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Clinical value of combined detection of thrombus precursor protein and P-selectin in the diagnosis of acute coronary syndrome.

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Key words: acute coronary syndrome; thrombus precursor protein; P-selectin; molecular markers of prethrombotic state.

Abstract. Acute coronary syndrome (ACS), including acute myocardial infarction (AMI) and unstable angina (UA), is the most threatening and lethal form of coronary heart disease. ACS has an abrupt onset and rapid development, which may lead to fatal conditions at any time. Thus, it is never too early to detect and diagnose patients with ACS. The objective of this work was to explore the significance of the combined detection of plasma thrombus precursor protein (TpP) and serum P-selectin (Ps), in the detection and diagnosis of patients with early ACS. A total of 126 subjects were included in the study, 64 ACS patients, 30 individuals with stable angina (SA) and 32 healthy persons who were selected as the control groups. There were no differences in gender, age, ethnicity, or blood glucolipid levels among the groups. Enzyme linked immunosorbent assay (Elisa) was used to quantitatively determine the plasma levels of TpP and Ps. The levels of the two biomarkers in the case group were significantly higher than those in the control groups. Among the ACS patients, the levels of TpP and Ps were higher in AMI patients than in the UA patients. In addition, there was no significant differences in the levels of Ps between SA patients and healthy persons. In conclusion, plasma TpP and serum Ps are remarkably increased in patients with ACS. Therefore, TpP and Ps may serve as ACS indicators, and their measurement may provide a support for an early clinical identification of ACS.

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Valor clínico de la detección combinada de proteína precursora de trombo y selectina P en el diagnóstico del síndrome coronario agudo.

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Palabras clave: síndrome coronario agudo; proteína precursora de trombo: P-selectina; marcadores moleculares de estado pretrombótico.

Resumen. El síndrome coronario agudo (SCA), que incluye el infarto agudo de miocardio (IAM) y la angina inestable (AI), es la forma más amenazante y letal de enfermedad coronaria. El SCA tiene un inicio abrupto y un desarrollo rápido, lo que puede conducir a condiciones fatales en cualquier momento. Por lo tanto, nunca es demasiado pronto para detectar y diagnosticar pacientes con SCA. El objetivo de este trabajo fue explorar la importancia de la detección combinada de la proteína precursora de trombos plasmáticos (TpP) y la selectina P sérica (Ps), en la detección y diagnóstico de pacientes con SCA precoz. Se incluyeron en el estudio un total de 126 sujetos, 64 pacientes con SCA, 30 individuos con angina estable (AE) y 32 personas sanas que fueron seleccionadas como grupos de control. No hubo diferencias en el género, la edad, el origen étnico o los niveles de glucolípidos en sangre entre los grupos. Se usó el ensayo inmunoabsorbente ligado a enzimas (Elisa) para determinar cuantitativamente los niveles plasmáticos de TpP y Ps. Los niveles de los dos biomarcadores en el grupo de casos (SCA) fueron significativamente más altos que los de los grupos de control. Entre los pacientes con SCA, los niveles de TpP y Ps fueron más altos en los pacientes con IAM que en los pacientes con AI. Además, no hubo diferencias significativas en los niveles de Ps entre pacientes con SA y personas sanas. En conclusión, la TpP plasmática y la Ps sérica están notablemente aumentadas en pacientes con SCA. Por lo tanto, TpP y Ps pueden servir como indicadores de SCA y su medición puede proporcionar un apoyo para una identificación clínica temprana de SCA.

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INTRODUCTION

Acute Coronary Syndrome (ACS) refers to a group of clinical syndromes with acute myocardial ischemic events due to a sudden obstruction of the coronary blood flow. It is almost always associated with the rupture of an atherosclerotic plaque and partial or complete thrombosis of the infarct-related artery.

ACS generally is divided into ST-segment elevation myocardial infarction (STEMI), non–ST-segment elevation myocardial infarction (NSTEMI) and unstable angina (UA). The occurrence of ACS is primarily due to plaque erosion or plaque rupture after coronary atherosclerosis, and can cause incomplete occlusion of blood vessels or secondary complete occlusive thrombosis, which ultimately leads to acute myocardial

ischemia[1]. ACS is easy to be missed in the clinical area due to the fact that its signs and symptoms usually begin abruptly. ACS progresses rapidly, which affects the survival time and quality of life of ACS patients [2,3]. Therefore, an early diagnosis is very important for ACS.

