 53 surviving patients and illustrate how these levels fell in a majority of the cases (**Figure 3**). Kaplan-Meier curves of myocardial infarction of deaths during the 30-day follow up were used to compare the survival group PlGF levels vs. the non-survival group levels and to illustrate prognostic value of the tests. As shown in **Figure 4**, dotted line = PLGF > 13.21 ng/L.

All patients received antiplatelet therapy (aspirin or clopidogrel), unfractionated heparin, GP IIb/IIIa inhibitors, beta blockers, and, if indicated, oral ACE inhibitors. Patients received no antiinflammatory drugs, except aspirin.

Placental growth factor was determined in 105 healthy volunteers, and the value of upper reference limit was 25.8 ng/L.

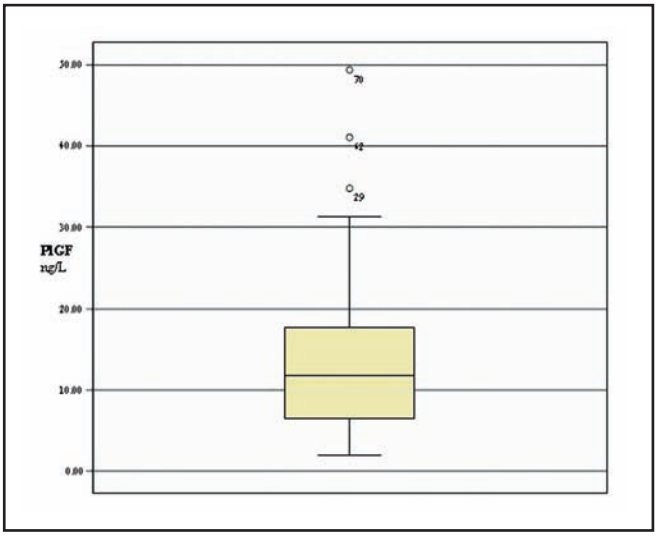

Figure 1_The PlGF levels for 102 observed patients at admission.

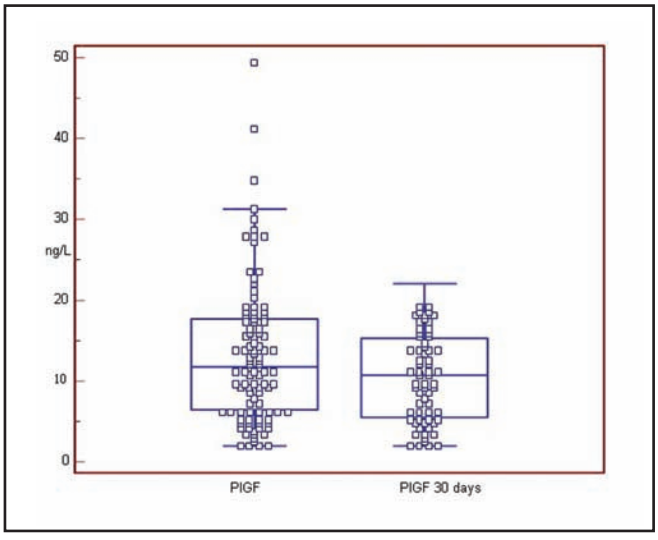

Figure 2_The comparison between PlGF concentrations at admission and after 30 days.

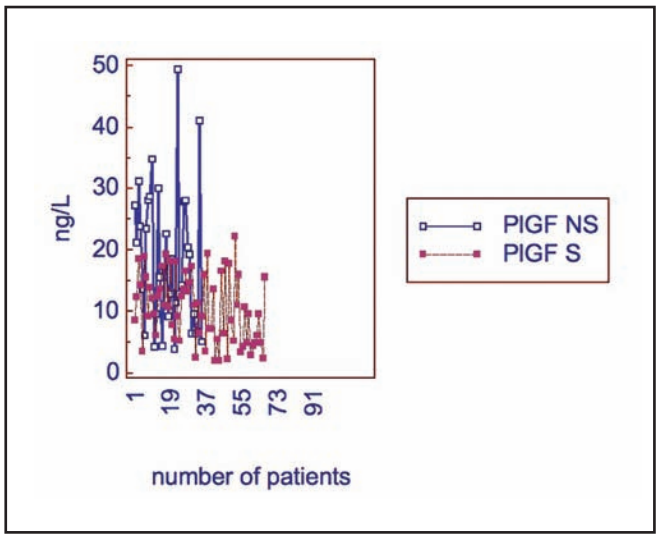

Figure 3_The PlGF levels of 53 surviving patients at the baseline.

