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Pregnancy-Associated Plasma Protein A and Proform Eosinophilic Major Basic Protein in the Detection of Different Types of Coronary Artery Disease

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Summary

Kryptor system was proven to be a rapid, standard method for pregnancy-associated plasma protein A and proform eosinophilic major basic protein (PAPP-A/proMBP) complex detection in coronary artery disease (CAD). No age and/or gender differences in 51 controls and 110 stable coronary artery disease (SCAD) patients were found. SCAD patients did not differ from controls and no difference in PAPP-A/proMBP levels with regards to the number of affected vessels was found. In 21 unstable angina pectoris (UAP), in 35 without and 66 with ST elevation acute myocardial infarctions (NSTEMI, STEMI respectively) patients PAPP-A/proMBP levels were increased ($P=0.004$ and $P<0.0005$, respectively). PAPP-A/proMBP levels did not correlate with cardiac troponin I (cTnI) in STEMI and NSTEMI patients. PAPP-A/proMBP increase was more frequent than cTnI ($P=0.036$) within the early phase of STEMI. In NSTEMI patients PAPP-A/proMBP positivity was present in 50 % of cTnI negative cases. Receiver operating characteristic (ROC) analysis revealed the highest diagnostic accuracy of PAPP-A/proMBP (0.919) in STEMI cTnI positive cases. The highest specificity/sensitivity PAPP-A/proMBP levels for particular acute coronary syndrome (ACS) types were 10.65-14.75 mIU/l. Combination of PAPP-A/proMBP with cTnI increases their diagnostic efficacy within the early phase of ACS. Our results suggest that PAPP-A/proMBP complex is involved in processes preceding vulnerable plaque development in ACS.

Key words

Coronary artery disease •PAPP-A/proMBP • Cardiac troponin I • Atherosclerosis

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Introduction

Pregnancy-associated plasma protein A (PAPP-A) belongs to the metzincin metalloproteinase superfamily. As an IGF-I-binding protein (IGFBP-4,5) metalloproteinase it cleaves inhibitory IGFBP-4,5 to amplify local insulin-like growth factor I (IGF-I) availability (Lawrence *et al.* 1999, Laursen *et al.* 2001). In pregnancy PAPP-A is linked as a heterotetrameric 2:2 complex to the proform of eosinophil major basic protein (proMBP) with a minor proportion of 2:1 linkage to proMBP or as an uncomplexed PAPP-A (Oxvig *et al.* 1993, Overgaard *et al.* 2000).

ProMBP is a heterogeneously glycosylated proteoglycane that is processed in maturing eosinophile granules to 14 kDA eosinophilic major basic protein (MBP) (Overgaard *et al.* 1999). In pregnancy, angiotensinogen forms 2:2 complex of angiotensinogen and proMBP. The other 2:2:2 complex is between proMBP, angiotensinogen and complement C3dg (Oxvig *et al.* 1995). This complex increases during blood coagulation suggesting blood complement activation (Christiansen *et al.* 2000).

PAPP-A was detected in the serum as a potentially early indicator of acute coronary syndrome (ACS) (Bayes-Genis *et al.* 2001a,b). Increased levels of PAPP-A protein were observed in subjects at high cardiovascular risk with increased echogenicity of carotid

plaques (Beaudeaux *et al.* 2003). The different evaluation of their impact in patients with acute myocardial infarction (AMI) (Qin *et al.* 2002, Dominguez-Rodriguez *et al.* 2005), in the prediction of ischemic cardiac events, need of revascularization (Lund *et al.* 2003, Laterza *et al.* 2004, Heeschen *et al.* 2005) and diagnostic relationship to elevated creatine kinase MB fraction (CK-MBmass) and cardiac troponin I (cTnI) in serial serum examinations (Khosravi *et al.* 2002) in the acute coronary syndrome substantiated further clinical studies.

