

Original Article

Pregnancy-associated plasma protein A and copeptin as markers in the early diagnosis of acute coronary syndrome

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Abstract: Objective: To evaluate the combined detection of pregnancy-associated plasma protein A (PAPP-A) and copeptin as a diagnostic marker in patients with acute coronary syndrome (ACS). Methods: Serum levels of PAPP-A, copeptin, creatine kinase isoenzyme MB (CK-MB) and cardiac troponin I (cTnI) were detected within 3 hours after acute chest pain in 120 patients who were suspected of having ACS and in 100 healthy control subjects. Electrocardiograms (ECG) were also performed for each subject. ACS was diagnosed using the current three-part gold standard (clinical manifestation, ECG result, biomarker levels). The sensitivity and selectivity of combined detection of PAPP-A and copeptin for the diagnosis of ACS were evaluated. Results: Serum levels of PAPP-A and copeptin were significantly higher in patients with ACS than in controls ($P < 0.01$). The sensitivity and negative predictive value of combined detection of PAPP-A and copeptin were 96.15% and 98.21%, respectively, both significantly higher than ECG, CK-MB, cTnI, PAPP-A alone, copeptin alone, and cTnI plus ECG ($P < 0.05$). Combined PAPP-A and copeptin detection also gave the highest diagnostic rate of all of these tests (89%). Conclusion: Combined detection of PAPP-A and copeptin is a highly effective tool for the early diagnosis of ACS.

Keywords: Acute coronary syndrome, pregnancy-associated plasma protein A, copeptin, diagnosis

Introduction

Acute coronary syndrome (ACS) is a group of acute myocardial ischemia conditions caused by rupture of coronary atherosclerotic plaque, secondary to complete or incomplete occlusive thrombosis. ACS is the most common cardiac disease with chest pain as the main manifestation [1] and is the main cause of death in patients with coronary heart disease. Good judgment and timely intervention could reduce the occurrence of cardiovascular accidents and improve the cure rate of ACS in the early stage [2, 3]. Diagnosis of ACS is usually based on clinical findings, electrocardiograph changes, biochemical markers of myocardial damage, and coronary arteriography [4, 5].

The most widely used biomarkers for ACS diagnosis are cardiac troponin (cTn), creatine kinase isoenzyme MB (CK-MB), pregnancy-associated plasma protein A (PAPP-A) and copeptin. Although cTn level is a highly sensitive

and specific marker for the diagnosis of myocardial necrosis, cTn cannot be detected in the blood until 6 to 9 h after the onset of chest pain [4]. CK-MB levels increases 3 to 8 hours after the onset of acute myocardial infarction, reaching a maximum 8 to 24 hours later [6]. Thus, like cTn, CK-MB is not detectable in the early stage of ACS. Therefore, other single markers or combined markers of myocardial necrosis should be evaluated.

PAPP-A, secreted by activated macrophages during the atherosclerotic process, plays a pivotal role in the pathophysiology of atherosclerotic plaque formation and development [7, 8]. PAPP-A is abundantly expressed in plaque cells and extracellular matrix of eroded and ruptured plaques [9]. Post-mortem evaluation of plaques in patients who died of cardiac causes showed an association between PAPP-A and unstable atherosclerotic plaques, and low levels of PAPP-A in stable plaques [10]. Cosin-Sales et al [11] confirmed that PAPP-A levels are signifi-

cantly higher in patients with complex plaques compared to those with smooth plaques. In addition, PAPP-A has been shown to be associated with recurrent cardiovascular events and mortality in patients with certain forms of ACS [12]. In patients with ischemic cardiac chest pain, high PAPP-A levels may be helpful in predicting long-term cardiovascular mortality [13]. PAPP-A is also a useful marker for early detection of ACS and identification of patients at risk for an acute ischemic cardiac event [14]. However, some studies have reported that PAPP-A alone is not sensitive or specific for acute myocardial infarction [6, 15].

Copeptin, a surrogate marker for arginine vasopressin secretion, is a novel biomarker that has shown great potential in diagnosis of cardiovascular diseases, especially ACS and chronic heart failure [16]. Copeptin levels have been found to be significantly higher in patients with acute myocardial infarction compared with patients having other diagnoses in the early stage [17]. Studies have shown that the diagnostic accuracy of cTn for the diagnosis of acute myocardial infarction was improved by combination with copeptin [18]. In patients with acute myocardial infarction, copeptin was also shown to predict left ventricular dysfunction and remodeling as well as clinical heart failure distant from the infarct period [19]. Copeptin has the advantages that are secreted soon after an infarction occurs and can be detected quickly, at a time when other biomarkers are still undetectable [20]. However, copeptin provides little clinical information when measured alone because of its nonspecific elevation in many pathophysiologic conditions [21].

