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Pregnancy-Associated Plasma Protein-A Levels in Patients With Acute Coronary Syndromes

Comparison With Markers of Systemic Inflammation, Platelet Activation, and Myocardial Necrosis

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OBJECTIVES The goal of this study was to determine the predictive value of pregnancy-associated plasma protein-A (PAPP-A) in patients with acute coronary syndromes (ACS).

BACKGROUND Pregnancy-associated plasma protein-A is a zinc-binding matrix metalloproteinase abundantly expressed in eroded and ruptured plaques and may serve as a marker of plaque destabilization.

METHODS In 547 patients with angiographically validated ACS and in a heterogeneous emergency room population of 644 patients with acute chest pain, respectively, PAPP-A as well as markers of myocardial necrosis (troponin T [TnT]), ischemia (vascular endothelial growth factor [VEGF]), inflammation (high-sensitivity C-reactive protein [hsCRP]), anti-inflammatory activity (interleukin [IL]-10), and platelet activation (soluble CD40 ligand [sCD40L]) were determined. Patients were followed for the occurrence of death or myocardial infarction.

RESULTS In patients with ACS, elevated PAPP-A levels (>12.6 mIU/l) indicated an increased risk (odds ratio 2.44 [95% confidence interval (CI) 1.43 to 4.15]; p = 0.001). When the analysis was restricted to TnT-negative patients, PAPP-A still identified a subgroup of high-risk patients (odds ratio [OR] 2.72 [95% confidence interval (CI) 1.25 to 5.89]; p = 0.009). In a multivariable model, PAPP-A (OR 2.01; p = 0.015), sCD40L (OR 2.37; p = 0.003), IL-10 (OR 0.43; p = 0.003), and VEGF (OR 2.19; p = 0.018) were independent predictors. Prospective validation in patients with chest pain confirmed that PAPP-A levels reliably identify high-risk patients (adjusted OR 2.32 [95% CI 1.32 to 4.26]; p = 0.008). Patients negative for all three markers (TnT, sCD40L, and PAPP-A) were at very low cardiac risk (30 days: 3.0% event rate; no death).

CONCLUSIONS The PAPP-A level as a marker of plaque instability is a strong independent predictor of cardiovascular events in patients with ACS. Simultaneous determination of biomarkers with distinct pathophysiological profiles appears to remarkably improve risk stratification in patients with ACS. (J Am Coll Cardiol 2005;45:229–37) © 2005 by the American College of Cardiology Foundation

Elevated levels of circulating cardiac troponin, a marker of myocardial necrosis, are found in about one-third of the patients with acute coronary syndromes (ACS) and are associated with an increased short-term risk of death and nonfatal myocardial infarction (1–4). Although the absolute short-term risk in troponin-negative patients is significantly lower as compared with troponin-positive patients, the large number of patients without troponin elevation remains clinically challenging with respect to risk assessment and therapeutic management. Specifically, the six-month risk of death or nonfatal myocardial infarction in troponin-negative patients was 8.4% in the c7E3 Anti Platelet Therapy in Unstable Refractory angina (CAPTURE) trial (5). Therefore, the simultaneous measurement of several biomarkers that reflect distinct pathophysiologic processes may improve risk stratification in patients without evidence for myocardial necrosis (6).

Convincing evidence suggests that both inflammatory as well as thrombotic mechanisms are involved in the pathophysiology of patients with ACS (7). The availability of a sensitive and specific early marker of plaque instability, whose levels become elevated before or even in the absence of myocardial necrosis, should improve diagnostic and therapeutic decision-making. Pregnancy-associated plasma protein-A (PAPP-A) is a high-molecular-weight, zinc-binding matrix metalloproteinase belonging to the metzincin superfamily of metalloproteinases (8,9) and was originally identified in the plasma of pregnant women (10). It is widely used for the screening of fetal trisomy in the first trimester of gestation; PAPP-A was also found to be abundantly expressed in eroded and ruptured plaques, respectively, but is only minimally expressed in stable plaques (11). However, a very recent study indicates that, even in patients with stable coronary heart disease, PAPP-A levels are associated with angiographic plaque complexity (12) and were suggested to predict recurrence of symptoms in pa-

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Abbreviations and Acronyms

ACS	= acute coronary syndromes
CAPTURE c7E3	= Anti Platelet Therapy in Unstable Refractory Angina trial
CI	= confidence interval
hsCRP	= high-sensitivity C-reactive protein
IL	= interleukin
PAPP-A	= pregnancy-associated plasma protein A
ROC	= receiver operating characteristic
sCD40L	= soluble CD40 ligand
TnT	= troponin T
VEGF	= vascular endothelial growth factor

tients with ACS (13). However, the precise role of circulating PAPP-A plasma levels for predicting hard end points like death or myocardial infarction in patients with ACS remains to be determined. Most importantly, whether PAPP-A plasma levels indeed provide additive and independent prognostic information compared with recently established biomarkers in patients with ACS is entirely unknown. Therefore, we compared the prognostic significance of PAPP-A plasma levels with markers of systemic inflammation, platelet activation, ischemia, and myocardial necrosis in two distinct study populations, a high-risk population with refractory ACS and a large heterogeneous group of patients with chest pain (6,14–16).

METHODS

Clinical trial population of patients with ACS. The CAPTURE trial included patients with recurrent chest pain at rest associated with electrocardiographic changes during treatment with intravenous heparin and glyceryl trinitrate (17). All patients underwent coronary angiography before randomization, indicating significant coronary artery disease with a culprit lesion ≥70% suitable for angioplasty. Heparin was administered from before randomization until at least 1 h after the percutaneous coronary intervention. For all patients, coronary angioplasty was scheduled between 18 and 24 h after beginning study treatment. The patients were randomly assigned to treatment with the glycoprotein IIb/IIIa receptor antagonist abciximab or placebo. Because other markers, such as troponin T (TnT) (5,18) and soluble CD40 ligand (sCD40L) (6), have been shown to interact with the treatment effect of abciximab, we separately analyzed patients with available blood samples enrolled in the placebo arm (n = 547; 86% of placebo patients) and patients enrolled in the abciximab arm (n = 547; 87% of abciximab patients). For all patients, the first available blood sample collected after a mean time of 8.7 ± 4.9 h after onset of symptoms was analyzed.