The aim of this study was to validate a standard, rapid, commercially available, diagnostic system measuring simultaneously PAPP-A/proMBP complex with regards to its possible pathogenetic role in the development of coronary artery disease (CAD) (Chen *et al.* 2003, Che *et al.* 2002, Spencer 2003, Resch *et al.* 2004). Therefore, this study was focused on the ascertainment of PAPP-A/proMBP complex diagnostic efficacy within the earliest clinical phase of ACS in comparison to cTnI representing the currently most important ACS biomarker in cohort of cases with exactly clinically and angiographically determined different types of CAD.

Methods

The Internal Ethics Review Board of the University Hospital Prague-Motol approved this prospective study and all study subjects signed respective informed consent forms prior to coronary angiography. Patients with different types of CAD were sampled without any selection bias. Exclusion criteria included advanced kidney, liver, overt heart failure and history of major surgery or trauma within the previous month, pregnancy, known or suspected inflammatory and/or neoplastic diseases.

Prolonged chest pain with ST elevation and increased CK-MBmass, more than twice above its upper limit (>9.2 μg/l), and the cTnI level of more than 0.5 μg/l characterized STEMI patients. NSTEMI patients differed by ST segment depressions or T wave inversions. Patients with unstable angina pectoris (UAP) were defined by 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, normal CK-MB, cTnI levels and CAD confirmed by angiography. Patients with stable CAD had effort-induced chest pain and/or positive stress test and severe CAD without ischemic episodes during the week preceding respective angiography (Table 1). PAPP-A and proMBP complex (PAPP-A/proMBP) was examined in 122 ACS patients. They comprised 66 STEMI (mean age ± SD was 62±10 years), 35 NSTEMI (67±10 years) and 21 UAP individuals (66±12 years). Obtained results were compared to 51 controls (59±9 years) with suspected CAD, but with normal coronary angiography or minimal vessel irregularities and 110 cases with stable coronary artery disease (64±9 years).

Age, gender, height, weight, blood pressure levels, cardiovascular risk factors as hypertension, diabetes mellitus, smoking, family history of CAD, hyperlipidemia and cardiac medication were evaluated (Table 1).

Blood samples were obtained during angiography and/or at routine admission examinations. The mean time ± SD from the last ischemic episode with chest pain to blood sampling in STEMI was 4±3.3 h within 1-12 h, in NSTEMI 9.1±2.1 h within 1-24 h and in UAP 15.5±9.5 h within 3-24 h.

Frozen sera (–20 °C), prepared within 1-2 h after the blood sampling, were used for examination of PAPP-A/proMBP by Kryptor™ system (Brahms-Germany), based on the time–resolved amplified cryptate emission, validated within the first trimester biochemical screening (Spencer 2003). cTnI was analyzed by the Immulite Turbo Troponin I kit and immulite analyzer (DPC USA) with the lowest measurable levels of 0.5 μg/l.

Angiography was performed in all patients to determine the extent of coronary atherosclerosis before heart surgery or because of CAD symptoms. Severe CAD was defined by the presence of one or more stenoses with at least 70 % diameter reduction on any major coronary artery.

Continuous variables were expressed as mean ± SD, discrete variables as counts and percentages. Non-parametric tests were used due to non-Gaussian distribution of PAPP-A/proMBP. These were compared among the groups using the Kruskal-Wallis test. In case of rejecting the hypothesis of all group equality, couples of groups were compared by the Mann-Whitney test. Bonferroni correction of statistical significance was applied in the case of multiple comparisons. Spearman's correlation coefficient was used to evaluate relationship of PAPP-A/proMBP to age and to cTnI. Chi-square test or Fisher's exact test were used to compare occurrence of the risk factors among the examined cohorts. McNemar test was used to compare frequency of increased levels of

