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Prospective Evaluation of Pregnancy-Associated Plasma Protein-A and Outcomes in Patients With Acute Coronary Syndromes

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Objectives This study sought to investigate whether pregnancy-associated plasma protein-A (PAPP-A) is useful for risk assessment in non–ST-segment elevation acute coronary syndrome (NSTE-ACS).

Background PAPP-A is a high molecular weight, zinc-binding metalloproteinase that is associated with vulnerable plaque and may be a predictor of cardiovascular disease and mortality.

Methods We measured PAPP-A at baseline in 3,782 patients with non NSTE-ACS randomized to ranolazine or placebo in the MERLIN–TIMI 36 (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary Syndromes) trial and followed for an average of 1 year. A cut point of 6.0 μIU/ml was chosen from pilot work in this cohort.

Results PAPP-A >6.0 μIU/ml at presentation was associated with higher rates of cardiovascular death (CVD) or myocardial infarction (MI) at 30 days (7.4% vs. 3.7%, hazard ratio [HR]: 2.01; 95% confidence interval [CI]: 1.43 to 2.82; $p < 0.001$) and at 1 year (14.9% vs. 9.7%, HR: 1.63; 95% CI: 1.29 to 2.05; $p < 0.001$). PAPP-A was also associated with higher rates of CVD (HR: 1.94; 95% CI: 1.07 to 3.52, $p = 0.027$) and myocardial infarction (HR: 1.82; 95% CI: 1.22 to 2.71, $p = 0.003$) individually at 30 days. There was no difference in the risk associated with PAPP-A stratified by baseline cardiac troponin I [Accu-TnI >0.04 μg/l], p interaction = 0.87). After adjustment for cardiac troponin I, ST-segment deviation, age, sex, diabetes, smoking, hypertension, and coronary artery disease, PAPP-A was independently associated with CVD/myocardial infarction at 30 days (adjusted HR: 1.62, 95% CI: 1.15 to 2.29; $p = 0.006$) and 1 year (adjusted HR: 1.35, 95% CI: 1.07 to 1.71; $p = 0.012$). PAPP-A also improved the net reclassification for CVD/MI ($p = 0.003$). There was no significant interaction with ranolazine.

Conclusions PAPP-A was independently associated with recurrent cardiovascular events in patients with NSTE-ACS. This finding supports PAPP-A as a candidate prognostic marker in patients with ACS and supports investigation of its therapeutic implications. (J Am Coll Cardiol 2012;60:332–8) © 2012 by the American College of Cardiology Foundation

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Metalloproteinases have been implicated in the destabilization of atherosclerotic plaque, specifically, degrading the proteins that maintain the integrity of the protective fibrous cap (1,2). Recognition of their biological proteolytic activity has led to the hypothesis that metalloproteinases might be markers of vulnerable or ruptured atherosclerotic plaque (2,3). Pregnancy-associated plasma protein-A (PAPP-A) is a high molecular weight, zinc-binding metalloproteinase (1). Originally identified in pregnant women, in whom it is produced in the placenta (4), PAPP-A is also found at lower concentration in nonpregnant women and men. This enzyme has been shown to cleave insulin-like growth factor binding protein-4 from insulin-like growth factor-1, an important regulatory protein in cell proliferation and metabolism (5).

The blood concentration of PAPP-A is increased in patients with acute coronary syndromes (ACS), and PAPP-A is present in vulnerable coronary plaque but not in stable plaques (3,6). Two initial studies observed that PAPP-A concentration is associated with recurrent ischemic events in patients with suspected ACS, including those with undetectable troponin (3,7). However, subsequent results have been mixed, and most of these studies were performed in the setting of less sensitive previous-generation assays for cardiac troponin (8).

We investigated whether PAPP-A, measured using a highly sensitive assay developed to detect low concentrations in nonpregnant individuals, would be useful in conjunction with a contemporary sensitive assay for cardiac troponin for assessing the risk of recurrent ischemic events in patients presenting with non–ST-segment elevation acute coronary syndromes (NSTE-ACS). In addition, we evaluated the relationship of PAPP-A with other biomarkers representing potentially different pathobiological processes.

Methods

Patient population. The MERLIN–TIMI 36 (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary Syndromes) trial enrolled 6,560 patients with NSTE-ACS and randomized them to treatment with either ranolazine or placebo in a 1:1 ratio and followed for a median of 348 days. The detailed entry criteria have been reported (9,10) and are summarized in the Online Appendix. The MERLIN–TIMI 36 trial, including this substudy, was approved by the relevant institutional review boards at all participating centers. Written informed consent was obtained from all patients.

