

PREGNANCY-ASSOCIATED PLASMA PROTEIN A AS A MARKER OF ACUTE CORONARY SYNDROMES

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ABSTRACT

Background Circulating markers indicating the instability of atherosclerotic plaques could have diagnostic value in unstable angina or acute myocardial infarction. We evaluated pregnancy-associated plasma protein A (PAPP-A), a potentially proatherosclerotic metalloproteinase, as a marker of acute coronary syndromes.

Methods We examined the level of expression of PAPP-A in eight culprit unstable coronary plaques and four stable plaques from eight patients who had died suddenly of cardiac causes. We also measured circulating levels of PAPP-A, C-reactive protein, and insulin-like growth factor I (IGF-I) in 17 patients with acute myocardial infarction, 20 with unstable angina, 19 with stable angina, and 13 controls without atherosclerosis.

Results PAPP-A was abundantly expressed in plaque cells and extracellular matrix of ruptured and eroded unstable plaques, but not in stable plaques. Circulating PAPP-A levels were significantly higher in patients with unstable angina or acute myocardial infarction than in patients with stable angina and controls ($P < 0.001$). A PAPP-A threshold value of 10 mIU per liter identified patients who had acute coronary syndromes with a sensitivity of 89.2 percent and a specificity of 81.3 percent. PAPP-A levels correlated with levels of C-reactive protein and free IGF-I, but not with markers of myocardial injury (troponin I and the MB isoform of creatine kinase).

Conclusions PAPP-A is present in unstable plaques, and circulating levels are elevated in acute coronary syndromes; these increased levels may reflect the instability of atherosclerotic plaques. PAPP-A is a new candidate marker of unstable angina and acute myocardial infarction. (N Engl J Med 2001;345:1022-9.)

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PATIENTS with acute coronary syndromes are at considerable risk for serious complications and death. Clinical outcomes might be improved by rapid and accurate diagnosis, followed by appropriate therapy. Diagnostic tests for myocardial injury include technetium-99m sestamibi scanning to identify defects in myocardial perfusion,¹ echocardiography to identify abnormalities in left ventricular wall motion,² and measurements of circulating levels of the MB isoform of creatine kinase,³ myoglobin,⁴ troponin I,⁵ and troponin T⁶ to identify myocardial necrosis. Each test reflects different steps of the pathway from coronary occlusion to myocardial ischemia: impairment of coronary-artery flow, ischemic my-

ocardial dysfunction, and eventually, myocardial-tissue necrosis. The availability of a sensitive and specific early marker of the instability of plaques, whose levels become elevated before or in the absence of an elevation of other markers of myocardial-cell injury, might improve diagnostic and therapeutic decision making and, possibly, the value of traditional tests.⁷

We hypothesized that pregnancy-associated plasma protein A (PAPP-A), which is found in both men and women, might be such a marker and could identify patients with unstable atherosclerotic plaques. PAPP-A is a high-molecular-weight, zinc-binding metalloproteinase.^{8,9} It is thus a potentially proatherosclerotic molecule and has recently been shown to be a specific activator of insulin-like growth factor I (IGF-I),⁹ a mediator of atherosclerosis.^{10,11} PAPP-A antigen is typically measured during pregnancy (levels increase to about 100 IU per liter at term) with the use of routine assays with limited sensitivity.¹²⁻¹⁴

We examined the level of expression of PAPP-A in unstable plaques from patients who died suddenly of cardiac causes. We also assessed circulating PAPP-A levels in patients with acute coronary syndromes (unstable angina and acute myocardial infarction), using a highly sensitive immunoassay.

METHODS

Collection and Analysis of Tissue

Atherosclerotic arteries were obtained at autopsy from eight patients within 24 hours after sudden death from cardiac causes, as defined previously.¹⁵ The characteristics of acute rupture of plaques, erosion of plaques, and stable plaques were also defined previously.¹⁵ Immunohistochemical staining was performed on 5- μ m-thick paraffin sections with use of a peroxidase-labeled streptavidin-biotin method.¹⁶ Monoclonal human antibody against PAPP-A (234-5)¹³ was used at a concentration of 20 μ g per milliliter. Sections were also stained with antibodies against macrophage CD68 (clone KP-1, Dako, Carpinteria, Calif.) at a dilution of 1:200 and with antibodies against smooth-muscle α -actin (clone 1A4, IgG2a, Dako) at a dilution of 1:500. The total area of plaque and the percentage of the area that stained for PAPP-A were evaluated. Quantitative immunohistochemical analysis was performed with use of a quantitative color-image analysis system (Diagnostic Instruments, Sterling Heights, Mich.).