Emergency room population of patients with acute chest pain. A heterogeneous group of 626 consecutive patients with acute chest pain (161 females and 465 males, mean age 61 years [range 38 to 82 years]) presenting to the emergency room at the University of Hamburg with acute chest pain lasting less than 12 h. Patients with characteristic ST-segment elevations were excluded. The presence of coronary artery disease was documented by one 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 a normal coronary angiogram. Blood samples were collected at the time of arrival in the emergency room (5.1 ± 3.4 h after onset of symptoms before initiation of treatment), and a second blood sample was drawn 4 h later.

Biochemical analysis. Determination of the cardiac markers was performed blinded to the patients' histories and the allocated treatment at the research laboratory of the University of Frankfurt. High-sensitivity interleukin (IL), vascular endothelial growth factor (VEGF), and sCD40L were measured by ELISA (both R&D Systems, Wiesbaden, Germany). We used the following, previously established diagnostic threshold values: 5.0 μg/l for sCD40L, 300 ng/l for VEGF, and 3.5 ng/l for IL-10 (6,14,15). Cardiac TnT and PAPP-A were determined using a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics, Mannheim, Germany). Using internal controls, total imprecision for PAPP-A over the eight-week period was 8.5%. High-sensitivity C-reactive protein (hsCRP) was measured using the Behring BN II Nephelometer (Dade-Behring, Deerfield, Illinois). A diagnostic threshold value of 10 mg/l was used (16,19).

Statistical methods. A logistic regression model was used to estimate the relative risk for cardiovascular events, and patients were categorized in quintiles of PAPP-A levels (20). Primary end points were mortality and nonfatal myocardial infarction after 30 days (chest pain study) and 6 months (CAPTURE trial), respectively. For each time point (24 h, 72 h, 30 days, and 6 months), the logistic regression model was used to estimate the relative risk for death and myocardial infarction. Post-hoc analysis of PAPP-A quintiles was performed using the logistic regression model, with PAPP-A quintiles as a categorical variable, and the first quintile served as the reference group. Receiver operating characteristics (ROC) curve analysis over the dynamic range of the PAPP-A assay was used to identify the threshold level for PAPP-A providing the highest predictive value. Cumulative survival was univariately evaluated by Kaplan-Meier analysis (log-rank test). The effect of baseline characteristics (with $p = 0.10$ necessary to enter a variable into the model) and other biochemical markers on any observed associations between PAPP-A levels and cardiovascular events was analyzed using stepwise logistic regression models. All results for continuous variables are expressed as means ± SD. Continuous variables were tested

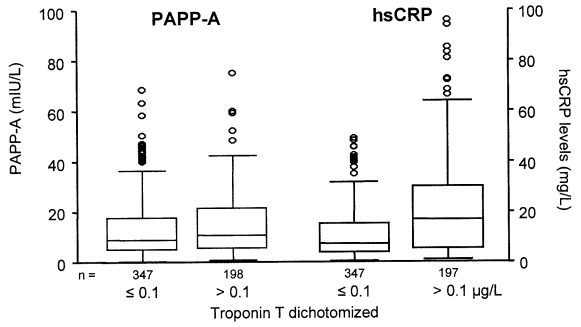

Figure 1. Pregnancy-associated plasma protein A (PAPP-A) and high-sensitivity C-reactive protein (hsCRP) plasma levels, respectively, according to the baseline troponin T status. Only for illustrative purposes, outliers are marked as **circles**. Importantly, all data points were included in the statistical analysis.

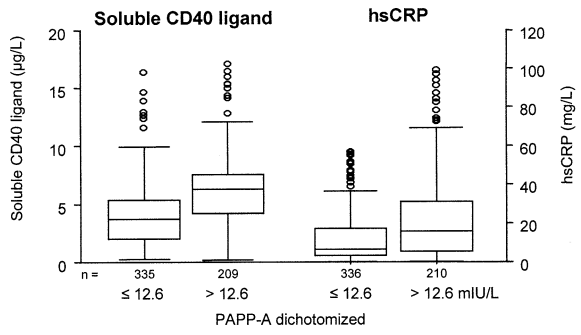

Figure 2. Soluble CD40 ligand and high-sensitivity C-reactive protein (hsCRP) plasma levels, respectively, according to the baseline pregnancy-associated plasma protein A (PAPP-A) status. Only for illustrative purposes, outliers are marked as **circles**. Importantly, all data points were included in the statistical analysis.

for normal distribution with the Kolmogorov-Smirnov test. Comparisons between groups were analyzed by *t* test (two-sided) or by Mann-Whitney *U* test for not normally distributed variables. Comparison of categorical variables was generated by the Pearson chi-square test. Values of $p < 0.05$ were considered statistically significant. All analyses were performed with SPSS 11.5 (SPSS Inc., Chicago, Illinois).

RESULTS

Baseline PAPP-A plasma levels showed a mean level of 14.2 mIU/l (median 9.3 mIU/l; range 0.2 to 105.4 mIU/l). When PAPP-A plasma levels were linked to traditional risk markers, PAPP-A concentrations did not correlate with TnT levels (Spearman rank correlation coefficient $r = 0.11$; $p = 0.16$) and were similar in patients with high TnT plasma levels and in patients with low TnT plasma levels (Fig. 1). Only for illustrative purposes, outliers are marked as circles, whereas all data points were included in the statistical analysis. Similarly, VEGF ($r = 0.08$; $p = 0.07$) and IL-10 plasma levels ($r = -0.04$; $p = 0.35$) did not show any association with PAPP-A plasma levels. In contrast, bivariate correlation analysis revealed a significant correlation between PAPP-A and hsCRP as well as between PAPP-A and sCD40L, although the correlation coefficients were low with $r = 0.21$ for hsCRP ($p < 0.001$) and $r = 0.18$ for sCD40L ($p < 0.001$). However, if the analysis was restricted to patients without myocardial necrosis (no troponin elevation), the correlation between hsCRP and PAPP-A became more obvious with an r value of 0.68 ($p < 0.001$). Consequently, patients with elevated PAPP-A levels had significantly higher hsCRP and sCD40L levels, respectively (Fig. 2).