Abbreviations and Acronyms

ACS = acute coronary syndrome(s)
BNP = B-type natriuretic peptide
CI = confidence interval
cTnI = cardiac troponin I
CV = cardiovascular
CVD = cardiovascular death
HR = hazard ratio
MI = myocardial infarction
MPO = myeloperoxidase
NRI = net reclassification improvement
NSTE-ACS = non–ST-segment elevation acute coronary syndrome(s)
PAPP-A = pregnancy-associated plasma protein-A

Biomarker testing. PAPP-A was measured in serum in batch (Active cPAPP-A ELISA, Beckman Coulter, Brea, California) by personnel blinded to treatment allocation and clinical events. Analytical sensitivity for the Active cPAPP-A assay is 0.18 μIU/ml, with total imprecision (cardiovascular [CV]) of 10% and 6% at concentrations of 2 and 11 μIU/ml, respectively. Troponin was measured using the AccuTnI (Beckman Coulter using the 99th percentile reference limit (0.04 μg/l). Details of sample collection and other biomarker testing are described in the Online Appendix.

Endpoints. The primary endpoint for this analysis was CV death or myocardial infarction (MI). All endpoints were adjudicated by an independent clinical endpoints committee, blinded to treatment allocation, according to definitions that were published previously (Online Appendix) (9,10).

Statistical methods. Baseline characteristics were compared using the chi-square test for categorical variables and the Wilcoxon rank sum test for continuous variables. Event rates presented are Kaplan-Meier failure rates at 30 days and 12 months. The assessment of the relationship between PAPP-A and outcome was performed using Cox regression. Interaction testing was performed for PAPP-A concentration and heparin administration based on previous studies demonstrating an interaction between heparin and PAPP-A concentration (11). Adjusted analyses included all elements of a well-validated risk model in NSTE-ACS (Thrombolysis In Myocardial Infarction risk score) (12). There was no interaction with the randomized therapy, and therefore treatment allocation was not included in the model.

A pilot study within a development set was performed to develop a prognostic cut point for validation and is described in the Online Appendix. The optimized cut point (6.0 μIU/ml) from the derivation set was then applied in the validation set as well as in the entire cohort. The increased discriminative value of PAPP-A in addition to clinical predictors, and troponin was examined with the method described by Harrell (13) to determine the net reclassification improvement (NRI) (13). NRI was calculated for comparison of the clinical model with cardiac troponin I (cTnI) alone and with PAPP-A using Harrell's technique, as programmed in R, which evaluates the change in estimated risk as a continuous variable and therefore is not dependent on a previous categorization

from CV Therapeutics and Sanofi-Aventis; grant support from Amgen, Critical Diagnostics, Integrated Therapeutics, Millennium Pharmaceuticals, Novelo, and Sanofi-Synthelab; and has served as a consultant for Beckman Coulter, Critical Diagnostics, Gilead, Instrumentation Laboratories, Menarini, Merck & Co., Ortho-Clinical Diagnostics, Roche, Sanofi-Aventis, Schering-Plough, and Siemens. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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JACC Vol. 60, No. 4, 2012
July 24, 2012:332–8

Table 1 Baseline Characteristics by PAPP-A Concentration

	PAPP-A <6.0 μIU/ml (n= 3,138)	PAPP-A ≥6.0 μIU/ml (n = 644)	p Value
Demographics			
Age, ≥75 yrs, %	14	25	<0.001
% Male	64	69	0.011
% White race	97	98	0.052
Weight, kg	82 (73–93)	80 (70–90)	<0.001
Risk factors, %			
Diabetes mellitus	34	27	0.002
Current smoker	25	22	0.067
History of hypertension	77	64	<0.001
History of dysplipidemia	69	62	<0.001
Previous myocardial infarction	36	36	0.79
Previous congestive heart failure	22	16	<0.001
Previous PCI or CABG	26	26	0.87
Creatinine clearance <60 ml/min	19	26	<0.001
Presentation, %			
Index NSTEMI	46	61	<0.001
Troponin I ≥0.04	58	73	<0.001
TIMI risk score ≥3	75	76	0.19
ST-segment depression ≥1 mm	36	39	0.13
B-type natriuretic peptide, >80 pg/ml	42	50	<0.001
High-sensitivity C-reactive protein ≥15	23	17	0.0047
Myeloperoxidase >670 pmol/l	35	65	<0.001
Randomized treatment			
Allocated to ranolazine, %	49	52	0.15

Values are % or median (interquartile range).