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Patient Population

The study groups consisted of 17 patients with acute myocardial infarction, 20 with unstable angina, 19 with stable angina, and 13 age-matched control patients without clinical or angiographic evidence of coronary atherosclerosis. All subjects were consecutively identified and approached for consent as they were scheduled to undergo coronary arteriography in the Mayo Clinic cardiac catheterization laboratory.

Acute myocardial infarction was defined as prolonged chest pain accompanied by ST-segment elevation or depression evolving into pathologic Q-wave or T-wave inversion and confirmed by a finding that the creatine kinase MB fraction was more than twice the upper limit of the normal range and by a troponin I level of more than 0.5 ng per milliliter. Unstable angina was defined as chest discomfort at rest with either ST-segment depression of at least 0.1 mV or T-wave inversion in two or more contiguous electrocardiographic leads, a creatine kinase MB fraction that was within normal limits, and angiographically confirmed coronary artery disease. Chronic, stable, effort-induced angina was diagnosed as chest pain of at least six months' duration accompanied by evidence of severe coronary artery disease on coronary angiography and by the absence of clinically evident ischemic episodes during the week preceding arteriography.

Exclusion criteria were advanced kidney or liver failure, overt heart failure, and a history of major surgery or trauma within the previous month. Patients with known or suspected systemic thrombotic disorders (other than those of coronary origin) or inflammatory diseases were excluded, as were those who were pregnant. Angiographically severe coronary artery disease was defined by the presence of one or more stenoses of at least 70 percent in any major coronary artery. To identify a possible association between PAPP-A levels and the extent and severity of coronary artery disease discovered at angiography, the Jenkins score was calculated for every patient.¹⁷ Scores can range from 0 to 32, with higher scores indicating more severe disease.

Blood samples were obtained at the time of coronary angiography, placed on ice, and centrifuged within 30 minutes at $1600 \times g$ for 5 minutes. All samples were analyzed by personnel who had no knowledge of the subjects' clinical data. The mean (\pm SD) time from the last ischemic episode to blood sampling was 8.4 ± 3.0 hours in the group with myocardial infarction and 9.4 ± 3.9 hours in the group with unstable angina.

The study was approved by the institutional review board of the Mayo Clinic and Foundation, and all patients gave oral informed consent.

Plasma Protein Assays

PAPP-A levels were determined by means of a biotin–tyramide–amplified enzyme immunoassay with a limit of detection of 0.03 mIU per liter and intraassay and interassay coefficients of variation of 10 percent and 15 percent, respectively. PAPP-A polyclonal antibodies were used for capture¹⁸ and a combination of monoclonal antibodies was used for detection.¹³ The assay was calibrated against the World Health Organization's international reference standard 78/610, which is the standard for pregnancy-associated proteins.

A highly sensitive latex-particle–enhanced immunoturbidimetric assay (Kamiya Biomedical, Seattle) was used to quantitate the level of C-reactive protein. Total IGF-I and free IGF-I were measured by commercially available immunoradiometric assays (Diagnostic Systems Laboratories, Webster, Tex.). We hypothesized that increased PAPP-A levels would lead to increased levels of free IGF-I as a result of proteolysis by IGF-binding protein 4.⁹ Creatine kinase MB and cardiac troponin I were measured with an immunochemiluminometric assay (Chiron, Emeryville, Calif.) in a standard fashion at the Mayo Clinic clinical laboratory.

Statistical Analysis

Results of analyses of histopathological data are presented as means \pm SD. We used Student's *t*-test to compare eroded and ruptured plaques with stable plaques. We used analysis of variance or

two-way cross-tabulation with the chi-square test, when appropriate, to compare differences between groups in demographic and angiographic characteristics. Data on PAPP-A, free IGF-I, total IGF-I, and C-reactive protein, which were not distributed normally, were summarized by medians and ranges and were compared among the groups with use of the Kruskal–Wallis test. When the results indicated that there were significant differences between groups, we made pairwise group comparisons using the Wilcoxon rank-sum statistic. Associations among circulating levels of these proteins were assessed by Spearman's rank-correlation coefficient. Associations of PAPP-A with risk factors and comparisons of PAPP-A levels in each group after adjustment for these risk factors were assessed with use of multiple linear regression, with the logarithm of PAPP-A as the dependent variable.

We used receiver-operating-characteristic (ROC) analysis on the levels of PAPP-A and C-reactive protein for myocardial infarction and unstable angina. The ROC curve is a common test to quantitate the diagnostic accuracy of medical tests. This strategy plots the true positive fraction, or sensitivity, against the false positive fraction (1 – specificity) by varying the threshold value for the test. The threshold is varied with increasingly stringent criteria for positivity. The ROC curve thus indicates the probability of a true positive result as a function of the probability of a false positive result for all possible threshold values. The area under the ROC curve assesses the relative accuracy of two diagnostic tests. An area of 0.5 indicates that the test results are no better than those obtained by chance, whereas an area of 1.0 indicates a perfectly sensitive and specific test. The areas under the curve for PAPP-A and C-reactive protein levels were compared according to the method of DeLong et al.¹⁹ *P* values less than 0.05 were considered to indicate statistical significance. All *P* values are two-sided.