Interaction between PAPP-A plasma levels and cardiac risk. Patients were stratified into quintiles according to their measured PAPP-A levels: (PAPP-A_1) <4.5 mIU/l (n = 111), (PAPP-A_2) 4.5 to 7.5 mIU/l (n = 108), (PAPP-A_3) 7.6 to 12.6 mIU/l (n = 109), (PAPP-A_4) 12.7 to 24.0 mIU/l (n = 110), and (PAPP-A_5) >24.0 mIU/l (n = 109), respectively. For the initial 24-h period, the combined end points of mortality and nonfatal myocardial infarction did not differ between the quintiles ($p = 0.69$) (Fig. 3). For the 72-h follow-up including periinterventional events, the difference in cardiac events between the quintiles reached a level of statistical significance ($p = 0.019$). During 30-day and 6-month follow-up, the event rate curves continued to diverge, resulting in highly significant differences between the quintiles both at 30 days ($p = 0.008$) and 6 months of follow-up ($p = 0.004$). For the 6-month follow-up data, post-hoc analysis of the PAPP-A quintiles using a logistic regression model revealed that only the upper two PAPP-A quintiles (4th quintile: $p = 0.034$; 5th quintile: $p = 0.002$) significantly differed from the first PAPP-A quintile, which served as a reference. Consistent with these results, ROC curve analysis verified a threshold level of 12.6 mIU/l PAPP-A for maximized predictive value (Fig. 4).

Therefore, the study population was dichotomized according to the calculated threshold level of 12.6 mIU/l,

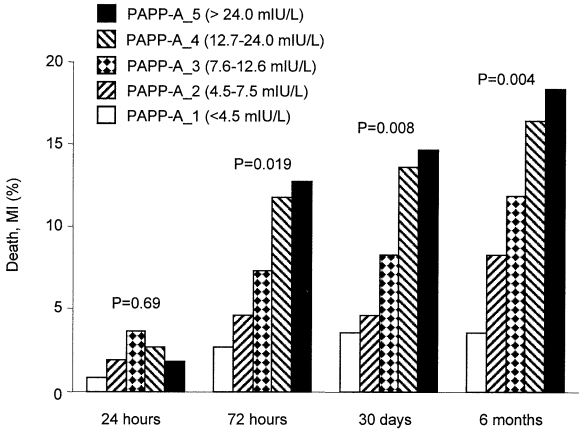

Figure 3. Association between the pregnancy-associated plasma protein A (PAPP-A) plasma levels and the cardiac event rate at 24 h, 72 h, 30 days, and 6 months according to the PAPP-A quintile in the placebo group of the Anti Platelet Therapy in Unstable Refractory Angina (CAPTURE) study (n = 547). Differences in event rates between the quintiles were significant at 72 h ($p = 0.019$), 30 days ($p = 0.008$), and 6 months ($p = 0.004$) of follow-up. MI = myocardial infarction.

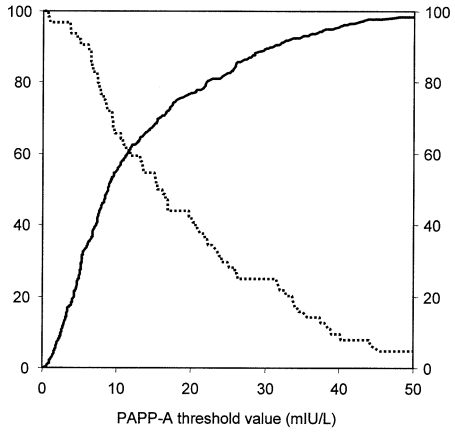

Figure 4. Receiver operating characteristic curve analysis for the predictive value of pregnancy-associated plasma protein A (PAPP-A) plasma levels for the occurrence of death or nonfatal myocardial infarction at six months of follow-up (n = 547). **Solid line** = sensitivity (%); **dotted line** = specificity (%).

resulting in 219 patients with elevated PAPP-A levels (40.0%). Besides higher levels of hsCRP and sCD40L, respectively, in patients with elevated PAPP-A plasma levels, baseline characteristics in patients with elevated PAPP-A plasma levels were not significantly different from patients with low PAPP-A plasma levels (Table 1). The odds ratios for death and myocardial infarction (adjusted for differences in baseline characteristics) were 1.07 (95% confidence interval [CI] 0.34 to 3.42; p = 0.91) at 24 h, 2.74 (95% CI 1.44 to 5.22; p = 0.002) at 72 h (Fig. 5a), 2.84 (95% CI 1.55 to 5.22; p = 0.001) at 30 days, and 2.44 (95% CI 1.43 to 4.15; p = 0.001) at 6 months (Fig. 5b). Six-month cumulative event rates in patients with low PAPP-A levels were 7.9% versus 17.4% for patients with high PAPP-A levels. This difference in event rates was also driven by a higher mortality in patients with elevated PAPP-A plasma levels (3.2% vs. 1.2%; p = 0.098). Consistently, urgent revascularization procedures including percutaneous coronary intervention and coronary artery bypass grafting were significantly higher in patients with elevated PAPP-A plasma levels (13.6% vs. 7.9%; p = 0.012). Non-urgent revascularization procedures during six months of follow-up showed a higher incidence in patients with high PAPP-A plasma levels as compared with patients with low PAPP-A plasma levels (34.4% vs. 19.7%; p = 0.005).