Thrombosis is one of the leading causes of atherosclerotic plaque formation. The detection of thrombus markers can identify whether the patient is in the prethrombotic state, which is of particular significance to assess the risk of ACS. The thrombus precursor protein (TpP) is a marker, and the most direct evidence, of impending thrombosis [4]. P-selectin (Ps) is a member of the selectin family of cell adhesion molecules, which plays a vital role in the initiation of inflammation, the mediation of leukocyte adhesion and aggregation on the endothelium, and the formation of thrombus [5]. In this study, through the determination of the above two biomarkers in healthy individuals and patients with ACS or SA in our hospital, we comprehensively analyzed the differences in the levels of the two factors in each studied group and investigated the significance of combined detection of TpP and Ps in the early diagnosis of ACS.

MATERIALS AND METHODS

Subjects and study design

Sixty-four patients with ACS treated in the Shanghai Xinhua Hospital Chongming Branch, from October 2019 to October 2020, were randomly selected as the case groups (including 30 patients with unstable angina (UA) and 34 patients with acute myocardial infarction (AMI) (18 patients with NSTEMI, 16 patients with STEMI)). Thirty patients with stable angina (SA) and 32 healthy people corresponding to the case group in terms of gender, age, ethnicity, etc. were selected as the control groups. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Xinhua Hospital Chongming Branch. All subjects gave their informed consent for inclusion before participating in the study (on admission). After admission, patients in each group received a routine electrocardiogram (or dynamic electrocardiogram), and the grouping was determined after the measurement of myocardial enzymes. Patients receiving platelet therapy, or with an abnormal coagulation function (DIC, hemophilia, leukemia, abnormal coagulopathy caused by severe liver disease, vitamin K deficiency) were excluded.

Baseline data were collected, including gender, age, nationality, blood glucose, blood lipids, and myocardial enzyme indexes. All the candidates had 4 ml of fasting venous blood drawn at 8:00 in the morning after admission. The Ps tube was immediately centrifuged at 3,000 rpm for 10 minutes to collect serum. The TpP tube was added with sodium citrate for anticoagulation and centrifuged at 3,000 rpm for 10 minutes to collect plasma. The collected serum and plasma were stored at –70° for testing.

Biomarker assays

Serum P-selectin was determined using a kit provided by Wuhan USCN Business Co., Ltd., and plasma TpP was measured with a kit provided by Wuhan Cusabio Technology LLC. The model of the microplate reader was Shanghai Kehua st-360.

Statistical analyses

The data obtained from the experiment were analyzed by SPSS 22.0 statistical software. The measurement data were expressed by $\bar{\chi} \pm$ SD. The comparison between means was made by the Student's t-test (analysis of variance was used to compare more than two groups). The counting data was represented by the composition ratio, and comparison between groups was by the χ^2 test. $P<0.05$ indicated that the difference was statistically significant.

RESULTS

Basic characteristics of the study participants

The details of demographic data of the 126 study participants are listed in Table 1. The results showed that there were no significant differences in age and sex among the groups.

Comparison of results between Stable Angina patients and healthy people

The results in Table 2, show that there were no statistically significant differences in age, blood glucose concentration, blood lipid concentration or the serum Ps levels between stable angina patients and healthy people ($P>0.05$). The serum TpP level of patients with SA was higher than that of the healthy people ($P<0.05$).

Comparison within case groups

In the case groups, there was no significant difference in the baseline data between patients with UA, STEMI and NSTEMI ($P>0.05$). The serum levels of Ps and TpP of patients with NSTEMI and STEMI were higher than those of patients with UA ($P<0.05$). See Table 3.

Comparison of results between case and control groups

The demographics, clinical, and laboratory data (age, gender, blood glucose, blood lipids, etc.) between case groups and control groups had no significant difference ($P>0.05$). The Ps and TpP levels of case groups were significantly higher than those of control groups ($P<0.05$). See Table 4.

Table 1
Basic information of patients.

Index		ACS			Control Groups		p
		UA (n=30)	NSTEMI (n=18)	STEMI (=16)	SA (n=30)	Healthy (n=32)	
Age		67.40±10.669	61.44±14.454	65.00±8.990	65.97±10.361	62.22±14.988	NS
Gender	Male (n/%)	21/70	13/72	22/73	22/73	19/60	NS
	Female (n/%)	9/30	5/28	8/27	8/27	19/60	

ACS: Acute coronary syndrome; UA: unstable angina; NSTEMI: non–ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction; SA: stable angina.

Table 2
Comparison of results between Stable Angina patients and healthy people.

Index	SA Group (30 cases)	Healthy Group (32 cases)	p
Age (years)	65.97±10.361	62.22±14.988	NS
Blood glucose (mmol/L)	6.54±1.946	6.30±2.257	NS
Blood lipid (mmol/L)	1.69±1.544	1.60±1.069	NS
Ps (ng/mL)	45.74±28.427	58.12±36.322	NS
TpP (ng/mL)	4.27±1.932	2.73±1.159	0.001

SA: stable angina Ps: P-selectin TpP: Thrombus precursor protein.