RESULTS

Expression of PAPP-A in Unstable Plaques

Four ruptured plaques and four eroded plaques were identified as culprit unstable lesions in the arteries obtained at autopsy from eight patients. Four stable plaques were also identified. There were no significant differences in the total area of the plaques among the ruptured plaques (mean, 7.1 ± 1.4 mm²), eroded plaques (mean, 8.0 ± 3.7 mm²), and stable plaques (mean, 5.7 ± 2.1 mm²). In plaques with large lipid cores and cap rupture, staining for PAPP-A occurred mostly in the inflammatory shoulder region (Fig. 1A), in areas surrounding the lipid core, and localized with CD68-positive cells (not shown). In fibrous plaques with superficial erosion, PAPP-A was identified within spindle-shaped smooth-muscle cells containing α -actin (not shown), in the extracellular matrix (Fig. 1B), and in noneroded endothelial cells (Fig. 1C). Quantitative image analysis showed that the level of expression of PAPP-A in fibrous eroded plaques exceeded that in ruptured plaques (28.3 ± 16.8 percent vs. 18.5 ± 8.0 percent), but the difference was not statistically significant (*P* = 0.34). PAPP-A was absent or only minimally expressed in stable plaques (Fig. 1D). Quantitative image analysis showed that the level of expression of PAPP-A was significantly lower in stable plaques (3.2 ± 1.9 percent) than in ruptured plaques (*P* = 0.01) or eroded plaques (*P* = 0.02).

Circulating Marker Proteins in Acute Coronary Syndromes

To determine whether the abundant expression of PAPP-A in unstable plaques might translate into ele-