Table 1. Clinical characteristics of studied patients

Clinical characteristics		Characterization of the sample under investigation				
		Control (n = 51)	**SCAD (n = 110)**	**UAP (n = 21)**	**NSTEMI (n = 35)**	**STEMI (n = 66)**
Mean age (years) (range	*Male*	55 (38-68)	63 (42-87)	65 (42-82)	65 (45-82)	59 (41-82)
	Female	61 (44-76)	65 (48-77)	68 (46-82)	69 (49-86)	70 (48-83)
Sex no.(%)	*Male*	17 (33.3)	81 (73.6)	13 (61.9)	24 (68.6)	51 (77.3)
	Female	34 (66.7)	29 (26.4)	8 (39.1)	11 (31.4)	15 (22.7)
Risk factors no. (%)	*Arterial hypertension*	25 (49)	69 (62.7)	17 (81) *‡	23 (65.7)	24 (38.1) †
	Smoking	15 (29.4)	28 (25.5)	2 (9.5) ‡	14 (40)	35 (54.7)*
	Hyperlipoproteinemia	23 (45.1)	72 (65.5) *§	7 (33.3)	10 (28.6) #	12 (18.8)*
	Diabetes mellitus	9 (17.6)	35 (31.8)	8 (38.1)	14 (40)*	8 (12.5) †
Therapy no. (%)	*Aspirin*	34 (68)	92 (84.4)*§	13 (65)‡	15 (46.9)† #	14 (21.5)*
	Beta-blockers	24 (48)	81 (74.3)*§	9 (45)‡	14 (43.8)† #	12 (18.5)*
	ACE inhibitors	22 (44)	59 (54.1)§	8 (40)‡	8 (25) #	10 (15.4)*
	Nitrates	22 (44)	66 (60.6)*§	9 (45)‡	9 (28.1) #	9 (13.8)*
	Statins	10 (20)	49 (45)*§	5 (25)	3 (9.4) #	6 (9.2)
	Calcium-channel blockers	12 (24)	18 (16.5)	3 (15)	4 (12.5)	6 (9.2)
Angiographic findings no.(%)	*One vessel disease*	0	40 (36.7)	6 (28.6)	8 (28.6)	26 (40.6)
	Two vessel disease	0	25 (22.9)	5 (23.8)	4 (14.3)	21 (32.6)
	Three vessel disease	0	44 (40.4)	10 (47.6)	16 (57.1)	17 (26.6)

* P<0.05 – comparison to controls; † P< 0.05 – comparison of NSTEMI versus STEMI; ‡ P<0.05 – comparison of UAP versus STEMI; # P<0.05 – comparison of SCAD versus NSTEMI; § P<0.05 – comparison of SCAD versus STEMI; SCAD – stable coronary artery disease, UAP – unstable angina pectoris, STEMI – acute myocardial infarction with ST elevation, NSTEMI – acute myocardial infarction without ST elevation.

PAPP-A/proMBP and cTnI.

Receiver operating characteristic (ROC) curves (Swets 1988) were applied for the evaluation of the diagnostic accuracy of PAPP-A/proMBP in ACS patients by the size of the area under ROC curve (AUC). AUC>0.9 indicates the highest diagnostic accuracy, 0.65-0.9 confident diagnostic utility, 0.5-0.65 low diagnostic accuracy. Areas under ROC curve for different tests were compared according to the overlap of their confidence intervals and according U-test. ROC analysis provided also ascertainment of the highest specificity and sensitivity (HSS) of PAPP-A/proMBP. The SPSS software for Windows (Release 10.0.7) was used. Percentiles were qualified using the weighted average method.

Results

Clinical characteristics, type of medication, mutual comparison of patients and controls (Table 1) reflect general health status of this age group. Chi square test revealed no differences in the number of affected vessels among CAD patients when all types were compared together.

Table 2. Levels of PAPP-A/proMBP (mIU/l) in different types of CAD

	Controls (n = 51)	SCAD (n = 110)	UAP (n = 21)	NSTEMI (n = 35)	STEMI (n = 66)
Mean ± SD	7.28±3.57	7.91±3.61	19.73±19.78	21.13±24.71	30.3±30.11
2 SD	7.14				
Median	7.5	8.1	11.4	14.3	17.75
Range	0-14.2	0-16.4	0-68.3	0-123	0-146.8
2 Medians	15				
P1		0.14	0.004	<0.0005	<0.0005
P2			0.011*	<0.0005	<0.0005
P3				0.5	0.045*
P4					0.08

P1 – comparison of SCAD, UAP, NSTEMI, STEMI vs. controls; P2 – comparison of UAP, NSTEMI, STEMI vs. SCAD; P3 – comparison of NSTEMI, STEMI vs. UAP; P4 – comparison of STEMI vs. NSTEMI; PAPP-A/proMBP was compared among the groups using the Kruskal-Wallis test. In case of rejecting the hypothesis of all groups equality, couples of groups were compared by the Mann-Whitney test. * according to Bonferroni correction labeled p values did not document statistical significance despite P≤0.05, because significant values corresponded to α<0.005 for ten groups compared. SCAD – stable coronary artery disease; UAP – unstable angina pectoris, STEMI – acute myocardial infarction patients with ST elevation; NSTEMI – acute myocardial infarction patients without ST elevation.