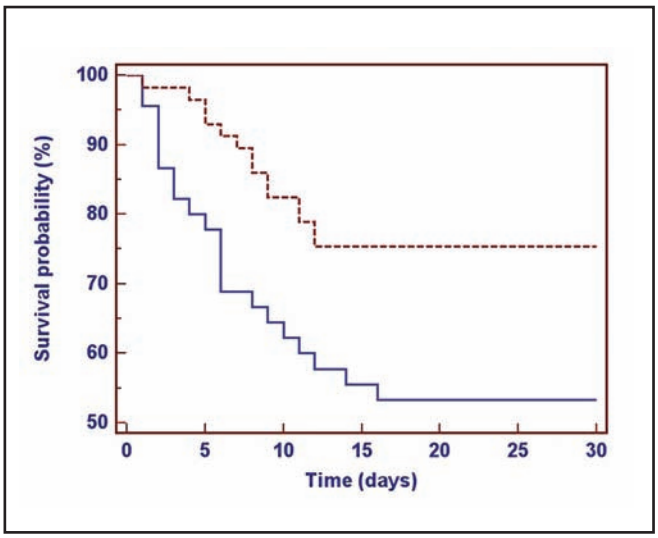

Figure 4_Kaplan-Meier curves of myocardial infarction or deaths during the 30-day follow up.

Discussion

It is estimated that in all patients, ACS is recognized by the ordinary cardiologist because of typical and extremely strong chest pains. For such patients the presence of thrombogenic contents in the circulation[15,16] could be responsible for the plaque rupture in these unstable coronary conditions. The most cases of ACS[17,18] are results of platelet activation and existing thrombus formation which could cause possible atherosclerosis. It is possible to take a lot of already familiar cardiac markers, but we decided to observe the role of PlGF as a member of the vascular endothelial growth factor (VEGF) family in patients with coronary heart disease. The present findings indicate that the concentration of PlGF, a more specific marker of vascular inflammation, is significantly changed at the admission in comparison to the history of previous cardiovascular disease in 56 patients, as well as in correlation with the obesity in 31 patients. At the same time, 62 patients showed a change of PlGF concentration associated with determined value of BMI risk factor. All these risk factors could potentially change PlGF serum concentrations and make it relevant at the admission to the ED. Pilarczyk et al[19] tested a hypothesis that PlGF-expression in human atherosclerotic carotid plaques is related to inflammation, vascularization, and clinical plaque instability mediates angiogenesis with inflammation. Namely, this biomarker could be released into vulnerable plaques from damaged cardiomyocites and the degree of injured vascular endothelium apparently determines the induction of PlGF very early after the onset of MI, which have been reported Iwama et al.[20] Since the pathway, which is leading to up-regulating of PlGF expression level, is not known yet, we could take the possibility that the concentration of this biomarker can be regulated by the duration of the acute phase of MI. A physiologically active biomarker, PlGF recruits macrophages that have important roles in removing necrotic tissue from an injured area and promote the mobilization of bone marrow monocytes into the same inflammatory atherosclerotic lesions causing the process of pathological angiogenesis. As it is already shown in some previous studies[21-24] serum PlGF concentrations in patients who presented to the ED with chest pain are involved in tissue repair of injured myocardium. It is evident that the degree of PlGF production released from the heart after a heart attack correlated with the improvement of cardiac function and therefore, PlGF might enhance wound healing and become important in the clinical treatment of MI.

Our results based on all selected NSTEMI patients suggest that altered serum PlGF levels may be a relevant predictor of plaque instability in the first few hours after admission to the ED. The predictive value of PlGF was independent of hsCRP, a biomarker of general inflammation. This means that PlGF provides prognostic value independently[25,26] of hsCRP and could serve as an important clinical factor in the prediction of the further development of MI providing useful information in determining future ACS risk.

Interestingly, baseline PlGF was also associated with an increased risk of cardiovascular complications during the 30 days of follow-up in survived patients. These data add to the growing evidence showing that hsCRP as a classic systemic acute-phase protein might not be specific enough for the inflammation process involved in vascular atherosclerosis.[27] Therefore, hsCRP levels collected in ACS patients seem to be less useful as a tool for cardiovascular risk stratification and patient management. In contrast, PlGF as a more specific marker of vascular inflammation, providing independent predictive value for the short-term cardiovascular outcome is not hampered by the occurrence of an ACS[28] and therefore playing a very important role in clinical decision making.

Our results confirmed that the plaque instability, represented by PlGF elevation upon admission, could have an important role in the pathogenesis of future coronary events in patients with NSTEMI. Future studies of the role of new cardiac markers implied in clinical practice should assess a potential long-term benefit of this biomarker in ACS patients.

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1. Boersma E, Akkerhuis KM, Theroux P, Califf RM, Topol EJ, Simoons ML. Platelet glycoprotein IIb/IIIa receptor inhibition in non-ST-elevation acute coronary syndromes: early benefit during medical treatment only, with additional protection during percutaneous coronary intervention. *Circulation*. 1999;100:2045-2048.