CABG = coronary artery bypass graft; IQR = interquartile range; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; PAPP-A = pregnancy-associated plasma protein-A; TIMI = Thrombolysis In Myocardial Infarction.

(13). All analyses, unless otherwise specified, were performed using STATA version 9.2 (StataCorp, College Station, Texas).

Results

Baseline characteristics. PAPP-A was measured in all available baseline serum samples (N = 3,782). The baseline characteristics of the study population are listed in Table 1. Patients with a baseline concentration of PAPP-A ≥6.0 μIU/ml (n = 644, 17%) were older, more frequently male, and more likely to have a creatinine clearance <60 ml/min; however, they were less likely to have diabetes mellitus, hypertension, or dysplipidemia.

Other correlates of PAPP-A. Patients with elevated baseline levels were more likely to have had an index presentation of non–ST-segment elevation MI and to have a TIMI risk score of ≥4. Nevertheless, PAPP-A was only weakly correlated with the concentrations of cTnI (r = 0.17), B-type natriuretic peptide (BNP) (r = 0.093), myeloperoxidase (MPO) (r = 0.39), and high-sensitivity C-reactive protein (r = −0.13) (all p < 0.001). There was no significant difference in left ventricular ejection fraction (p = 0.68) or severity of coronary artery disease (p = 0.66) among patients with and without elevated PAPP-A.

Cut-point determination. The derivation cohort of 543 patients was randomly selected for cut-point derivation. Baseline characteristics and the distribution of PAPP-A were similar between the derivation and validation sets and are shown separately in Online Table 1, with the exception of troponin elevation. A cut point of 6.0 μIU/ml showed the strongest relationship with CV death or MI and was prospectively tested in the validation cohort (Online Table 2). The prognostic performance of PAPP-A was highly similar in the derivation and validation cohorts (Online Tables 3 and 4), and therefore the results of the overall cohort are presented from this point forward.

Unadjusted relationship between PAPP-A and CV outcomes. In unadjusted analyses, patients with a baseline PAPP-A ≥6.0 μIU/ml had an approximately 2-fold higher risk of CV death or MI at 30 days compared with those with PAPP-A concentrations below the cut point (HR: 2.01; 95% CI: 1.43 to 2.82) (Table 2). The adverse CV risk associated with an elevated PAPP-A persisted through 12 months (HR: 1.63; 95% CI: 1.29 to 2.05) (Fig. 1 and Table 2). Moreover, patients with an elevated baseline PAPP-A had significantly higher rates individually of 12-month recurrent ischemic events (MI, p = 0.0049 and severe recurrent ischemia, p = 0.032), as well as CV death (p = 0.0028) compared with patients with a baseline PAPP-A <6.0 μIU/ml (Table 2). There was no significant relationship between PAPP-A and the risk of hospitalization for heart failure (Table 2).

Table 2 PAPP-A Concentration and Cardiovascular Outcomes

	30-Day Outcomes				12-Month Outcomes			
	PAPP-A <6.0 μIU/ml (n = 3,138), %	PAPP-A ≥6.0 μIU/ml (n = 644), %	HR (95% CI)	p Value	PAPP-A <6.0 μIU/ml (n = 3,138), %	PAPP-A ≥6.0 μIU/ml (n = 644), %	HR (95% CI)	p Value
CVD	1.2	2.3	1.94 (1.07–3.52)	0.027	4.0	6.9	1.68 (1.19–2.37)	0.0028
MI	2.9	5.2	1.82 (1.22–2.71)	0.0029	7.2	9.9	1.49 (1.13–1.98)	0.0049
SRI	3.4	6.0	1.78 (1.23–2.58)	0.0019	10.3	13.2	1.31 (1.02–1.68)	0.032
HF	1.8	2.4	1.29 (0.73–2.28)	0.38	3.8	4.6	1.19 (0.79–1.79)	0.42
CVD/MI	3.7	7.4	2.01 (1.43–2.82)	<0.001	9.7	14.9	1.63 (1.29–2.05)	<0.001
CVD/MI/SRI	6.9	11.9	1.78 (1.37–2.31)	<0.001	18.1	23.2	1.38 (1.15–1.66)	<0.001

CI = confidence interval; CVD = cardiovascular death; HF = heart failure; HR = hazard ratio; MI = myocardial infarction; PAPP-A = pregnancy-associated plasma protein-A; SRI = severe recurrent ischemia.