Early diagnostic assessment of patients with acute chest pain remains crucial for risk stratification and selection of treatment strategy. No single criterion has a 100% diagnostic rate for ACS; therefore, the evaluation of multiple criteria might be a better approach to ACS diagnosis. The purpose of the present study was to evaluate the combination of PAPP-A and copeptin levels as a tool for early diagnosis of ACS.

Materials and methods

Study design and study subjects

Patients who came to People's Hospital of Weifang, Shangdong province, China complain-

ing of acute chest pain from February, 2014 to October, 2015 were recruited to this study. 120 patients complaining of acute chest pain were seen in that time frame; 102 of them were diagnosed as ACS. Among these 102 subjects with ACS, 68 were male and 34 were female, and the average age was 64.5 ± 8.7 years. During the same period, 100 healthy people were recruited as normal control group. This group included 64 males and 36 females, and the average age was 58.4 ± 7.6 years. There was no significant difference in gender distribution and age between the two groups ($P > 0.05$). People with history of malignancy, current pregnancy, chronic renal failure, chronic liver failure, stroke, endocrine disease, chronic obstructive pulmonary disease, acute or chronic infection, sepsis or severe systemic disease or trauma were excluded from the study. Additionally, people with history of heart failure were excluded from the control group. Subjects were informed about the study and gave written informed consent. The study was approved by the Clinical Research Ethics Committee of People's Hospital of Weifang, Shangdong province, China.

Diagnosis of ACS using gold standard criteria

According to the American Heart Association (AHA) guidelines, there are three gold standards for the diagnosis of ACS [22]: (1) clinical symptom (ischemic chest pain); (2) specific changes in ECG (ST elevation or depression, ST-segment depression, T wave changes, newly developed left bundle branch block); (3) increased levels of specific biomarkers cTnI and CK-MB. The diagnosis of ACS can be confirmed when the two of these three criteria are positive. ACS includes three subgroups, ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP).

Detection of PAPP-A, copeptin, CK-MB and cTnI

Venous blood samples (10 ml) were taken from all subjects for the detection of PAPP-A, copeptin, CK-MB and cTnI. For potential members of the study group (patients complaining of acute chest pain), blood samples were taken within 3 hours of the onset of acute chest pain. Within 30 minutes after being drawn, blood samples were centrifuged at 1600 rpm for 5 min, and sera were stored at -80 until analysis. Samples were thawed only once. Briefly, the

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Table 1. ACS-related risk factors in study subjects

Parameter	ACS (n=102)	Control (n=100)	P
Age (yr)	64.5±8.7	58.4±7.6	NS
Family history of CAD	35	22	NS
Hypertension	32	30	NS
History of hyperlipidemia	15	14	NS
Diabetes mellitus	12	11	NS
Smoking	32	37	NS
Sedentary life style	34	25	NS
Serum glucose (mg/dl)	140.39±45.72	110.34±24.21	<0.01
Serum total cholesterol (mg/dl)	184.22±23.56	153.73±17.93	<0.01
Serum total triglycerides (mg/dl)	167.67±61.32	144.24±31.47	<0.01
Serum LDL-cholesterol (mg/dl)	119.72±31.94	84.54±26.17	<0.01
Serum HDL-cholesterol (mg/dl)	36.41±5.76	45.42±8.76	<0.01
Serum urea (mg/dl)	26.67±5.16	25.39±4.48	NS
Serum creatinine (mg/dl)	0.82±0.11	0.84±0.15	NS
Diastolic blood pressure (mmHg)	88.35±14.71	77.15±13.75	<0.01
Body mass index (kg/m ²)	26.74±4.35	25.79±3.82	NS

Data are expressed as mean ± SD for continuous variables and percentage (%) for categorical variables. CAD: coronary artery disease, HDL: high density lipoprotein, LDL: low density lipoprotein.

concentration of PAPP-A was measured by ELISA according to the manufacturer's instructions (Demeditec Diagnostics, Germany) Serum CK-MB was detected by Immuno-inhibition using a Stat Fax 3300 Biochemistry analyser (Awareness Technology Inc., Florida, USA), with a 24 IU/L threshold of positivity, as recommended by the manufacturer. cTnI was measured using an electrochemiluminescence MODULAR ANALYTICS E170 detector (ROCHE Diagnostics, Mannheim, Germany), and the threshold of positivity was set at 0.014 µg/L as recommended by the manufacturer. Copeptin was measured by immune fluorescence, using Time Resolved Amplified Cryptate Emission technology and KRYPTOR instrumentation (BRAHMS, Hennigsdorf, Germany), with a 14.1 pmol/L threshold of positivity as recommended by the manufacturer.

Statistical analysis

Statistical data were expressed as the mean ± SD, and the frequency was expressed as a percentage. The data were analyzed with SPSS13.1 software. Two samples were compared with t test, the mean of the multiply samples were compared with the analysis of variance, the comparison between groups was performed

with q test. Statistical significance was defined as $P < 0.05$.