Table 3
Comparison of the results of UA, NSTEMI and STEMI within the case group.

Index	UA (30 cases)	NSTEMI (18 cases)	STEMI (16 cases)	p		
				(a and c1)	(a and c2)	(c1 and c2)
Blood glucose (mmol/L)	6.57±2.253	8.52±4.150	7.09±2.041	NS	NS	NS
Blood lipids (mmol/L)	1.81±1.504	1.91±1.198	1.99±1.168	NS	NS	NS
Ps (ng/mL)	39.09±12.139	87.40±37.413	93.39±39.000	<0.001	<0.0010	NS
TpP (ng/mL)	6.03±2.033	9.82±3.659	10.93±3.725	0.001	0.001	NS

UA: unstable angina NSTEMI: non–ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction Ps: P-selectin TpP: Thrombus precursor protein.

Table 4
Comparison of results between case and control groups.

Index	Case	Control	p
Age (years)	65.23±11.396	64.03±12.990	0.581
Blood glucose (mmol/L)	7.20±2.886	6.41±2.103	0.090
Blood lipids (mmol/L)	1.89±1.326	1.64±1.318	0.319
Ps (ng/mL)	66.25±38.39	52.13±33.07	0.029
TpP (ng/mL)	8.32±3.69	3.47±1.746	<0.001

Ps: P-selectin TpP: Thrombus precursor protein.

DISCUSSION

Many studies have confirmed that ACS is caused by unstable plaque, surface rupture, and breakage in the coronary arteries, which cause bleeding and thrombosis, leading to partial or complete occlusion of the coronary arteries. Platelet adhesion, activation, aggregation, and thrombosis are central to its pathogenesis [6]. Through the detection of related factors involved in thrombosis, the early recognition of ACS can be improved.

TpP is a high molecular weight soluble fibrin polymer, formed by the polymerization of fibrin monomers produced by the action of thrombin on fibrinogen and is the direct precursor of insoluble fibrin. Studies have confirmed that when the TpP level rises, it means that the thrombus has started, and the fibrin monomer has begun to polymerize, which can be used as a predictor of thrombosis [7]. Because of the specific antigenic determinants on the structure, this antigenic determinant does not exist on fibrinogen and fibrin degradation products. It may be more clinically significant than other indicators for diagnosing various thrombotic diseases [8].

Ps is a member of the selectin family of adhesion molecules. It plays a vital role in the process from leukocyte recruitment to plaque rupture. Related experiments have shown that Ps expression in platelets near the ruptured plaque was significantly increased, and there were a large number of mononuclear macrophages and T lymphocytes around it [9]. Many studies have also confirmed that the level of Ps can be used

to determine the incidence and severity of coronary heart disease [10,11].

The levels of the two indicators in the case group, were generally higher than those of healthy individuals and SA patients, which were similar to the results of Atalar [12] and Kayikcioglu et al. [13]. In addition, this study found that the levels of Ps and TpP in patients with acute myocardial infarction were significantly higher than those in patients with unstable angina. This indicated that there is a significant correlation between the changes in the levels of the two and AMI, but there is little difference between petients with STEMI and NSTEMI. There was no significant difference between healthy people and SA patients. Ps and TpP may play a role in early detection of ACS, but they cannot distinguish between NSTEMI and STEMI.

The concentration of TpP in plasma reflects the activity of thrombin in circulation. The increase of TpP indicates that the fibrin monomer has polymerized, which indicates that the thrombus is about to form (and is an indicator of thrombus activity this is a repetition). Stimulated by hypoxia, free radicals, and thrombin, the expression of Ps increases, which mediates the adhesion of leukocytes and endothelial cells and plays a central role in thrombosis [14,15]. Therefore, in patients with ACS caused by thrombosis, the expression levels of TpP and Ps may be significantly up-regulated. TpP is of great significance not only for the early diagnosis but also for the severity and prognosis of ACS [16]. Thus, TpP cannot only be used for the diagnosis but also for the classification of ACS in the future.

In summary, through the detection of molecular markers of the prethrombotic state, the early diagnosis of ACS can be achieved. It provides crucial guidance for further decisions on treatment options in clinical work, reduces the risk of premature death, improves the survival rate of patients, and brings greater benefits to the clinical response in the occurrence of acute cardiovascular events.

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Conflict of interests

All of the authors had no any personal, financial, commercial or academic conflicts of interest separately.