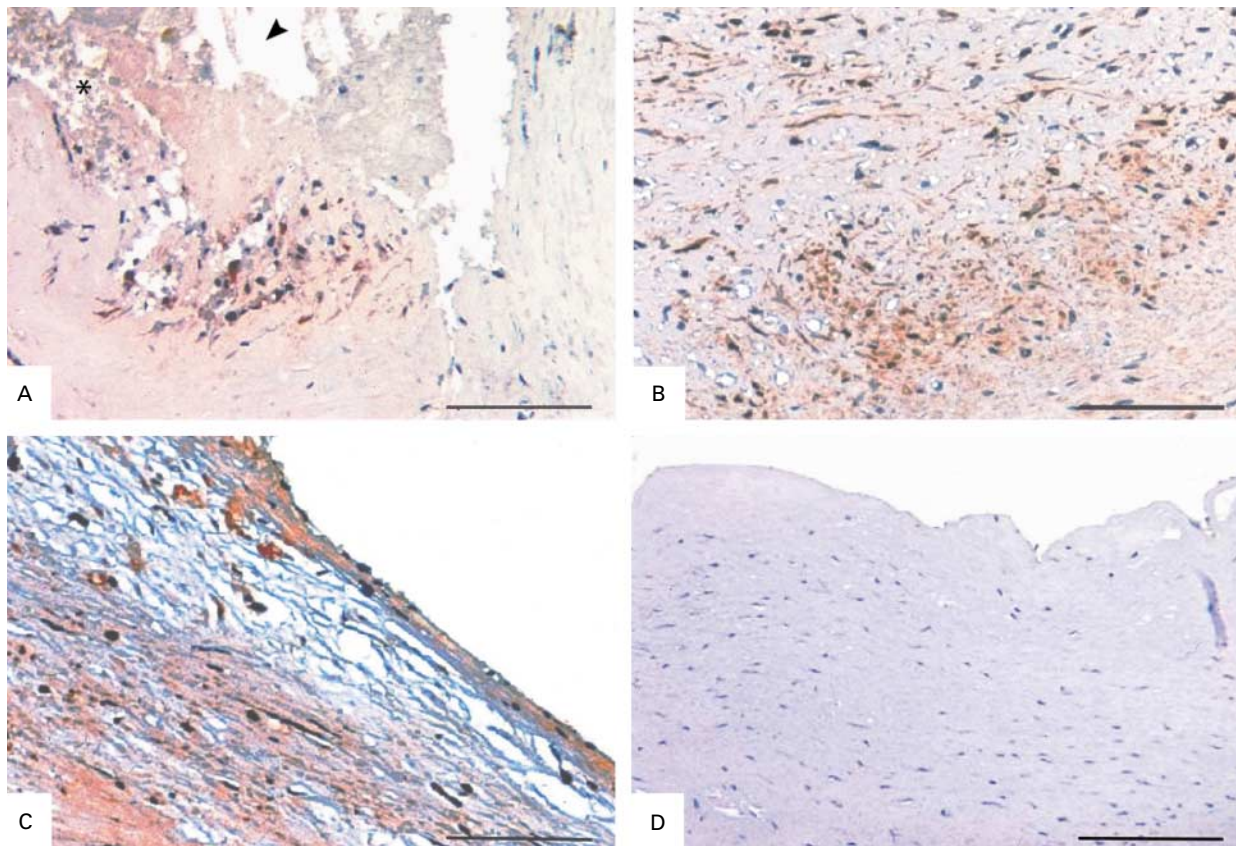


Figure 1. Expression of Pregnancy-Associated Plasma Protein A (PAPP-A) in Unstable and Stable Coronary Atherosclerotic Plaques. The expression of PAPP-A was determined immunohistochemically by staining with antihuman PAPP-A monoclonal antibodies (reddish-brown areas). Panel A shows PAPP-A in an inflammatory shoulder area densely infiltrated by macrophages. The inflammatory infiltrate is present between the cholesterol core (arrowhead) and the luminal thrombus (asterisk). In Panel B there is intense staining for PAPP-A within spindle-shaped smooth-muscle cells and in the extracellular matrix of an eroded plaque. In Panel C PAPP-A is present in noneroded endothelial cells of an eroded plaque. In Panel D PAPP-A is absent in a stable plaque. The bar equals 50 μm .

vated circulating levels, we measured PAPP-A levels in patients with acute coronary syndromes (myocardial infarction and unstable angina) and in those in stable condition (patients with stable angina and controls without atherosclerosis). Table 1 shows the age, sex, risk factors, base-line therapy, and angiographic results in the four groups. Patients with stable angina had three-vessel disease significantly more frequently than did patients with myocardial infarction ($P=0.004$), but no significant differences were observed among the groups with stable angina, unstable angina, and myocardial infarction regarding the coronary atherosclerotic burden, as evaluated by the Jenkins score ($P=0.88$). The control subjects had lower levels of risk factors than the three other groups, but the levels were similar among these three groups.

Circulating PAPP-A Levels

The data on PAPP-A levels are shown in Table 2. The Kruskal–Wallis test indicated that the differenc-

es among the groups were as follows. The median PAPP-A levels in control subjects (7.4 mIU per liter; range, 3.8 to 10.4) were not significantly different from those in patients with stable angina (8.4 mIU per liter; range, 4.4 to 22.5; $P=0.07$). In the group of patients with unstable angina, the median PAPP-A levels (14.9 mIU per liter; range, 6.3 to 63.4) were significantly higher than those in the control group ($P<0.001$) or the group with stable angina ($P<0.001$). In the group of patients with myocardial infarction, the median PAPP-A levels (20.6 mIU per liter; range, 9.2 to 46.6) were also significantly higher than those in the control group ($P<0.001$) or the group with stable angina ($P<0.001$). The PAPP-A levels did not differ significantly between the group with unstable angina and the group with myocardial infarction ($P=0.75$).

Multiple-regression models showed that the PAPP-A level was not associated with age, sex, risk factors, or medications. Among the three groups with atherosclerosis, the PAPP-A level was significantly and inversely

TABLE 1. CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	CONTROLS (N=13)	PATIENTS WITH STABLE ANGINA (N=19)	PATIENTS WITH UNSTABLE ANGINA (N=20)	PATIENTS WITH MYOCARDIAL INFARCTION (N=17)
Age — yr	58.5±13.2	66.4±10.9	67.7±11.7	63.4±10.4
Sex — no.				
Male	5	15	13	10
Female	8	4	7	7
Risk factors — no. (%)				
Hypertension	4 (31)	9 (47)	6 (30)	8 (47)
Smoking	0	4 (21)	6 (30)	5 (29)
Hypercholesterolemia	2 (15)†	12 (63)	13 (65)	11 (65)
Diabetes	0	6 (32)	6 (30)	2 (12)
Therapy — no. (%)				
Aspirin	3 (23)†	17 (89)	18 (90)	16 (94)
Beta-blockers	3 (23)†	15 (79)	13 (65)	13 (76)
ACE inhibitors	1 (8)†	2 (11)‡	10 (50)	7 (41)
Nitrates	1 (8)	2 (11)	4 (20)	1 (6)
Statins	2 (15)†	13 (68)	13 (65)	11 (65)
Calcium-channel blockers	2 (15)†	8 (42)	6 (30)	0§
Angiographic findings — no. (%)				
1-Vessel disease	0†	4 (21)	6 (30)	7 (41)
2-Vessel disease	0†	4 (21)	7 (35)	8 (47)
3-Vessel disease	0†	11 (58)	7 (35)	2 (12)§
Jenkins score¶	0†	13.3±6.8	13.2±9.9	12.1±4.9

*Plus-minus values are means ±SD. ACE denotes angiotensin-converting enzyme.