Table 1. Baseline Characteristics According to the PAPP-A Status

	PAPP-A Low	PAPP-A High	p Value
n	328	219	
Male	70.2%	71.9%	0.62
Age (yrs)	60.5 ± 11.0	62.2 ± 10.4	0.39
Troponin T ≥0.1 μg/l	36.4%	39.2%	0.23
VEGF ≥300 ng/l	50.8%	54.3%	0.43
CRP ≥10 mg/l	37.3%	56.2%	**<0.001**
IL-10 <3.5 ng/l	57.3%	53.4%	0.38
sCD40L ≥5.0 μg/l	33.4%	51.4%	**<0.001**
ST-segment depression	45.1%	53.6%	0.062
T-wave inversion	51.7%	51.7%	1.00
History of			
Angina >4 weeks	55.5%	56.3%	0.64
Infarction <30 days	13.3%	12.5%	0.89
Infarction ≥30 days	20.3%	20.6%	0.90
PTCA	17.6%	17.4%	0.75
CABG	3.4%	3.5%	0.98
Risk factors			
Diabetes	9.5%	10.7%	0.97
Hypertension	34.6%	37.4%	0.64
Current smokers	40.9%	42.6%	0.41
Medication			
Aspirin	98.1%	97.8%	1.00
Heparin IV	99.0%	98.8%	1.00
Nitrates IV	99.5%	99.2%	1.00
Beta-blockers	63.5%	62.9%	0.94

Bold values indicate significant differences.

CABG = coronary artery bypass grafting; CRP = C-reactive protein; IL-10 = interleukin-10; IV = intravenous; PAPP-A = pregnancy-associated plasma protein A; PTCA = percutaneous transluminal coronary angioplasty; SCD40L = soluble CD40 ligand; VEGF = vascular endothelial growth factor.

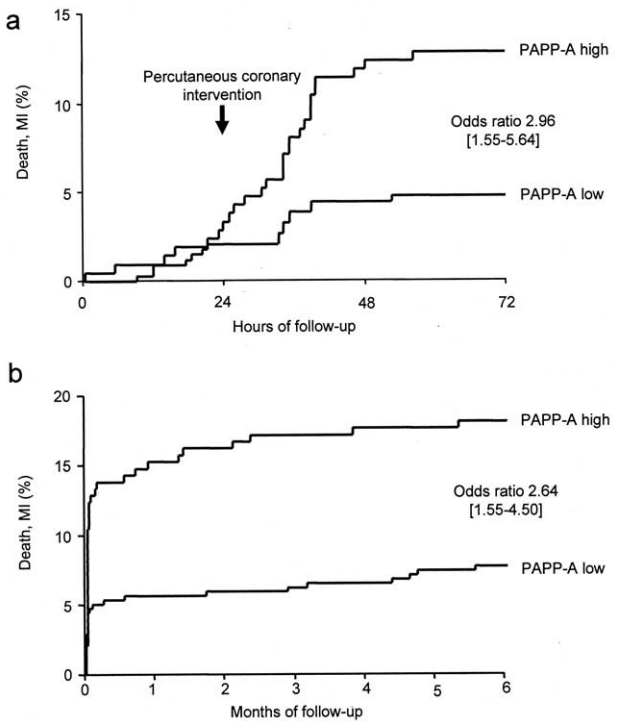

Figure 5. Kaplan-Meier event rate curves showing the cumulative incidence of death or nonfatal myocardial infarction (MI) at 72 h **(a)** and 6 months of follow-up **(b)** according to the baseline pregnancy-associated plasma protein A (PAPP-A) plasma level (diagnostic threshold 12.6 mIU/l; n = 547).

Effect of abciximab treatment on the predictive value of PAPP-A levels. All patients with available blood samples treated with either placebo or abciximab were included in this analysis (n = 1,090; 86.2% of the entire study population). The association between cardiovascular risk and PAPP-A quartiles remained significant in the abciximab group (p = 0.014), albeit on a lower level of statistical significance as compared with the placebo group (p < 0.001). Consistently, logistic regression analysis indicated a borderline significant interaction between the efficacy of

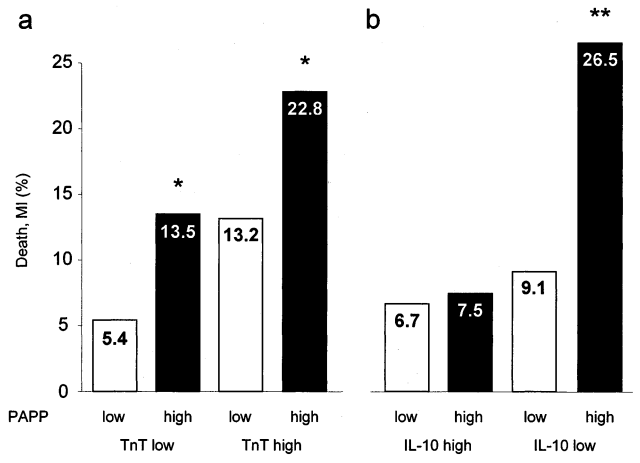

Figure 6. Predictive value of pregnancy-associated plasma protein A (PAPP-A) for the incidence of death and nonfatal myocardial infarction (MI) at six months of follow-up was also apparent in patients without troponin T (TnT) elevation **(a)** and was restricted to patients with low levels of the anti-inflammatory cytokine interleukin (IL)-10 **(b)**. Diagnostic thresholds were 12.6 mIU/l for PAPP-A, 10 mg/l for high-sensitivity C-reactive protein, and 3.5 ng/l for IL-10 (n = 547). *p < 0.01 versus PAPP-A low; **p < 0.01 versus PAPP-A low.

treatment with abciximab and PAPP-A values (p = 0.025). Subsequently, in a multivariable model including TnT, sCD40L, and PAPP-A, sCD40L (p < 0.001) remained the only independent predictor of the effect of abciximab.

Multimarker considerations. The predictive value of PAPP-A was also apparent in patients without evidence for myocardial necrosis; TnT-negative patients (threshold level 0.1 μg/l) with elevated PAPP-A levels were at significantly higher risk as compared with TnT-negative patients with low PAPP-A levels (adjusted odds ratio 2.72 [95% CI 1.25 to 5.89]; p = 0.009) (Fig. 6a). The predictive value of PAPP-A was also observed for a reduced threshold level of 0.01 μg/l for TnT (adjusted odds ratio 3.97 [95% CI 1.24 to 12.68]; p = 0.016). Troponin-T-positive patients suffered from increased cardiovascular risk, and this was particularly true for patients who also had elevated PAPP-A plasma levels (adjusted odds ratio 1.94 [95% CI 1.02 to 4.09]; p = 0.027).