Table 3. Percentile values of PAPP-A/proMBP (mIU/l) in controls and patients with different types of CAD – sensitivity of PAPP-A/proMBP for diagnosis of particular ACS types.

Studied group	75^{th} percentile	sensitivity	90^{th} percentile	sensitivity	95^{th} percentile	sensitivity
Controls	9.6		12.32		13.84	
SCAD	10		12.3		13.6	
UAP	27.4	0.57	60.38	0.43	67.86	0.38
NSTEMI	20.75	0.71	45.15	0.62	102.37	0.59
STEMI	45.05	0.77	61.95	0.72	102.4	0.66

SCAD – stable coronary artery disease, UAP – unstable angina pectoris, NSTEMI – acute myocardial infarction without ST elevation, STEMI – acute myocardial infarction with ST elevation.

The PAPP-A/proMBP values in controls are shown in Table 2. No gender (P=0.11) and age (P=0.5) differences were revealed. Mean levels were 7.28±3.57 mIU/l in agreement with medians. The lowest measurable value was 3.42 mIU/l. The 75-95^{th} percentile PAPP-A/proMBP levels are in the Table 3. Non-measurable levels were found in 5 of 51 individuals (9.8 %).

A single control patient (1/51) had higher level (38.5 mIU/l) than mean ± 2 SD/2 medians with elevated hs-CRP (5 mg/l). This female was under long-term oncogynecology surveillance after hysterectomy. In 9 out of 51 control patients the PAPP-A/proMBP levels were within the range 10-14.2 mIU/l. In 5 of them nephrosclerosis, recovery after bronchopneumonia and gastrointestinal bleeding, chronic duodenal ulcer, ulcerative colitis and chronic obstructive pulmonary disease were detected.

Mean levels of PAPP-A/proMBP in stable CAD (7.91±3.61 mIU/l) were not significantly different from controls (P=0.14) (Table 2). No gender (P=0.35) and age (P=0.98) differences were revealed within range of 42-87 years. Males had higher levels than females (8.19±3.42 mIU/l vs. 6.96±3.76 mIU/l, P=0.034), only when controls and SCAD patients levels were pooled. The number of individuals with non-measurable levels was 11/110 (10.0 %). No differences in PAPP-A/proMBP levels between patients with one (7.91±3.87 mIU/l) and two or three affected vessels (7.90±3.52 mIU/l) were disclosed.

Highly increased levels of PAPP-A/proMBP in ACS (Table 2) were detected in UAP, NSTEMI and STEMI cases compared to controls. NSTEMI and STEMI patients differed highly significantly from the SCAD cohort. No differences were disclosed between NSTEMI, STEMI and UAP and between NSTEMI and STEMI

Table 4. Area under receiver operating characteristic (ROC) curve of PAPP-A/proMBP in ACS.