Results and discussion

ACS-related risk factors in study subjects are shown in **Table 1**. Serum glucose, total cholesterol, total triglycerides, LDL-cholesterol, and diastolic blood pressure were significantly higher in subjects with ACS compared to subjects in the control group. Serum HDL-cholesterol was significantly higher in control subjects compared to those with ACS.

Study subjects with ACS were classified as having STEMI, NSTEMI or UAP ACS, as described in Materials and Methods, and levels of the ACS biomarkers PAPP-A, copeptin, CK-MB and cTnI were

compared between subjects in these ACS sub-groups and the control group. The levels of PAPP-A, copeptin, CK-MB and cTnI in subjects with ACS (STEMI, NSTEMI and UAP) were significantly higher than in control subjects ($P < 0.01$, **Table 2**). No significant differences in levels of these biomarkers were observed among subjects with different sub-classes of ACS.

Of the 120 study subjects hospitalized for chest painless than 3 hours after onset and suspected of having ACS, 58 had significant ECG findings, 72 had elevated cTnI, 44 had elevated CK-MB, 92 had elevated PAPP-A, 88 had elevated copeptin, had elevated cTnI and copeptin, and had elevated PAPP-A and copeptin (**Table 3**). A total of 102 patients were diagnosed with ACS, using the gold standard diagnosis criteria. Of these 26 had significant ECG findings, 28 had elevated cTnI, 21 had elevated CK-MB (+), 79 had elevated PAPP-A, 71 had elevated copeptin, had elevated cTnI and copeptin, and had elevated PAPP-A and copeptin.

Sensitivity, specificity, predictive value and diagnostic rate of ECG, cTnI, CK-MB, PAPP-A and copeptin alone, and combined cTnI and ECG or PAPP-A and copeptin for ACS are shown

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Table 2. Serum levels of ACS diagnostic biomarkers in study subjects

Biomarker	STEMI ACS (n=68)	NSTEMI ACS (n=21)	UAP ACS (n=13)	Control (n=100)	P value
PAPP-A (mIU/L)	16.32±9.12	11.54±4.22	13.28±8.51	7.16±1.41	<0.01
Copeptin (pmol/L)	38.44±9.62	34.59±10.74	31.63±10.38	10.24±5.47	<0.01
CK-MB (IU/L)	18.46±8.45	17.93±7.92	18.06±7.51	10.16±7.55	<0.01
cTn I (ug/L)	0.38±0.02	0.35±0.01	0.40±0.01	0.11±0.02	<0.01

NSTEMI: non-ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction; UAP: unstable angina pectoris PAPP-A: pregnancy associated plasma protein-; CK-MB: creatine kinase-MB; cTnI: cardiac troponin I.

Table 3. Sensitivity, specificity, predictive value and diagnostic rate of single and combined factors in the diagnosis of ACS

Test (s)	# of subjects with suspected ACS testing positive	# of subjects with confirmed ACS testing positive	Sensitiv- ity %	Specific- ity %	Positive Predictive Value %	Negative Predictive Value %	Diagnos- tic rate %
ECG	58	26	44.82	92.54	92.24	89.19	64
cTnI	72	28	38.89	96.74	93.55	44.58	55
CK-MB	44	21	47.72	93.25	79.41	58.23	68
PAPP-A	92	79	85.86	91.81	95.21	93.75	83
Copeptin	88	71	80.68	84.16	92.59	75.48	84
PAPP-A + Copeptin	106	101	95.28	82.73	89.26	98.21	89
cTnI + ECG	83	48	57.83	91.73	84.57	93.35	74

PAPP-A: pregnancy associated plasma protein-A; cTnI: cardiac troponin I; CK-MB: creatine kinase-MB; ECG: electrocardiogram.

in **Table 3**. Cardiac troponins still serve as a “gold standard” in acute chest pain evaluation, although their sensitivity during the first few hours after the onset of chest pain is relatively low. In the present study, the sensitivity of cTnI was 38.89%, and its specificity was 93.55%, similar to previous reports [23, 24]. The sensitivity and negative predictive value of PAPP-A + copeptin were significantly higher than all of the other diagnostic tests investigated in this study ($P<0.05$). PAPP-A + copeptin also gave the highest diagnostic rate. Positive predictive value of PAPP-A + copeptin was significantly higher than CK-MB ($P<0.05$), and similar to all other tests investigated. The specificity of PAPP-A + copeptin was 82.73%, lower than the other tests, but still an acceptable level. Overall, PAPP-A + copeptin seems to be the best choice among the diagnostic tests investigated in this study for early diagnosis of ACS.

Potential limitations of this study merit consideration. First, this was a single-center study with limited population size, so there may be statistical bias. Second, biomarker levels were only measured at one time point. Third, this study did not address whether PAPP-A and copeptin levels are related to ACS prognosis.

In conclusion, the combined detection of PAPP-A and copeptin might improve the diagnostic rate of ACS in patients with chest pain.

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Disclosure of conflict of interest

None.

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