†P<0.05 for the comparison with the other three groups.

‡P<0.05 for the comparison with the group with unstable angina and the group with myocardial infarction.

§P<0.05 for the comparison with the group with stable angina.

¶Scores can range from 0 to 32, with higher scores indicating more severe disease.

TABLE 2. MEDIAN LEVELS OF PREGNANCY-ASSOCIATED PLASMA PROTEIN A, C-REACTIVE PROTEIN, AND FREE AND TOTAL INSULIN-LIKE GROWTH FACTOR I.*

VARIABLE	CONTROLS (N=13)	PATIENTS WITH STABLE ANGINA (N=19)	PATIENTS WITH UNSTABLE ANGINA (N=20)	PATIENTS WITH MYOCARDIAL INFARCTION (N=17)
PAPP-A (mIU/liter)				
Median	7.4	8.4	14.9	20.6
Range	3.8–10.4	4.4–22.5	6.3–63.4	9.2–46.6
C-reactive protein (mg/dl)†				
Median	0.28	0.16	0.3	1.03
Range	0.08–0.8	0.02–5.2	0.03–9.7	0.07–9.9
Free IGF-I (ng/ml)				
Median	0.9	0.8	1.2	1.2
Range	0.3–3.9	0.4–1.7	0.1–3.5	0.2–5.2
Total IGF-I (ng/ml)				
Median	108.8	151.2	141.3	112.5
Range	45.1–374.3	60.2–262.4	21.6–317.2	12.4–251.0

*PAPP-A denotes pregnancy-associated plasma protein A, and IGF-I insulin-like growth factor I.

†P=0.002 for the comparison among the groups.

associated with the extent of atherosclerosis, expressed as the number of vessels with clinically significant luminal stenosis (one, two, or three vessels) ($P=0.04$), but it was not associated with the Jenkins score ($P=0.27$). This result probably reflects the coexistence of quiescent atherosclerotic plaques with active, vulnerable, or fissured plaques in the coronary tree.

Circulating Levels of C-Reactive Protein, IGF-I, and Markers of Myocardial Injury

The Kruskal–Wallis analysis of C-reactive protein levels indicated that there were significant differences among the groups. Levels of C-reactive protein were significantly higher in the patients who had had a myocardial infarction than in the patients with unstable and stable angina ($P=0.02$ and $P=0.001$, respectively), and the levels were slightly but significantly higher in the patients with unstable angina than in those with stable angina ($P=0.045$). In the control group the levels of C-reactive protein were significantly lower than those in the myocardial-infarction group ($P=0.006$). The level of C-reactive protein was significantly associated with the PAPP-A level in patients with acute coronary syndromes (Spearman's $\rho=0.61$, $P<0.001$) (Fig. 2). The levels of C-reactive protein were not associated with age, sex, risk factors, medications, or the extent of coronary atherosclerosis.

No significant differences in free IGF-I levels were found among the groups; however, a weak but significant correlation was observed between free IGF-I levels and PAPP-A levels in patients with acute coronary syndromes (Spearman's $\rho=0.39$, $P=0.02$) (Fig. 2). No significant differences were found in total IGF-I levels between patients with acute coronary syndromes and patients in stable condition (Table 2), and no correlation with PAPP-A levels was observed.

Creatine kinase MB levels were not elevated in blood samples from patients with unstable angina, and only 3 of 20 patients with unstable angina had troponin I levels above normal (mean, 1.6 ± 0.7 ng per milliliter). In the group of patients with myocardial infarction, the peak levels of troponin I and creatine kinase MB were 60.9 ng per milliliter (range, 1.3 to 368) and 76.3 ng per milliliter (range, 4.4 to 341), respectively. In this group, there were no significant correlations between the level of PAPP-A and the level of either troponin I (Spearman's $\rho=0.33$, $P=0.18$) or creatine kinase MB (Spearman's $\rho=0.23$, $P=0.36$) (Fig. 3). Even when the patients with unstable angina and the patients with myocardial infarction were combined, there was no significant association between the level of PAPP-A and the level of either troponin I (Spearman's $\rho=0.07$, $P=0.69$) or creatine kinase MB (Spearman's $\rho=0.10$, $P=0.57$). Therefore, the elevated PAPP-A levels in these patients cannot be attributed to myocardial necrosis.