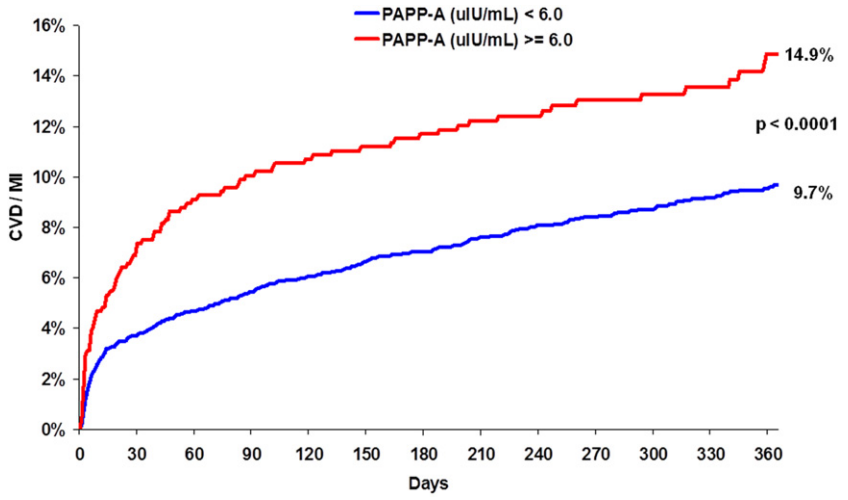

Figure 1 **CVD or MI by Baseline PAPP-A Concentration**

Kaplan-Meier estimated cumulative incidence of cardiovascular death (CVD) or myocardial infarction (MI) stratified by baseline pregnancy-associated plasma protein-A (PAPP-A) concentration at 6.0 μIU/ml.

Application in conjunction with a sensitive troponin and natriuretic peptide. The risk of CV death or MI associated with baseline PAPP-A concentration was not modified by troponin status as measured using a contemporary sensitive assay both at 30 days (p interaction = 0.93) and 12 months (p interaction = 0.87) (Fig. 2). A similar relationship between PAPP-A and CV death or MI was seen among patients with elevated BNP (Online Table 4 and Fig. 3).

Multivariable assessment of PAPP-A and CV outcomes. When applied in conjunction with established clinical risk predictors in ACS (age, sex, ST-segment deviation, diabetes, smoking, hypertension, and history of coronary artery disease) as well as established biomarkers (cTnI), PAPP-A was independently associated with the risk of both 30-day CV death/MI (adjusted HR: 1.62; 95% CI: 1.15 to 2.29; p = 0.006) and 30 day CV death, MI, or severe recurrent ischemia

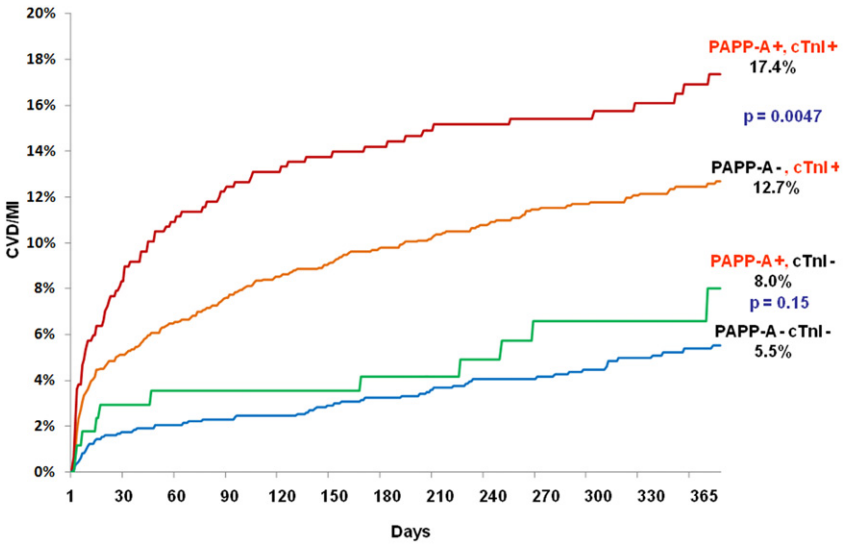

Figure 2 **CVD or MI Baseline PAPP-A and cTnI Concentration**

Kaplan-Meier estimated cumulative incidence of CVD or MI stratified by baseline PAPP-A concentration at 6.0 μIU/ml and cardiac troponin I (cTnI) using the 99th percentile reference limit (0.04 μg/l). Other abbreviations as in Figure 1.