Most notably, the predictive value of PAPP-A was restricted to patients with reduced levels of the anti-inflammatory cytokine IL-10 (Fig. 6b). Logistic regression analysis indicated a significant interaction between the predictive value of PAPP-A values and IL-10 levels (p = 0.002). If IL-10 levels were increased (≥3.5 ng/l), patients with PAPP levels above the calculated threshold value of 12.6 mIU/l were protected from an increased cardiac risk (odds ratio 1.13 [95% CI 0.44 to 2.90]; p = 0.81). However, for patients with IL-10 values below 3.5 ng/l, increased PAPP-A levels were associated with a marked increase in cardiovascular risk (odds ratio 3.61 [95% CI 1.84 to 7.10]; p < 0.001).

In a stepwise multivariable logistic regression analysis including biochemical markers as well as baseline characteristics, none of the established risk factors that were significant predictors in a univariable model was an independent predictor after the dichotomized biochemical markers TnT, hsCRP, and sCD40L were introduced into the model (Table 2). Therefore, the stepwise multivariable analysis was restricted to biochemical markers. Troponin T (p = 0.008) and PAPP-A (p = 0.007) remained independent significant predictors of the patients' outcome, whereas hsCRP lost significance after PAPP-A was introduced into the model (p = 0.003 without PAPP-A; p = 0.16 after introduction of PAPP-A) (Table 3; step I). Pregnancy-associated plasma protein A remained a significant predictor of the patients' outcome after the inclusion of the anti-inflammatory cytokine IL-10 (Table 3; step II) (p = 0.006) and after inclusion of sCD40L as a marker of platelet activation (Table 3; step III) (p = 0.015). After the introduction of VEGF as a marker of myocardial ischemia, TnT lost its predictive value for the six-month outcome (Table 3; step IV) (p = 0.24 after the introduction of VEGF vs. p = 0.16 before the

Table 2. Multivariable Logistic Regression Model for Death and Nonfatal Myocardial Infarction During Six Months of Follow-Up

Variables	Odds Ratio	95% CI	p Value
Gender	0.91	0.68 to 1.39	0.16
Age >65 yrs	1.36	0.91 to 1.82	0.34
Diabetes mellitus	1.22	0.83 to 1.49	0.61
Hypercholesterolemia	0.90	0.68 to 1.13	0.59
Hypertension	1.00	0.89 to 1.04	1.00
History of CHD	0.86	0.65 to 1.19	0.72
ST-segment depression	1.29	0.72 to 2.31	0.39
Troponin T ≥0.1 μg/l	2.23	1.25 to 3.98	**0.007**
CRP ≥10.0 mg/l	2.03	1.11 to 3.59	**0.018**
PAPP-A ≥12.6 mIU/l	2.33	1.30 to 4.17	**0.005**

Bold values indicate significant differences.
CHD = coronary heart disease; CI = confidence interval; other abbreviations as in Table 1.

Table 3. Multimarker Assessment: Stepwise Multivariable Logistic Regression Model for Death and Nonfatal Myocardial Infarction During Six Months of Follow-Up

Step	Variables in the Model	Odds Ratio	95% CI	p Value
I	CRP ≥10 mg/l	1.49	0.86 to 2.59	0.16
	TnT ≥0.1 μg/l	2.07	1.21 to 3.56	**0.008**
	PAPP-A ≥12.6 mIU/l	2.13	1.24 to 3.68	**0.007**
II	CRP ≥10 mg/l	1.44	0.67 to 2.17	0.50
	TnT ≥0.1 μg/l	2.13	1.24 to 3.69	**0.006**
	PAPP-A ≥12.6 mIU/l	2.18	1.25 to 3.78	**0.006**
	IL-10 ≥3.5 ng/l	0.47	0.26 to 0.82	**0.008**
III	CRP ≥10 mg/l	1.23	0.68 to 2.19	0.49
	TnT ≥0.1 μg/l	1.93	1.12 to 3.35	**0.019**
	PAPP-A ≥12.6 mIU/l	2.05	1.18 to 3.32	**0.015**
	IL-10 ≥3.5 ng/l	0.42	0.24 to 0.74	**0.002**
	sCD40L ≥5 μg/l	2.45	1.39 to 4.32	**0.002**
IV	TnT ≥0.1 μg/l	1.43	0.79 to 2.60	0.24
	PAPP-A ≥12.6 mIU/l	2.01	1.14 to 3.49	**0.014**
	IL-10 ≥3.5 ng/l	0.43	0.25 to 0.76	**0.003**
	sCD40L ≥5 μg/l	2.37	1.34 to 4.18	**0.003**
	VEGF >300 ng/l	2.19	1.14 to 4.18	**0.018**

Bold values indicate significant differences.
TnT = troponin T; other abbreviations as in Tables 1 and 2.

JACC Vol. 45, No. 2, 2005
January 18, 2005:229–37

introduction of VEGF), whereas PAPP-A remained a significant independent predictor ($p = 0.014$).

Prospective validation in emergency room patients with acute chest pain. To investigate whether the predictive value of PAPP-A can be used for risk stratification of a more heterogeneous population of patients with acute chest pain presenting to the emergency room, we prospectively enrolled a total of 644 patients suspicious for having an ACS. When the first blood sample was drawn, none of the chest pain patients had received anticoagulant treatment, which may have affected the inflammatory-thrombotic cascade. Despite similar clinical presentation at the time of arrival, patients were finally classified as follows: 323 patients with ACS, 105 patients with stable angina, 19 patients with congestive heart failure, and 197 patients with no evidence of coronary heart disease.