Types of ACS		Area	Std. Error	Asymptotic 95 % confidence interval		HSS (mIU/l)
				Lower band	Upper band	
STEMI (cTnI+,0)	*vs (C)*	0.865	0.034	0.798	0.932	12.05
STEMI (cTnI+,0)	*vs (C+SCAD)*	0.849	0.034	0.782	0.816	12.05
STEMI (cTnI+)	*vs (C)*	0.919	0.036	0.849	0.989	12.30
STEMI (cTnI 0)	*vs C*	0.812	0.052	0.711	0.914	14.75
NSTEMI (cTnI+,0)	*vs (C)*	0.803	0.054	0.696	0.909	14.25
NSTEMI (cTnI +)	*vs (C)*	0.828	0.063	0.704	0.952	12.35
NSTEMI (cTnI 0)	*vs (C)*	0.755	0.092	0.574	0.936	14.25
STEMI+NSTEMI (cTnI+,0)	*vs (C)*	0.843	0.031	0.782	0.805	14.25
STEMI+NSTEMI (cTnI+,0)	*vs UAP*	0.613	0.071	0.474	0.752	12.75
UAP	*vs (C)*	0.718	0.072	0.577	0.860	10.65
UAP+STEMI+NSTEMI	*vs (C)*	0.821	0.031	0.760	0.883	14.25
STEMI (cTnI+,0)	*vs (SCAD)*	0.841	0.036	0.771	0.912	12.00
NSTEMI (cTnI+,0)	*vs (SCAD)*	0.775	0.056	0.664	0.885	13.5
UAP	*vs (SCAD)*	0.675	0.076	0.525	0.825	11.15

HSS – best sensitivity/specificity PAPP-A/proMBP levels; (cTnI+/0) – troponin positivity (>0.5 μg/l)/negativity (<0.5 μg/l) in AMI patients; (cTnI+,0) – troponin positivity (>0.5 μg/l) and negativity (<0.5 μg/l) alltogether in AMI patients; (C) – control PAPP-A/proMBP levels; (C + SCAD) – pooled control and SCAD PAPP-A/proMBP levels; (SCAD) – SCAD PAPP-A/proMBP levels. Areas under ROC curves were compared by the U-test in STEMI (cTnI+,0) vs. C to UAP vs. C, NSTEMI (cTnI +,0) vs. C to UAP vs. C, STEMI (cTnI+,0) vs. C to NSTEMI (cTnI+,0) vs. C, STEMI+NSTEMI) vs. UAP to UAP x C and STEMI (cTnI+) vs. C to UAP vs. C; U-test disclosed statistical significance for the comparison of STEMI (cTnI+) vs. C to UAP vs. C only (U-test = 2.49, P=0.0127). SCAD – stable coronary artery disease, UAP – unstable angina pectoris; NSTEMI – acute myocardial infarction patients without ST elevation, STEMI – acute myocardial infarction patients with ST elevation.

patients. The number of individuals with non-measurable levels was 1/21 (4.8 %) in UAP, 2/35 (5.7 %) in NSTEMI and 1/66 (1.51 %) in STEMI patients. Percentile values with corresponding diagnostic sensitivity are listed in Table 3.

PAPP-A/proMBP ROC curves analysis in different types of ACS are shown in Table 4 and Fig. 1. ROC curves demonstrate that PAPP-A/proMBP levels provided a confident diagnostic utility for the discrimination of all ACS types from control levels as well as from controls combined with SCAD patients. The best specificity/sensitivity (HSS) PAPP-A/proMBP levels correspond to the range 10.65-14.75 mIU/l (comparison to control levels) for particular ACS types. The highest diagnostic accuracy (AUC=0.919) was found in cTnI positive (cTnI+) STEMI patients (Table 4). The lowest AUC was in discrimination of AMI from UAP individuals (AUC=0.613).

A comparison of AUC in different ACS patient groups indicates that significant difference, evaluated by the U-test, was detected only between troponin-positive STEMI (cTnI+ vs. controls) and UAP patients (UAP vs. controls) (P=0.01 U-test = 2.49). Tendencies towards significant differences were found between STEMI troponin-positive (cTnI+) and STEMI troponin-negative (cTnI 0) patients (P=0.09 U-test = 1.69) and STEMI and NSTEMI troponin-positive patients (P=0.07 U-test = 1.79). The same was true for the comparison of all STEMI (cTnI+,0) and UAP patients (P=0.06 U-test = 1.84). No statistical significant differences were disclosed between other different ACS groups.

The comparison of PAPP-A/proMBP and cTnI in AMI patients indicated that in STEMI patients the levels of cTnI were 6.6±18.2 μg/l at the time of the blood sampling for PAPP-A/proMBP and maximal cTnI levels was 146±163 μg/l. In NSTEMI patients the first levels of cTnI were 5.8±8.9 μg/l and maximal cTnI levels were 27.9±35.4 μg/l.