JACC Vol. 60, No. 4, 2012
July 24, 2012:332–8

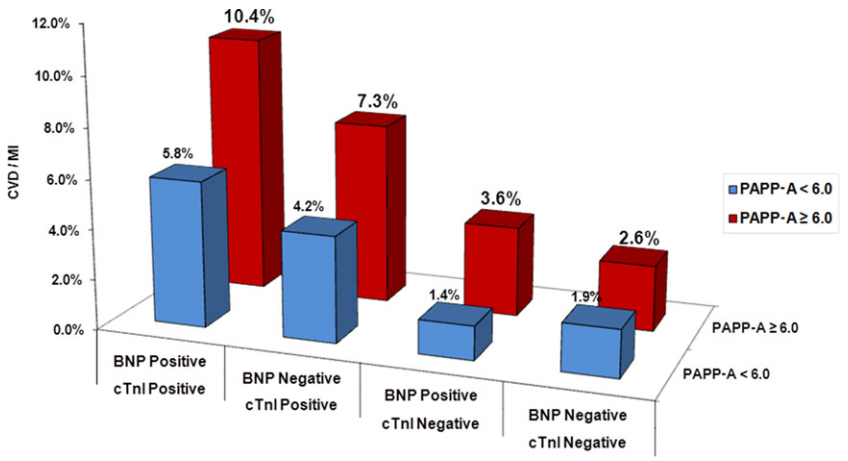

Figure 3 CVD or MI at 30 Days by Baseline PAPP-A, BNP, and cTnI Concentration

Kaplan-Meier estimated cumulative incidence of CVD or MI at 30 days stratified by PAPP-A concentration at 6.0 μIU/ml, cTnI using the 99th percentile reference limit (0.04 μg/l), and B-type natriuretic peptide (BNP) at 80 pg/ml at baseline. Abbreviations as in Figures 1 and 2.

(adjusted HR: 1.52; 95% CI: 1.16 to 1.98; p = 0.002) (Fig. 4). Moreover, when other inflammatory biomarkers, MPO, and high-sensitivity C-reactive protein, and BNP were added to the model in addition to TnI, only PAPP-A remained a predictor of CV death or MI at 30 days (adjusted HR: 1.56; 95% CI: 1.09 to 2.24; p = 0.015) and 1 year (adjusted HR: 1.37; 95% CI: 1.07 to 1.75; p = 0.011) (Fig. 5). There was no significant interaction between PAPP-A and heparin use for CV death/MI or CV death, MI, or severe recurrent ischemia at 30 days or 1 year (p > 0.65 for each interaction).

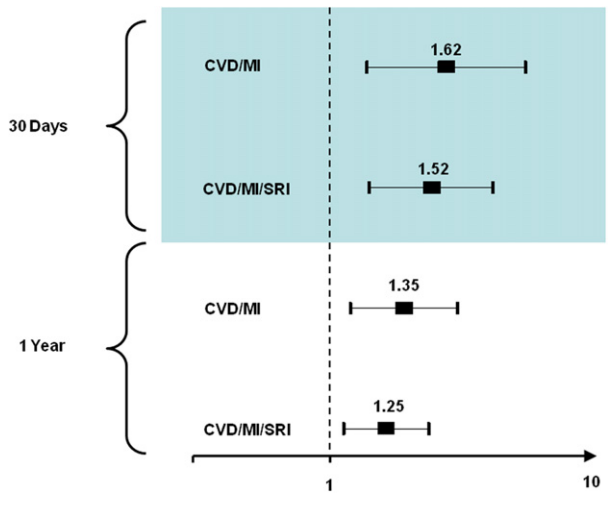

Figure 4 Adjusted Risk of Cardiovascular Events at 30 Days and 1 Year

Adjusted hazard ratios for CVD or MI and CVD, MI, or severe recurrent ischemia (SRI) at both 30 days and 12 months. After adjusting for established clinical predictors of outcome, patients who had a baseline PAPP-A concentration ≥6 μIU/ml were consistently at higher risk of recurrent cardiovascular events compared with patients who had baseline PAPP-A <6 μIU/ml. Adjusted for elements of Thrombolysis In Myocardial Infarction risk score (age, multiple risk factors, known coronary disease, aspirin use, severe angina, cTnI using the 99th percentile reference limit [0.04 μg/l] and ST-segment deviation), and sex. Abbreviations as in Figure 1.