Pregnancy-associated plasma protein A levels were significantly higher in patients with ACS (mean 20.9 mIU/l; median 4.9 mIU/l [range 0.1 to 362.5 mIU/l]) as compared with patients with stable angina (mean 8.7 mIU/l; median 1.9 mIU/l [range 0.1 to 113.9 mIU/l]; $p = 0.007$) and patients without evidence for coronary heart disease (mean 5.2 mIU/l; median 1.4 mIU/l [range 0.1 to 54.1 mIU/l]; $p < 0.001$ vs. patients with ACS; $p = 0.044$ vs. patients with stable angina), respectively. Mean PAPP-A levels in patients with non–ST-segment elevation myocardial infarction did not significantly differ from mean PAPP-A levels in patients with unstable angina (non–ST-segment elevation myocardial infarction: mean 27.4 mIU/l; median 4.0 mIU/l [range 0.1 to 362.5 mIU/l]; unstable angina: mean 18.1 mIU/l; median 5.3 mIU/l [range 0.1 to 221.1 mIU/l]; $p = 0.50$).

A total of 65 events (including 18 fatal events) were recorded during 30 days of follow-up. Using the prespecified threshold value for PAPP-A of 12.6 mIU/l, patients with high PAPP-A levels were at significantly increased risk as compared with patients with low PAPP-A levels (event rate 16.9% vs. 7.9%; univariate analysis: odds ratio, 2.38 [95% CI 1.40 to 4.05]; $p = 0.001$; multivariable analysis: adjusted odds ratio, 2.32 [95% CI 1.32 to 4.26]; $p = 0.008$). However, maximized predictive value as derived from ROC curve analysis was obtained at a threshold levels as low as 7.0 mIU/l (event rate 18.4% vs. 6.6%; univariate analysis: odds ratio, 3.19 [95% CI 1.90 to 5.37]; $p < 0.001$; multivariable analysis: adjusted odds ratio, 3.08 [95% CI 1.85 to 5.02; $p < 0.001$). Pregnancy-associated plasma protein A was a powerful predictor both in patients with low TnT levels and in patients with high TnT levels (Fig. 7a). Using a multimarker approach, TnT (odds ratio 9.24 [95% CI 5.13 to 16.64]; $p < 0.001$), sCD40L (odds ratio 2.93 [95% CI 1.65 to 5.22]; $p < 0.001$), and PAPP-A (odds ratio 3.11 [95% CI 1.74 to 5.56]; $p < 0.001$) emerged as independent powerful predictors of cardiovascular events during 30 days of follow-up. Patients who were negative for all three markers (TnT, sCD40L, and PAPP-A) were at very low cardiac risk (30 days of follow-up: no death; 3.0% rate for nonfatal myocardial infarction) (Fig. 7b).

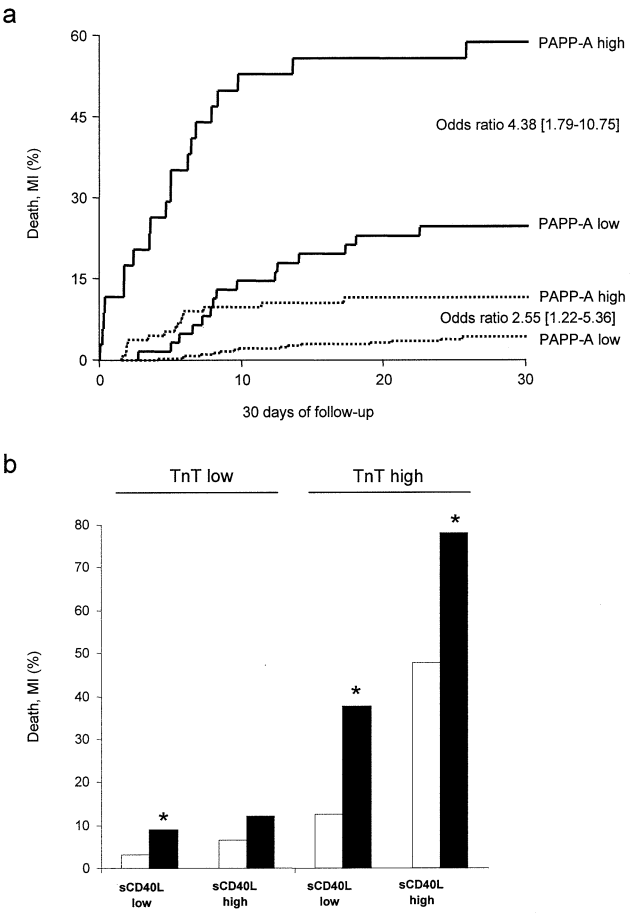

Figure 7. Predictive value of pregnancy-associated plasma protein A (PAPP-A) in patients with acute chest pain for the incidence of death and nonfatal myocardial infarction (MI) during 30 days of follow-up **(a)**. In patients that were negative both for troponin T (TnT) and soluble CD40 ligand (sCD40L), PAPP identified a subgroup suffering from increased cardiovascular risk during 30 days of follow-up **(b)**. Diagnostic thresholds were 12.6 mIU/l for PAPP-A, 0.1 μg/l for TnT, and 5.0 μg/l for sCD40L (n = 644). **Solid lines** = TnT high; **dotted lines** = TnT low; **open bars** = PAPP-A low; **solid bars** = PAPP-A high.

DISCUSSION

The results of the present study demonstrate that elevated blood levels of the metalloproteinase PAPP-A are associated with adverse outcome in patients with ACS. In line with a very recent study (13), the predictive value of PAPP-A plasma levels was also prominent in patients without troponin elevation. Thus, an increased PAPP-A plasma level is not only a marker of plaque instability favoring the development of ACS, but—more importantly—is indicative of a poor prognosis before the occurrence of an acute ischemic event caused by plaque instability. The finding that the predictive value of PAPP-A was restricted to patients with low levels of the anti-inflammatory cytokine IL-10 further supports the concept that the balance between pro- and anti-inflammatory cytokines is a major determinant of patients' outcome in ACS (15). By

multivariable regression analysis, several biochemical markers including TnT, sCD40L, IL-10, and PAPP-A were identified as independent predictors of the patients' outcome during the subsequent six months of follow-up.