PAPP-A as a Diagnostic Marker of Acute Coronary Syndromes

The mean (\pm SE) area under the ROC curve for PAPP-A was 0.94 ± 0.03 among the patients with acute myocardial infarction and 0.88 ± 0.05 among the patients with unstable angina, with control patients and patients with stable angina serving as the ROC control group. In a parallel analysis, C-reactive protein had a mean area under the curve of 0.81 ± 0.07 among the patients with myocardial infarction and 0.67 ± 0.08 among the patients with unstable angina. These differences between the two markers were significant both for the group with myocardial infarction ($P=0.03$) and for the group with unstable angina ($P=0.01$) (Fig. 4). These data suggest that PAPP-A is a valuable mark-

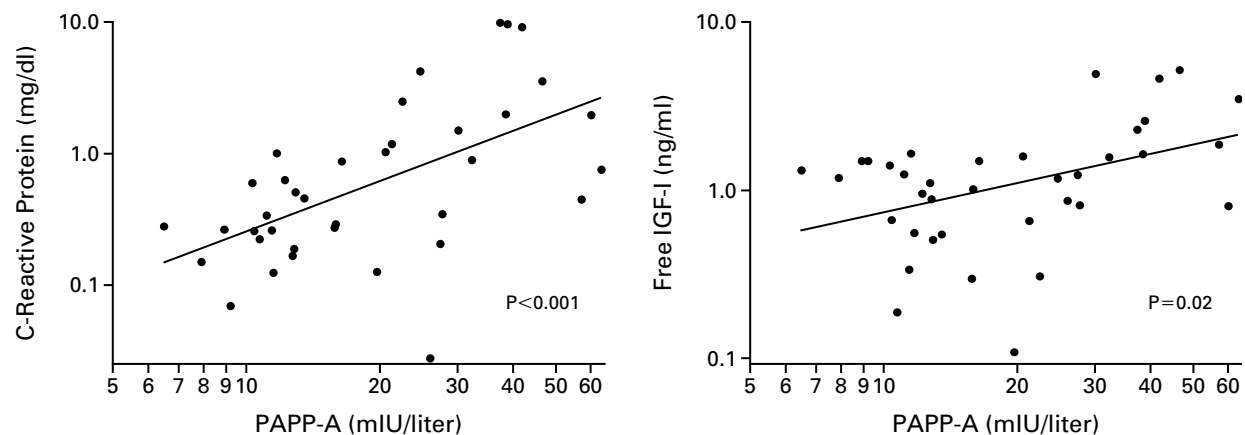


Figure 2. Correlation between Levels of Pregnancy-Associated Plasma Protein A (PAPP-A) and Levels of C-Reactive Protein and Free Insulin-like Growth Factor I (IGF-I) in 17 Patients with Myocardial Infarction and 20 Patients with Unstable Angina.

A significant association was found between the levels of PAPP-A and C-reactive protein (Spearman's $\rho=0.61$, $P<0.001$) and the levels of PAPP-A and free IGF-I (Spearman's $\rho=0.39$, $P=0.02$).

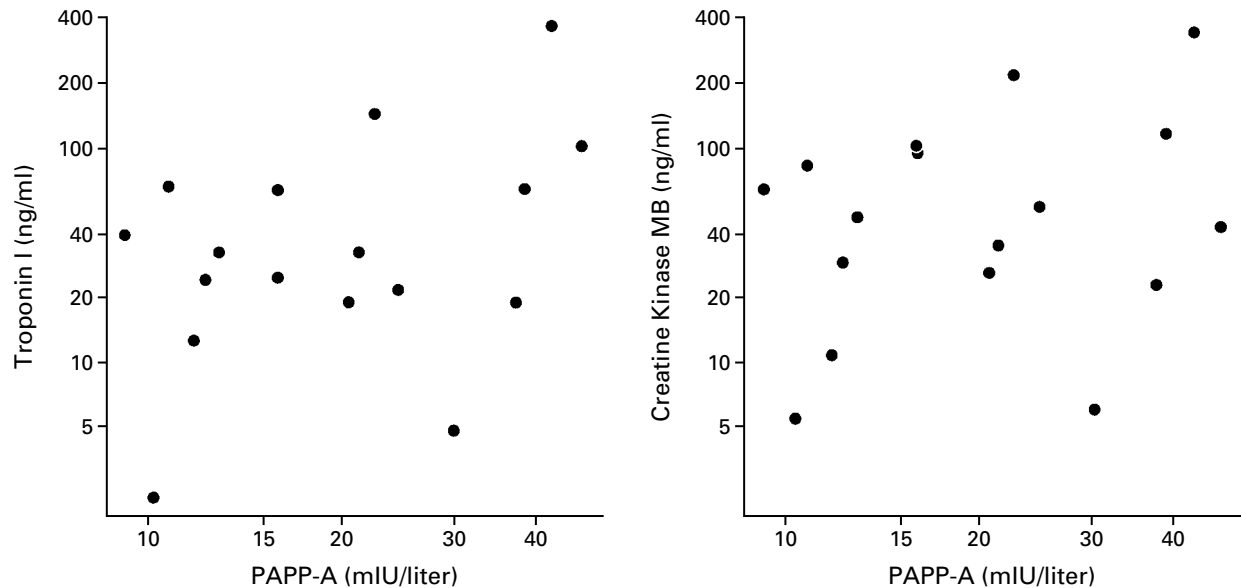


Figure 3. Correlation between Pregnancy-Associated Plasma Protein A (PAPP-A) Levels and the Levels of the Cardiac Necrosis Markers Troponin I and Creatine Kinase MB in 17 Patients with Acute Myocardial Infarction.