Reclassification of risk. The incremental improvement in risk stratification beyond clinical risk predictors was evaluated using NRI as a measure of reclassification. The addition of PAPP-A dichotomized at 6.0 μIU/ml significantly improved reclassification for cardiovascular death (CVD)/MI (p = 0.003) and for CVD/MI/severe recurrent ischemia (p = 0.021) at 12 months. Furthermore, when cTnI was added to clinical predictors, the NRI remained significant for both CVD/MI (p = 0.0028) and for CVD/MI/severe recurrent ischemia (p = 0.021). In contrast to reclassification, there was no significant improvement in the c-statistic for CVD/MI by adding PAPP-A to clinical predictors and TnI (0.706 vs. 0.709, p = 0.23).

Discussion

We found that an elevated PAPP-A concentration in patients with NSTE-ACS identified individuals at significantly higher short and long-term risk of adverse CV events when used independently and in conjunction with clinical characteristics and contemporary sensitive troponin and natriuretic peptide assays. Importantly, PAPP-A was predictive of the risk of recurrent MI rather than new or worsening heart failure. Consistent with the hypothesized pathobiology, these findings support PAPP-A specifically as a candidate marker of risk of recurrent atherothrombosis and differentiates PAPP-A from other novel markers such as natriuretic peptides and MPO, which are markers of all-cause mortality and heart failure but show variable association with recurrent ischemic events (14–16).

Clinical and research implications. Hospitalization for recurrent ischemic events occurs in as many as 1 in every 5

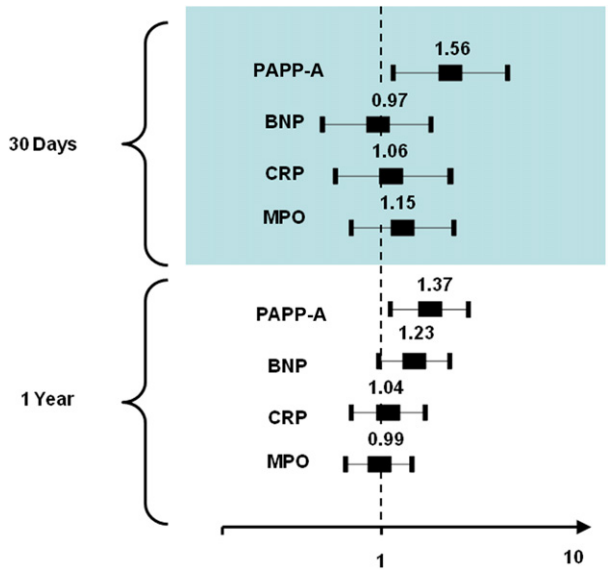

Figure 5 **Adjusted Risk of CVD/MI With Concurrent Assessment of Novel Biomarkers**

Adjusted hazard ratios for CVD or MI at both 30 days and 12 months by baseline PAPP-A, myeloperoxidase (MPO), C-reactive protein (CRP), and BNP concentration. After adjusting for established clinical predictors and established prognostic markers, patients who had a baseline PAPP-A concentration ≥6 μIU/ml were consistently at higher risk of recurrent cardiovascular events compared with patients who had baseline PAPP-A <6 μIU/ml. Adjusted for elements of Thrombolysis In Myocardial Infarction risk score (age, multiple risk factors, known coronary disease, aspirin use, severe angina, cTnI using the 99th percentile reference limit [0.04 μg/l] and ST-segment deviation), sex, PAPPA ≥6 μIU/ml, MPO >670 pmol/l, high-sensitivity CRP >15 mg/l, and BNP >80 pg/ml. Abbreviations as in Figures 1 and 3.

patients during the first year after NSTE-ACS (17). Although clinical models for the prediction of CV mortality after ACS have been developed and are used clinically, prediction of recurrent ischemic events with similarly high discrimination has been difficult (18), as evident by the significant decrement in the c-statistic of the TIMI risk score for patients with NSTE-ACS when predicting MI (0.66) versus mortality (0.74) (12) and for the Global Registry of Adverse Coronary Events score when predicting death or MI (0.70) compared with death alone (0.82) (19). Cardiac troponin is strongly and reproducibly associated with the risk of recurrent ischemic events after ACS. However, other newer established or emerging biomarkers have proven to have stronger associations with heart failure events (20). As we have shown previously in this population, BNP is an independent predictor of death and heart failure; however, it is not strongly predictive of recurrent ischemic events (21). In this context, our findings with PAPP-A are intriguing, both for its potential role as a clinical risk predictor and for therapeutic guidance.