In STEMI patients no correlations between PAPP-A/proMBP and cTnI levels were revealed (Spearman's rho=0.038, P=0.768), as in both STEMI and NSTEMI patients (Spearman's rho=0.07, P=0.47 for

Table 5. Comparison of c-Troponin I and PAPP-A/proMBP in STEMI patients in the range of >75 to >95 percentiles.

c-Troponin I	PAPP-A/proMBP (mIU/l)					
	>75 percentile (>9.6 mIU/l)		>90 percentile (>12.3 mIU/l)		>95 percentile (>13.84 mIU/l)	
	<75 percentile	>75 percentile	<90 percentile	>90 percentile	<95 percentile	>95 percentile
Negativity *(<0.5 μg/l)*	11 (17.46 %)	23 (36.51 %)	14 (22.22 %)	20 (31.75 %)	14 (22.22 %)	20 (31.75 %)
Positivity *(>0.5 μg/l)*	4 (6.35 %)	25 (39.68 %)	4 (6.35 %)	25 (39.68 %)	8 (12.70 %)	21 (33.33 %)
P	0.0003		0.002		0.036	

% – from total number of 63 patients examined, P – statistical significance (evaluated by McNemar test) between the number of either PAPP-A/proMBP or c-Troponin I only positive patients. It was ascertained in the group of c-Troponin I negative patients (<0.5 μg/l) and positive patients (>0.5 μg/l) and PAPP-A/proMBP levels. PAPP-A/proMBP positivity in the range of >75 percentiles corresponds to the levels >9.6 mIU/l, in the range >90 percentiles corresponds to >12.3 mIU/l and in the range > 95 percentiles corresponds to >13.84 mIU/l.

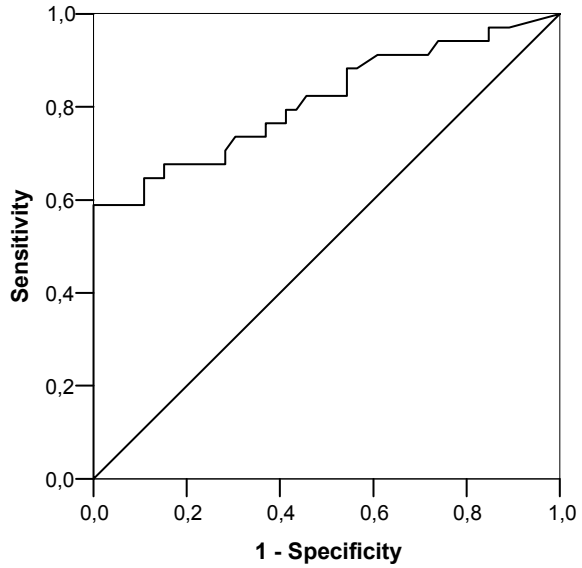

Fig. 1. ROC curve of PAPP-A/proMBP in STEMI cTnI negative patients. This figure documents diagnostic impact of PAPP-A/proMBP in the first hours after the onset of chest pain; in these STEMI patients cTnI increase was not revealed yet. STEMI – acute myocardial infarction patients with ST elevation, AUC – area under ROC curve, cTnI – Troponin I.

cTnI, Spearman's rho=0.11, P=0.26 for cTnI max).

The analysis of cTnI and PAPP-A/proMBP levels in STEMI patients (Table 5) in the range of >75 to >95 percentiles revealed that in the group with levels >75 percentiles there is highly significant prevalence (McNemar test, P=0.0003) of PAPP-A/proMBP only positive patients compared to cTnI only positive cases (>0.5 μg/l). Such a significant difference also remains in patients with levels >90 and >95 percentiles (McNemar test, P=0.002 and 0.036, respectively). PAPP-A/proMBP increased levels were disclosed earlier in 20 out of 34 (58.8 %) STEMI cTnI-negative patients (Table 5) within the range of >95 percentiles.