No significant association was found between the levels of PAPP-A and the levels of troponin I (Spearman's $\rho=0.33$, $P=0.18$) or creatine kinase MB (Spearman's $\rho=0.23$, $P=0.36$).

er — better than C-reactive protein — for the identification of patients with acute coronary syndromes.

A threshold level of 10 mIU of PAPP-A per liter had the highest combined sensitivity and specificity for the identification of acute coronary syndromes. The sensitivity and specificity of PAPP-A levels above 10 mIU per liter were 89.2 percent and 81.3 percent, respectively. The sensitivity of PAPP-A levels above 10 mIU per liter was 94.1 percent in the case of myocardial infarction and 85.0 percent in the case of unstable angina. In the case of the groups without atherosclerosis, only 1 of 13 control subjects (8 percent) and 5 of 19 patients with stable angina (26 percent) had PAPP-A levels of more than 10 mIU per liter.

DISCUSSION

We found a relation between unstable coronary disease and the levels of PAPP-A. PAPP-A is a member of the metzincin superfamily of metalloproteinases⁹ originally identified in the serum of pregnant women.²⁰ Circulating PAPP-A levels are used in the fetal diagnosis of Down's syndrome.¹⁴ Only recently has PAPP-A been identified outside pregnancy in nonplacental tissue.^{9,21}

Histologic evidence of an association between PAPP-A and acute coronary syndromes comes from the evaluation of unstable plaques in patients who have died suddenly of cardiac causes. Using specific monoclonal antibodies, we found that PAPP-A was abundantly expressed in both eroded and ruptured plaques but was only minimally expressed in stable plaques.

Our findings suggest that PAPP-A may be produced by activated cells in unstable plaques and released into the extracellular matrix. Whether PAPP-A can degrade extracellular matrix remains unclear. Other metalloproteinases have been associated with macrophage-rich shoulder regions of atherosclerotic lesions²² and circumstantially implicated in plaque rupture.²³

The finding of a high level of expression of PAPP-A in unstable atherosclerotic plaques prompted us to assess circulating PAPP-A levels in patients with acute coronary syndromes. Circulating levels of PAPP-A were significantly elevated in patients with unstable angina and patients with myocardial infarction. We determined that a PAPP-A threshold of 10 mIU per liter accurately identified patients with acute coronary syndromes.

Several studies have assessed other serum markers in patients with unstable angina. Hamm and colleagues found that the diagnostic sensitivity of cardiac-specific troponins in unstable angina is low, since only 22 percent of patients had positive troponin T tests and 36 percent had positive troponin I tests.²⁴ C-reactive protein is also a suboptimal diagnostic marker of unstable angina. Liuzzo et al. found that only 20 of 31 patients with unstable angina (65 percent) had elevated levels of C-reactive protein on admission.²⁵ Both markers are nonetheless associated with unfavorable outcomes when they are elevated.^{6,25} In our study, troponin I levels were elevated in 3 patients with unstable angina (15 percent) and C-reactive protein levels were elevated in 10 (50 percent) of these patients. By comparison,