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Contribution of each author in the development of the work and writing the paper

HXJ and HDM conceived of the study, and WZX and ZJH participated in its design and coordination and LHQ and LYM helped to draft the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. **Saremi F. Cardiac MR.** Imaging in acute coronary syndrome: application and image interpretation. Radiology 2017; 282(1):17-32.
2. **Emergency Physician Branch of Chinese Medical Doctor Association**. 2015 China Emergency Acute Coronary Syndrome Clinical Practice Guidelines (2)—Diagnosis. Chin Emerg Med 2016; 36(1): 9-11.

3. **Giustino G, Baber U, Stefanini GG, Aquino M, Stone GW, Sartori S, Steg PG, Wijns W, Smits PC, Jeger RV, Leon MB, Windecker S, Serruys PW, Morice MC, Camenzind E, Weisz G, Kandzari D, Dangas GD, Mastoris I, Von Birgelen C, Galatius S, Kimura T, Mikhail G, Itchhaporia D, Mehta L, Ortega R, Kim HS, Valgimigli M, Kastrati A, Chieffo A, Mehran R.** Impact of clinical presentation (Stable Angina Pectoris vs Unstable Angina Pectoris or Non-ST-Elevation Myocardial Infarction vs ST-Elevation Myocardial Infarction) on long-term outcomes in women undergoing percutaneous coronary intervention with drug-eluting stents. Am J Cardiol 2015; 116(6):845-852.
4. **Song Z, Wang XP**. The significance of thrombus precursor protein in the diagnosis of thrombotic diseases. Tianjin Med J 2007,35(4):312-314.
5. **Semenov AV, Kogan-Ponomarev MI, Ruda MIa, Komarov AL, Panchenko EP, Chazova IE, Mazurov AV**. Soluble P-selectin a marker of platelet activation and vessel wall injury: increase of soluble P-selectin in plasma of patients with myocardial infarction, massive atherosclerosis and primary pulmonary hypertension. Ter Arkh 2000; 72(4): 15-20.
6. **Li-Sha G, Peng C, Yue-Chun L**. Recurrent acute coronary syndrome and restenosis after percutaneous coronary intervention in a patient with idiopathic thrombocytopenic purpura: a case report and literature review. BMC Cardiovasc Disord 2015; 15:101.
7. **Song YP, Wang WJ, Xu ZC, Zhang YH**. The prognostic value of d-dimer, high-sensitivity C-reactive protein and brain natriuretic peptide in acute coronary syndrome. Chin Gen Pract 2007,10(10):801-803.
8. **Chu J, He XD, Ye SL, Zhai ZM**. Detection of thrombus precursor protein and creatine kinase in patients with acute myocardial infarction and unstable angina. Chin J Arterios 2002; 10: 165-166.
9. **Kabbani SS, Watkins MW, Holoch PA, Terrien EF, Sobel BE, Schneider DJ**. Platelet reactivity in coronary ostial blood: a reflection of the thrombotic state accompanying plaque rupture and of the adequacy of antithrombotic therapy. J Thromb Thrombolysis 2001; 12: 171- 176.
10. **Jiang JC, Rong QJ**. The significance of P-selectin, C-reactive protein and troponin I on the short-term prognosis of patients with acute coronary syndrome. Mod Pract Med 2011; 2: 182-183.
11. **Chen ZQ, Hong L, Wang H, Yin QL, Lai HL, Lu LX, Ge YZ.** Changes and clinical significance of plasma P-selectin, glycoprotein/Ⅲa, fibrinogen and high-sensitivity C-reactive protein in elderly patients with acute coronary syndrome. Chin Gerontol Mag 2010,30(22):3269-3270.
12. **Atalar E, Aytemir K, Haznedaroğlu I, Ozer N, Ovünç K, Aksöyek S, Kes S, Kirazli S, Ozmen F**. Increased plasma levels of soluble selectins in patients with unstable angina. Int J Cardiol 2001; 78: 69-73.
13. **Kayikçioglu M, Can L, Mete-Erdem N, Kültürsay H, Payzin S, Kokuludag A, Türkoglu C**. Soluble p-selectin and the success of thrombolysis in acute myocardial infarction. Int J Cardiol 2001; 79: 223-229.
14. **Shebuski RJ, Kilgore KS**. Role of inflammatory mediators in thrombogenesis. J Pharmacol Exp Ther 2002; 300(3):729-735.
15. **Carter AM, Anagnostopoulou K, Mansfield MW, Grant PJ**. Soluble P-select in levels, P-selectin polymorphisms and cardiovasculardisease. J Thromb Haemost 2003, 1(8):1718-1723.
16. **Zhao GQ, Guo ZF**. Correlation analysis of fibrinogen, thrombus precursor protein and severity of coronary artery disease in patients with coronary heart disease. J pract clin med 2018; 22 (22): 1-4.