Cardiac troponins are sensitive and specific markers of myocardial necrosis secondary to thrombotic complications during an ACS and are highly predictive of the early clinical course after the onset of ACS. However, risk stratification in troponin-negative patients with ACS remains challenging. About two-thirds of the patients with ACS but no ST-segment elevations have normal troponin values, and more than half of the patients have inconclusive electrocardiographic findings (21). During the first weeks after the onset of an ACS, the risk of death or nonfatal myocardial infarction in troponin-negative patients is about 5% to 8% (5,18). The present study demonstrates that PAPP-A levels identify a subgroup of patients without troponin elevation who are at substantially higher risk for cardiac events during the early time course after onset of symptoms (30 days: OR 3.33; 6 months: OR 2.72).

The progression and subsequent destabilization of atherosclerotic plaques involves major change in the structure of the arterial wall. The occurrence of a local inflammatory state in patients with ACS is well-established, as revealed by inflammatory markers such as C-reactive protein (22–24). Metalloproteinases are also potential indicators of arterial inflammation, and, by degrading extracellular matrix, may contribute to the fragility of the lipid-rich, atherosclerotic plaque and eventually to its rupture. As previously described for several other metalloproteinases (MMP-1, -3, -12, or -13) (25,26), PAPP-A was only recently found to be abundantly expressed in eroded and ruptured plaques, whereas PAPP-A expression was undetectable in stable plaques (11). Other studies have also demonstrated that patients with hyperechoic or isoechoic carotid plaques exhibit significantly higher PAPP-A levels than those with hypoechoic early carotid lesions (27). The exact role of PAPP-A in the pathophysiology of ACS remains unclear; PAPP-A has been shown to be a specific activator of insulin-like growth factor-I (9), a potent mediator of atherosclerosis (28,29). As a matrix metalloproteinase, PAPP-A may be involved in processing the plaques' extracellular matrix and, consequently, weakening the fibrous cap. This may result in a plaque morphology that is more susceptible to erosion, rupture, and subsequent thrombosis. The present study demonstrates that a single PAPP-A measurement provides significant predictive value for the incidence of death and nonfatal myocardial infarction in a high-risk population of patients with ACS as well as in a heterogeneous group of patients presenting with acute chest pain. These data suggest that PAPP-A plays an important pathophysiologic role in destabilization of the atherosclerotic plaque during ACS.

The production of PAPP-A by activated cells within the atherosclerotic lesion and its release into the extracellular matrix appear to be strongly linked to the local inflammatory process occurring within the arterial wall, as indicated by the significant positive correlation observed between CRP and PAPP-A levels. Consistent with our previous data on the important role of the inflammatory balance for the prognosis of patients with coronary heart disease (15,30), the predictive value of PAPP-A was almost entirely restricted to patients with low levels of the anti-inflammatory cytokine IL-10 (Fig. 6b). However, whereas CRP levels are linked to troponin elevation (14), PAPP-A levels appear to be less sensitive to minor myocardial injury, which might be specifically important in patients with ACS, of whom approximately one-third are positive for troponin at the time of arrival in the hospital (31). In addition, PAPP-A levels neither interfered with nor compromise the predictive power of sCD40L, a marker of platelet activation. By multivariable analysis, PAPP-A, sCD40L, and TnT all emerged as independent predictors of adverse outcome (Table 2). Combining PAPP-A and sCD40L was especially revealing in patients negative for TnT, suggesting that both markers reflect distinct pathways, which eventually contribute to a proinflammatory and procoagulant milieu in the coronary circulation. Supportive of a complementary rather than a competing role to predict adverse outcome in patients with ACS are our findings that aggressive inhibition of platelet aggregation by abciximab was specifically useful in patients with elevated sCD40L levels (6).

Only recently we have identified placental growth factor plasma levels as a powerful clinical biomarker of vascular inflammation and adverse outcome in patients with ACS (32). Even though both placental growth factor and PAPP-A are assumed to represent markers of vascular inflammation, they are involved in distinct pathophysiologic processes within atherosclerotic plaques. Therefore, it is not surprising that they negatively influence the predictive value of each other (data now shown). Whereas placental growth factor was the stronger predictor in the CAPTURE cohort, PAPP-A was the more powerful and independent predictor in the chest pain cohort. Because we utilized an advanced high-sensitivity prototype assay for measurement of PAPP-A that is suitable for routine use on an automatic electrochemiluminescent immunoassay system, whereas placental growth factor levels were determined with a research ELISA system developed for quantification of considerably higher levels of placental growth factor during pregnancy (33,34), it is not appropriate to directly compare the PAPP-A results with the placental growth factor results, and future studies using an advanced placental growth factor ELISA system are required.

Potential limitations of the present study merit consideration. First, because our blood samples were stored at −80°C until analysis, we cannot exclude the possibility of protein degradation. According to our experience, however, the delay from sampling to biochemical analysis in the core lab had no critical impact on the study results, and the plasma samples of our study were only thawed once for the present analysis. However, even if unaccounted for sources of protein instability

were present, their effects would minimally impact the validity of the present results, because all samples were handled identically. Secondly, because of the retrospective nature of the present analysis of the CAPTURE study and the selection of high-risk patients with documented coronary heart disease, results from the CAPTURE trial may not be generalizable to more heterogeneous populations of patients with chest pain presenting in the emergency room. We, therefore, prospectively tested the prognostic value of PAPP-A in conjunction with other markers (TnT and soluble CD40L) in a "real-world" cohort of patients presenting with acute chest pain to the emergency room. While we were able to confirm our results for the predictive value of PAPP-A in this validation set of patients, additional studies are needed to understand how various markers including placental growth factor may compliment each other in risk stratification.

In summary, the results of the present study demonstrate that elevated levels of PAPP-A are associated with increased cardiac risk. The predictive value of PAPP-A plasma levels was independent of elevated troponin levels, which reflect the acute risk secondary to thrombotic complications leading to myocardial injury during an ACS. Thus, an increased PAPP-A level is not only a marker of plaque instability favoring the progression to myocardial infarction, but is indicative of a poor prognosis even after the occurrence of an acute ischemic event caused by plaque instability. Because PAPP-A levels are relatively stable and no specific sampling conditions are required for PAPP-A, this marker may indeed be suitable for routine clinical use, but further studies are mandatory to truly identify the most reliable and powerful marker of plaque instability. Although limitations inherent to markers that are not specific to the coronary arteries and/or myocardium remain, PAPP-A may represent an important tool for diagnostic and therapeutic stratification of patients with ACS without evidence for myocardial necrosis.