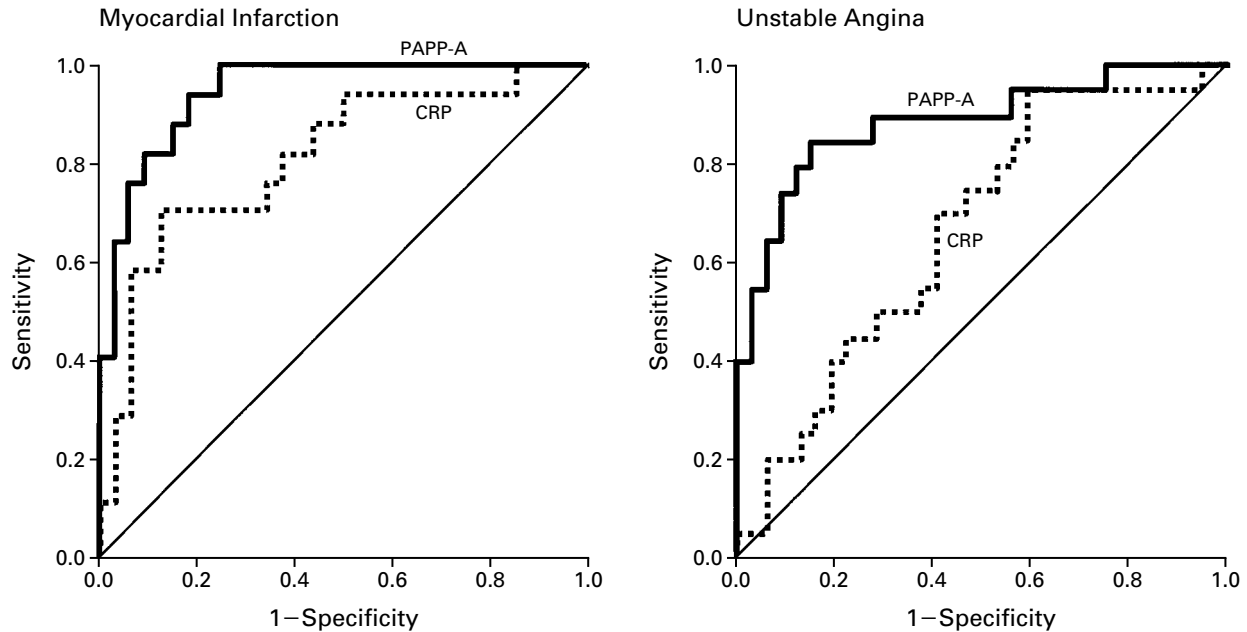


Figure 4. Receiver-Operating-Characteristic (ROC) Curves for the Levels of Pregnancy-Associated Plasma Protein A (PAPP-A) and C-Reactive Protein (CRP) in 17 Patients with Acute Myocardial Infarction and 20 Patients with Unstable Angina.

The mean (\pm SE) area under the curve for PAPP-A was 0.94 ± 0.03 among the patients with acute myocardial infarction and 0.88 ± 0.05 among the patients with unstable angina. The mean areas under the curve for C-reactive protein were 0.81 ± 0.07 and 0.67 ± 0.08 , respectively. There were significant differences between the areas under the curve for the two markers in the group of patients with acute myocardial infarction ($P=0.03$) and in the group of patients with unstable angina ($P=0.01$), with control patients and patients with stable angina serving as the ROC control group.

PAPP-A levels of more than 10 mIU per liter were present in 17 of 20 patients with unstable angina (85 percent), and in 16 of 17 patients with myocardial infarction (94 percent). Thus, PAPP-A levels appear to be valuable for detecting unstable coronary disease even when the levels of troponins and C-reactive protein are not elevated, thus potentially identifying high-risk patients whose disease might otherwise remain undiagnosed.

In our study, the levels of troponin I and creatine kinase MB were not associated with PAPP-A levels in patients with acute coronary syndromes. This finding indicates that PAPP-A is not produced in response to myocardial necrosis, a potent stimulus for the synthesis of inflammatory cytokines and acute-phase reactants. The magnitude of injury may reflect the size of the infarction, which may not be related to the size or magnitude of the inflammatory response associated with an active plaque in the infarcted artery.

The significant association between PAPP-A levels and C-reactive protein levels in patients with acute coronary syndromes is consistent with growing evidence suggesting that inflammation has a pivotal role in these syndromes.^{26,27} The accumulation of macrophages in unstable atherosclerotic lesions stimulates the production of metalloproteinases²⁸ and may be associated with increases in the plasma levels of C-reactive

protein.²⁹ We excluded patients with nonatherosclerotic inflammatory disorders in order to avoid potentially confounding results with respect to PAPP-A.

We also found an association between PAPP-A levels and free IGF-I levels. The recent identification of PAPP-A as the enzyme cleaving IGF-binding protein 4, an inhibitor of the action of IGF, suggests that PAPP-A increases the availability of IGF-I,⁹ thus contributing to the progression of both coronary atherosclerosis and restenosis.³⁰ The free fraction of circulating and locally synthesized IGF-I induces the migration of vascular smooth-muscle cells³¹ and is important for monocyte chemotaxis and the activation and release of cytokines within the atherosclerotic lesion.³²

Our study cannot answer questions such as whether PAPP-A is a primary or secondary component of acute coronary syndromes. Further studies are required to characterize the importance of PAPP-A within unstable coronary plaques and to elucidate the diagnostic and prognostic significance of elevated PAPP-A levels in patients with acute coronary syndromes. Our study was limited in terms of the number of patients examined, and it is important to confirm the findings with studies of more patients.

In conclusion, PAPP-A is a new candidate marker for the early diagnosis of acute coronary syndromes.

Our results demonstrate that circulating PAPP-A levels can identify patients early in the process of plaque instability, when it might still be possible to avert myocardial injury. In addition to their practical clinical importance, our observations point out new avenues of investigation into the causes of unstable angina and myocardial infarction.

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