Smaller previous studies have found mixed results regarding the association between PAPP-A and recurrent ischemic events independent of TnI or in patients with undetectable TnI (3,7). Lund et al. (7) found that increasing PAPP-A was an independent predictor of future ischemic events in 136 consecutive patients presenting to the emergency department with suspected ACS found to be cTnI negative. Heeschen et al. (22) found similar results in a larger cohort of 644 patients with chest pain. These studies, however, used insensitive TnI assays by current standards or cut points above those currently recommended, leading to recommendations for assessment using contemporary assays and cut points (8,23). Notably, our current study prospectively demonstrated a significant relationship between PAPP-A and CV death or recurrent ischemic events in 3,782 patients in conjunction with a contemporary sensitive assay for cTnI. Moreover, our large sample size adds substantially, more than doubling, the previous experience with PAPP-A for risk assessment and also permitted us to build a separate derivation set for development and internal validation of a prognostic decision limit.

The potential complementary nature of PAPP-A to other biomarkers that reflect distinct pathways underlying the development of recurrent events is further supported by the weak correlation between PAPP-A and markers of myonecrosis, hemodynamic stress, and even nonspecific markers of inflammation. Although each of these dimensions of performance that we observed with PAPP-A are interesting, further research is required to establish clear therapeutic ties (24).

Although the prognostic implications of increased PAPP-A appear reproducible and a plausible role in plaque destabilization exists, the precise pathways and contribution, if any, of PAPP-A relative to other metalloproteinases in plaque instability have yet to be definitively established. Our findings support additional mechanistic investigation of these pathways. Alternative interpretations of the association of PAPP-A and outcomes have also been proposed including a protective effect mediated through insulin-like growth factor-1 that is elicited in response to ischemic injury (6,25). Such an investigation is also likely to be valuable in elucidating the potential for PAPP-A as a therapeutic target (26). Previous work has shown that PAPP-A concentrations are not influenced by statin therapy, at least in stable patients with hypercholesterolemia (27), and the current analysis nested in MERLIN–TIMI 36 trial found no interaction with ranolazine.

Study limitations. This study was limited to patients selected for participation in a clinical trial who presented with suspected ACS and had samples available for analysis. As such, these results do not address the question of prognostic significance in the broader group of patients presenting with nontraumatic chest pain, including those patients with symptoms atypical of myocardial ischemia or presenting with other acute illnesses that may be inflammatory in nature. Although samples were handled using standardized procedures, it is possible that pre-analytical issues (i.e., storage) could have affected the stability of PAPP-A. Any such effect would be expected to have led us to underestimate the magnitude of the risk relationship. In addition, external validation of our proposed cut point in separate datasets

JACC Vol. 60, No. 4, 2012
July 24, 2012:332–8

would be valuable. Finally, we used an available contemporary sensitive troponin assay in this investigation, and different results might be observed if repeated using a third-generation highly sensitive assay.

Conclusions

PAPP-A was independently associated with the short- and long-term risk of CV death and recurrent ischemic events in patients with NSTE-ACS, along with clinical predictors and cTnI. The relationship with severe recurrent ischemic events differentiates PAPP-A from other novel markers that reliably predict only all-cause mortality and heart failure. Our findings add to emerging evidence supporting the metalloproteinase PAPP-A as a candidate prognostic marker of recurrent ischemia and CV death in patients with ACS and supports continued investigation of its potential therapeutic implications.