In NSTEMI patients no differences in the prevalence of cTnI and PAPP-A/proMBP positivities within the range of >75, >90 and >95 percentiles (McNemar test, P=0.8-1.0, respectively) were observed. Nevertheless, PAPP-A/proMBP positivity with levels >75 percentiles was detected in 7 out of 12 (58.3 %), in >90 and >95 percentiles in 6 out of 12 (50 %) cTnI-negative patients.

Discussion

To our best knowledge this is the first study where PAPP-A and proMBP proteins were examined simultaneously in randomly sampled controls and all types of CAD patients utilizing commercial automatic Kryptor detection system, which was verified in prenatal biochemical screening (Spencer *et al.* 2003). Analysis is completed within 30 min and thus it is feasible for clinical practice compared to the use of more demanding techniques (Bayes-Genis *et al.* 2001a, Dominguez-Rodriguez *et al.* 2005, Lund *et al.* 2003, Laterza *et al.* 2004, Heeschen *et al.* 2005). Low frequency of non-measurable levels, decreasing with clinical severity of CAD types, did not influence statistical significance and clinical impact of this study, since medians, non-

parametric tests and other appropriate tests were used for comparisons.

Recent results of Qin *et al.* (2005) indicate, that circulating ACS-related PAPP-A is non-complexed with proMBP and/or both types of complexed and uncomplexed PAPP-A proteins are present in ACS patients (Qin *et al.* 2006). This might explain non-measurable/normal and high variability of PAPP-A/proMBP levels in some our ACS patients (Table 2). Furthermore, some fatal AMI cases (Farb *et al.* 1996) are caused by thrombosis on a deendotelized, but otherwise intact plaque.

Our results prove that the Kryptor system provides relevant detection of serum PAPP-A complexed and uncomplexed with proMBP within the great majority of ACS patients. Our unpublished results with Perkin-Elmer ELISA kits applied in prenatal screening provided the same diagnostic utility in these CAD patients. Our data indicate that normal serum PAPP-A/proMBP levels decrease the risk of an unstable plaque presence.

Our control levels of PAPP-A/proMBP complex (Tables 2 and 3) are slightly higher than PAPP-A levels reported by Qin *et al.* (2002) – 5.68 mIU/l or Cosin-Sales *et al.* (2004) – 4.62 vs. 7.28 mIU/l. PAPP-A/proMBP levels were increased in the range of levels of different ACS type in 10 out of 51 control patients. In six of them it might be explained by reparative processes accompanying also other pathologic affections including neoplasia with known PAPP-A/proMBP involvement in healing (Chen *et al.* 2003) and angiogenesis (Jadlowiec *et al.* 2005). That has to be taken into the account in diagnostic evaluation of increased PAPP-A/proMBP levels in different types of CAD.

Different even contradictory observations concerning the clinical implementation of PAPP-A in ACS (Dominguez-Rodriguez *et al.* 2005, Laterza *et al.* 2004) might also be due to the use of different detection methods. Standard/feasible methods to ascertain the difference and ratios between different types of PAPP-A in CAD (Qin *et al.* 2005, 2006) are necessary to be developed and validated in subsequent studies.

So far only Cosin-Sales *et al.* (2004) in stable CAD studied ratio of PAPP-A to proMBP. Our results indicate that simultaneous PAPP-A/proMBP detection might improve PAPP-A diagnostic and possible prognostic validity in all CAD types. It is also supported by the fact that proMBP partially inhibits proteolytic PAPP-A activity (Overgaard *et al.* 1999, 2000) and thus its presumed role in the atherosclerotic process (Qin *et al.* 2005, Bayes-Genis *et al.* 2000, Aso *et al.* 2004, Apple *et al.* 2005), in inflammation and plaque disruption risk (Che *et al.* 2002, Resch *et al.* 2004). The diagnostic/prognostic value of this protein complex corresponds to the observation that its formation is also enhanced by altered redox potentials under pathological conditions (Glerup *et al.* 2005) and by angiotensinogen and complement C3dg binding to the proMBP (Oxvig *et al.* 1995, Christiansen *et al.* 2000). ProMBP is also a carrier of active molecules for tissue remodeling and differentiation and is believed to neutralize cytotoxicity of MBP (Bonno *et al.* 1994), what was confirmed in eosinophilic endomyocarditis and in myocardial infarction (Tai *et al.* 1987, Rauch *et al.* 1997). PAPP-A decreases the risk of thromboembolism due to the inhibition of proMBP maturation to MBP. MBP promotes thrombosis by binding to the anionic endothelial protein thrombomodulin and impairing its anticoagulant activities (Slungaard *et al.* 1993).