2. Storrow AB, Gibler WB. Chest pain centers: Diagnosis of acute coronary syndromes. *Ann Emerg Med*. 2000;35:449-461.
3. American Heart Association. Heart disease and stroke statistics: 2004 update. Dallas: American Heart Association. 2004.
4. Stone PH, et al. Influence of race, sex, and age on management of unstable angina and non-Q-wave myocardial infarction: The TIMI III registry. *JAMA*. 1996;275:1104-1112.
5. Svetlana I. Strategy of IFCC standardization and use of cardiac markers in acute coronary syndromes. *Jugoslav Med Biochem*. 2003;22:303-309.
6. Wu AH, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes R Jr. National Academy of Clinical Biochemistry standards of laboratory practice: Recommendations for the use of cardiac markers in coronary artery diseases. *Clin Chem*. 1999;45:1104-1121.
7. Apple FS, Wu AHB, Mair J, Ravkilde J, Panteghini M, Tate J, Pagani F, Christenson RH, Mockel M, Danne O, Jaffe AS. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. *Clin Chem*. 2005;51:810-824.
8. Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, Wu AHB, Christenson RH. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Clinical characteistics and utilization of biochemical markers in acute coronary syndromes. *Clin Chem*. 2007;53:552-574.
9. Ogedegbe HO. Biochemical markers in risk stratification and diagnosis of acute coronary syndromes. *LabMed*. 2002;33:42-53.
10. Carreiro-Lewandowski E. Update on cardiac biomarkers. *LabMed*. 2006; 37:597-605.
11. Lenderink T, Heeschen C, Fichtischerer S, Dimmeler S, Hamm CW, Zeicher AM, Simmons ML, Boersma E, for the CAPTURE Investigators. Elevated placental growth factor levels are associated with adverse outcomes at four-year follow-up in patients with acute coronary syndromes. *J Am Coll Cardiol*. 2006;47:307-311.
12. Heeschen C, Dimmeler S, Fichtlscherer S, Hamm CW, Berger J, Simmons ML, Zeiher AM, for the CAPTURE Investigators. Prognostic value of placental growth factor in patients with acute chest pain. *JAMA*. 2004;291:435-441.
13. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H, Scholz D, Acker T, DiPalma T, Dewerchin M, Noel A, Stalmans I, Barra A, Biacher S, Vandendriessche T, Ponten A, Eriksson U, Plate KH, Foidart JM, Schaper W, Charnock-Jones DS, Hicklin DJ, Herbert JM, Collen D, Persico MG. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med*. 2001;7:575-583.
14. Quantikine Human PlGF Immunoasay for the quantitative determination of human placenta growth factor (PlGF) concentrations in cell culture supernates, serum, plasma and urine. R&D Systems, Inc, Minneapolis, MN.
15. Farb A, Burke AP, Tang AL, Liang Y, Mannan P, Smialek J, Virmani R. Coronary plaque erosion without rupture into a lipid core. *Circulation*. 1996;93:1354-1363.
16. Daries MJ. The composition of coronary-artery plaques. *N Engl J Med*. 1997; 336:1312-1313.
17. Falk E. Unstable angina with fatal outcome: Dynamic coronary thrombosis leading to infraction and/or sudden death. Autopsy evidence of recurrent mural thrombosis with peripheral embolisation culminating in total vascular occlusion. *Circulation*. 1985;711:699-708.
18. Davies MJ, Thomas AC, Knapman PA, Hangartner JR. Intramyocardial platelet aggregation in patients with unstable angina suffering sudden ischemic cardiac mortality. *Circulation*. 1986;73:418-427.
19. Pilarczyk K, Sattler KJ, Galili O, Versari D, Olson MZ, Meyer FB, Zhy XY, Lerman LO, Lerman A. Placenta growth factor expression in human atherosclerotic carotid plaques is related to plaque destabilisation. *Atherosclerosis*. 2008;196:333-340.
20. Iwama H, Uemura S, Naya N, Imagawa K, Takemoto Y, Asai O, Onoue K, Okayama S, Somekawa S, Kida Y, Takeda Y, Nakatani K, Takaoka M, Kawata H, Horii M, Nakajima T, Doi N, Saito Y. Cardiac expression of placental growth factor predicts the improvement of chronic phase left ventricular function in patients with acute myocardial infarction. *J Am Coll Cardiol*. 2006;47:1559-1967.
21. Heil M, Ziegelhoeffer T, Pipp F. Blood monocyte concentration is critical for enhancement of collateral artery growth. *Am J Physiol Heart Circ Physiol*. 2002; 283:H2411-H2419.
22. Apple FS, Pearce LA, Chung A, Ler R, Murakami MAM. Multiple biomarker use for detection of adverse events in patients presenting with symptoms suggestive of acute coronary syndrome. *Clin Chem*. 2007;53:874-881.
23. Selvaraj SK, Giri RK, Perelman N, Johnson C, Malik P, Kalra VK. Mechanism of monocyte activation and expression of proinflammatory cytochemokines by placenta growth factor. *Blood.* 2003;102:1515-1524.
24. Semenza GL. Regulation of hypoxia-induced angiogenesisa chaperone escorts VEGF to the dance. *J Clin Invest.* 2001;108:39-40.
25. Koenig W, Khuseyinova N. Biomarkers of atherosclerotic plague instability and rupture. *Arterioscl Thromb Vasc Biol*. 2007;27:15-26.
26. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes. *Circulation*. 2006;113:e289-e292.
27. Biasucci LM, Liuzzo G, Colizzi C, Rizzello V. Clinical use of C-reactive protein for the prognostic stratification of patients with ischemic heart disease. *Ital Heart J.* 2001;2:164-171.
28. Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, Nagy JA, Hooper A, Priller J, De Klerck B. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med.* 2002;8:831-840.