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REFERENCES

1. Laursen LS, Overgaard MT, Nielsen CG, et al. Substrate specificity of the metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) assessed by mutagenesis and analysis of synthetic peptides: substrate residues distant from the scissile bond are critical for proteolysis. Biochem J 2002;367:31–40.
2. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
3. 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.
4. Wald NJ, George L, Smith D, Densem JW, Petterson K. Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. International Prenatal Screening Research Group. Br J Obstet Gynaecol 1996;103:407–12.
5. 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 U S A 1999;96:3149–53.
6. Bayes-Genis A, Schwartz RS, Lewis DA, et al. Insulin-like growth factor binding protein-4 protease produced by smooth muscle cells increases in the coronary artery after angioplasty. Arterioscler Thromb Vasc Biol 2001;21:335–41.
7. 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.
8. Apple FS, Wu AH, Mair J, et al. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem 2005;51:810–24.
9. Morrow DA, Scirica BM, Karwatowska-Prokopczuk E, et al. Evaluation of a novel anti-ischemic agent in acute coronary syndromes: design and rationale for the Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndromes (MERLIN)-TIMI 36 trial. Am Heart J 2006;151:1186.e1-9.
10. Morrow DA, Scirica BM, Karwatowska-Prokopczuk E, et al. Effects of ranolazine on recurrent cardiovascular events in patients with non-ST-elevation acute coronary syndromes: the MERLIN-TIMI 36 randomized trial. JAMA 2007;297:1775–83.
11. Tertti R, Wittfooth S, Porela P, Airaksinen KE, Metsarinne K, Pettersson K. Intravenous administration of low molecular weight and unfractionated heparin elicits a rapid increase in serum pregnancy-associated plasma protein A. Clin Chem 2009;55:1214–7.
12. Antman EM, Cohen M, Bernink PJ, et al. The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. JAMA 2000;284:835–42.
13. Harrell FE Jr. Harrell Miscellaneous Package for R. 2008. 3.7–0. Available at: http://biostat.mc. vanderbilt.edu/s/Hmisc. Accessed August 1, 2011.
14. Scirica BM, Sabatine MS, Jarolim P, et al. Myeloperoxidase levels associated with risk of cardiovascular death and heart failure after non-ST elevation acute coronary syndrome. J Am Coll Cardiol 2008;51:A218.
15. Scirica BM, Cannon CP, Sabatine MS, et al. Concentrations of C-reactive protein and B-type natriuretic peptide 30 days after acute coronary syndromes independently predict hospitalization for heart failure and cardiovascular death. Clin Chem 2009;55:265–73.
16. Scirica BM, Sabatine MS, Jarolim P, et al. Assessment of multiple cardiac biomarkers in non-ST-segment elevation acute coronary syndromes: observations from the MERLIN-TIMI 36 trial. Eur Heart J 2011;32:697–705.
17. Yan AT, Steg PG, Fitzgerald G, et al. Recurrent ischemia across the spectrum of acute coronary syndromes: prevalence and prognostic significance of (re-)infarction and ST-segment changes in a large contemporary registry. Int J Cardiol 2010;145:15–20.
18. Morrow DA. Cardiovascular risk prediction in patients with stable and unstable coronary heart disease. Circulation 2010;121:2681–91.
19. Fox KA, Dabbous OH, Goldberg RJ, et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). BMJ 2006;333:1091.
20. Christenson RH, National Academy of Clinical Biochemistry. National academy of clinical biochemistry laboratory medicine practice guidelines for utilization of biochemical markers in acute coronary syndromes and heart failure. Clin Chem 2007;53:545–6.
21. Morrow DA, Scirica BM, Sabatine MS, et al. B-type natriuretic peptide and the effect of ranolazine in patients with non-ST-segment elevation acute coronary syndromes: observations from the MERLIN-TIMI 36 (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary-Thrombolysis in Myocardial Infarction 36) trial. J Am Coll Cardiol 2010;55:1189–96.
22. Heeschen C, Dimmeler S, Hamm CW, et al. Pregnancy-associated plasma protein-A levels in patients with acute coronary syndromes: comparison with markers of systemic inflammation, platelet activation, and myocardial necrosis. J Am Coll Cardiol 2005;45:229–37.
23. Jaffe AS, Katus H. Acute coronary syndrome biomarkers: the need for more adequate reporting. Circulation 2004;110:104–6.
24. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation 2007;115:949–52.
25. Conti E, Andreotti F, Zuppi C. Pregnancy-associated plasma protein a as predictor of outcome in patients with suspected acute coronary syndromes. Circulation 2004;109:e211–2; author reply e211–2.
26. Lindeman JH, Abdul-Hussien H, van Bockel JH, Wolterbeek R, Kleemann R. Clinical trial of doxycycline for matrix metalloproteinase-9 inhibition in patients with an abdominal aneurysm: doxycycline selectively depletes aortic wall neutrophils and cytotoxic T cells. Circulation 2009;119:2209–16.
27. Stulc T, Malbohan I, Malik J, Fialova L, Soukupova J, Ceska R. Increased levels of pregnancy-associated plasma protein-A in patients with hypercholesterolemia: the effect of atorvastatin treatment. Am Heart J 2003;146:E21.

Key Words: acute coronary syndrome(s) ■ atherothrombosis ■ ischemia ■ metalloproteinase ■ PAPP-A.

APPENDIX

For supplemental material, please see the online version of this article.