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REFERENCES

1. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342–9.
2. Hamm CW, Ravkilde J, Gerhardt W, et al. The prognostic value of serum troponin T in unstable angina. N Engl J Med 1992;327:146–50.
3. Hamm CW, Goldmann BU, Heeschen C, Kreymann G, Berger J, Meinertz T. Emergency room triage of patients with acute chest pain by means of rapid testing for cardiac troponin T or troponin I. N Engl J Med 1997;337:1648–53.
4. Ohman EM, Armstrong PW, Christenson RH, et al. Cardiac troponin T levels for risk stratification in acute myocardial ischemia. GUSTO IIA Investigators. N Engl J Med 1996;335:1333–41.
5. Hamm CW, Heeschen C, Goldmann B, et al. Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. c7E3 Fab Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) Study Investigators. N Engl J Med 1999;340:1623–9.
6. Heeschen C, Dimmeler S, Hamm CW, et al. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003;348:1104–11.
7. Braunwald E. Unstable angina: an etiologic approach to management. Circulation 1998;98:2219–22.
8. Oxvig C, Sand O, Kristensen T, Kristensen L, Sottrup-Jensen L. Isolation and characterization of circulating complex between human pregnancy-associated plasma protein-A and proform of eosinophil major basic protein. Biochim Biophys Acta 1994;1201:415–23.
9. Lawrence JB, Oxvig C, Overgaard MT, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc Natl Acad Sci USA 1999;96:3149–53.
10. Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four human pregnancy-associated plasma proteins. Am J Obstet Gynecol 1974;118:223–36.
11. Bayes-Genis A, Conover CA, Overgaard MT, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med 2001;345:1022–9.
12. Cosin-Sales J, Christiansen M, Kaminski P, et al. Pregnancy-associated plasma protein A and its endogenous inhibitor, the proform of eosinophil major basic protein (proMBP), are related to complex stenosis morphology in patients with stable angina pectoris. Circulation 2004;109:1724–8.
13. Lund J, Qin QP, Ilva T, et al. Circulating pregnancy-associated plasma protein a predicts outcome in patients with acute coronary syndrome but no troponin I elevation. Circulation 2003;108:1924–6.
14. Heeschen C, Dimmeler S, Hamm CW, Boersma E, Zeiher AM, Simoons ML. Prognostic significance of angiogenic growth factor serum levels in patients with acute coronary syndromes. Circulation 2003;107:524–30.
15. Heeschen C, Dimmeler S, Hamm CW, et al. Serum level of the anti-inflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 2003;107:2109–14.
16. Heeschen C, Hamm CW, Bruemmer J, Simoons ML. Predictive value of C-reactive protein and troponin T in patients with unstable angina: a comparative analysis. CAPTURE Investigators. Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment trial. J Am Coll Cardiol 2000;35:1535–42.
17. The CAPTURE Investigators. Randomised placebo-controlled trial of abciximab before and during coronary intervention in refractory unstable angina: the CAPTURE study. Lancet 1997;349:1429–35.
18. Heeschen C, Hamm CW, Goldmann B, Deu A, Langenbrink L, White HD. Troponin concentrations for stratification of patients with acute coronary syndromes in relation to therapeutic efficacy of tirofiban. PRISM Study Investigators. Platelet Receptor Inhibition in Ischemic Syndrome Management. Lancet 1999;354:1757–62.
19. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med 2000;343:1139–47.
20. Cox DR. Regression models and life-tables. J R Stat Soc [B] 1972;34:187–220.
21. Cannon CP, McCabe CH, Stone PH, et al. The electrocardiogram predicts one-year outcome of patients with unstable angina and non–Q-wave myocardial infarction: results of the TIMI III registry ECG ancillary study. Thrombolysis in Myocardial Ischemia. J Am Coll Cardiol 1997;30:133–40.
22. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N Engl J Med 1994;331:417–24.
23. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
24. Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91:2844–50.

25. Prescott MF, Sawyer WK, Von Linden-Reed J, et al. Effect of matrix metalloproteinase inhibition on progression of atherosclerosis and aneurysm in LDL receptor-deficient mice overexpressing MMP-3, MMP-12, and MMP-13 and on restenosis in rats after balloon injury. Ann NY Acad Sci 1999;878:179–90.
26. Nikkari ST, Hoyhtya M, Isola J, Nikkari T. Macrophages contain 92-kd gelatinase (MMP-9) at the site of degenerated internal elastic lamina in temporal arteritis. Am J Pathol 1996;149:1427–33.
27. Beaudeux JL, Burc L, Imbert-Bismut F, et al. Serum plasma pregnancy-associated protein A: a potential marker of echogenic carotid atherosclerotic plaques in asymptomatic hyperlipidemic subjects at high cardiovascular risk. Arterioscler Thromb Vasc Biol 2003;23:e7–10.
28. Du J, Delafontaine P. Inhibition of vascular smooth muscle cell growth through antisense transcription of a rat insulin-like growth factor I receptor cDNA. Circ Res 1995;76:963–72.
29. Nichols TC, du Laney T, Zheng B, et al. Reduction in atherosclerotic lesion size in pigs by alphaVbeta3 inhibitors is associated with inhibition of insulin-like growth factor-I-mediated signaling. Circ Res 1999;85:1040–5.
30. Fichtlscherer S, Breuer S, Heeschen C, Dimmeler S, Zeiher AM. Interleukin-10 serum levels and systemic endothelial vasoreactivity in patients with coronary artery disease. J Am Coll Cardiol 2004;44:44–9.
31. Hamm CW, Braunwald E. A classification of unstable angina revisited. Circulation 2000;102:118–22.
32. Heeschen C, Dimmeler S, Fichtlscherer S, et al. Prognostic value of placental growth factor in patients with acute chest pain. JAMA 2004;291:435–41.
33. Maynard SE, Jiang-Yong Min J-Y, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1)may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649–58.
34. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.