In agreement with Bayes-Genis *et al.* (2001a,b), we observed that PAPP-A/proMBP values were not dependent on sex and age in controls and did not differ from controls in SCAD cases. Cosin-Sales *et al.* (2004, 2005) described increased PAPP-A levels correlating with age, male gender, hypertension, CAD extent, and complexity of coronary stenoses. We confirmed correlation with male gender only in pooled controls and SCAD PAPP-A/proMBP despite the fact that our PAPP-A/proMBP levels were similar (7.3±3.6 vs. 5.5±1.6 mIU/l). This observation could be explained by different detection methods, simultaneous measurements of PAPP-A and proMBP and/or lower number of our patients with established diagnostic limits of angiography.

The increased PAPP-A/proMBP levels in ACS patients with highest diagnostic validity in STEMI cTnI positive patients (Tables 2 and 4) suggest an association with the degree of coronary vessels alteration. It corresponds to increased PAPP-A levels in unstable plaques (Bayes-Genis *et al.* 2001a,b), including experimental restenosis by inflammatory cytokines, to its proatherogenic role (Lawrence *et al.* 1999) and promotion of the instability of the atherosclerotic plaque (Che *et al.* 2002).

PAPP-A/proMBP positivity in ACS patients corresponds to the cut-off levels higher than the 75th percentile (Tables 2 and 3). The highest sensitivity and specificity PAPP-A/proMBP values ascertained by ROC analysis (10.65-14.75 mIU/l) in different ACS types (Table 4) correspond also to 12.6 mIU/l of PAPP-A for

maximized predictive value in ACS patients (Heeschen *et al.* 2005).

We revealed that in STEMI patients increased PAPP-A/proMBP levels are more frequent and an earlier biomarker than cTnI. This evidence suggests involvement of PAPP-A/proMBP complex in the preceding processes of plaque formation, instability development including proliferative changes of coronary vessels. Furthermore, this is supported by the notion that increased PAPP-A may be a marker of atherosclerotic burden in asymptomatic patients with hypercholesterolemia (Štulc *et al.* 2003), of intimal hyperplasia only in hypercholesterolemic type II diabetics with carotid atherosclerosis (Aso *et al.* 2004) and of increased cardiovascular risk in hemodialyzed patients (Kalousová *et al.* 2004). These clinical data agree to experimental observations that PAPP-A is involved in matrix mineralization and angiogenesis (Jadlowiec *et al.* 2005). No correlations between PAPP-A/proMBP and cTnI levels in AMI patients also suggest their different role in AMI pathogenesis and its development.

Despite the fact, that PAPP-A/proMBP increased levels (>95 percentiles) were in 50 % of cTnI-negative NSTEMI patients, the absence of its significant increased prevalence compared to cTnI, can be explained because of longer time between onset of chest pain and first blood sampling for simultaneous PAPP-A/proMBP and cTnI examinations. Nevertheless, simultaneous assessment of PAPP-A and cTnI increased diagnostic sensitivity in NSTEMI patients similarly as in early phase STEMI to 82 % compared to the sensitivity of cTnI only (corresponding to 65 % in NSTEMI and 46 % in STEMI patients).

ROC analysis revealed that simultaneous assessment of PAPP-A/proMBP combined with cTnI (Tables 4 and 5, Fig. 1) will increase the diagnostic sensitivity/specificity of these biomarkers in the early phase of ACS development especially in the first hours after the onset of chest pain characterized by cTnI negativity (Fig. 1). Further studies focused on dynamic changes of PAPP-A/proMBP might contribute to the better understanding of ACS pathogenesis and diagnosis, therapeutic success and early prevention of adverse complications.

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