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Assay of Ischemia-Modified Albumin and C-Reactive Protein for Early Diagnosis of Acute Coronary Syndromes

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Diagnosis of cardiac ischemia in patients coming to emergency departments (ED) with symptoms of acute chest pain is often difficult. Many markers are sensitive and specific for the detection of myocardial necrosis but may not rise during reversible myocardial ischemia. Ischemia-modified albumin (IMA) has recently been shown to be a sensitive and early biochemical marker of ischemia. The variation laws were observed by measuring IMA and C-reactive protein (CRP) of 113 patients in ED within 12 hr after onset of chest pain. In the observation, blood was taken for IMA and CRP. Patients underwent standardized triage, diagnostic procedures, and treatment. Results of IMA and CRP were correlated with final diagnoses of nonischemic chest pain (NICP) and acute coronary syndrome (ACS). There were obvious distinction of IMA and CRP levels between the NICP and ACS groups. Receiver operator characteristic (ROC) curve analysis was used to determine the optimal cutoff of this assay for identifying individuals with ACS patients from NICP. The area under the curves of IMA is 0.948. The sensitivity and specificity of albumin cobalt binding (ACB) at a cutoff value of 70.0 units/mL were 94.4% and 82.6%, respectively. The area under the curves of CRP is 0.746. Sensitivity and specificity of CRP at a cutoff value of 3.16 mg/L were 70.0% and 73.9%, respectively. Negative predictive value (NPV) of IMA and CRP for ischemia origin was 79.2% and 38.6%, respectively. IMA may make an early diagnosis of acute coronary ischemia, and will improve the early diagnostic sensitivity and specificity of ACS. J. Clin. Lab. Anal. 22:45–49, 2008.

Key words: C reactive protein (CRP); myocardial ischemia; ROC curve

INTRODUCTION

Patients presently classified with the term acute coronary syndrome (ACS) in the emergency departments (ED) are difficult to assess because the symptoms can also be attributed to nonacute etiologies such as hypertension and anemia (1), or noncardiac etiologies including pulmonary and gastrointestinal disorders, infection, or other malignancies. Studies have shown that such vague clinical symptoms may cause patients with very real cardiovascular disease being erroneously discharged from the ED (2).

ACSs, such as unstable angina (UA) and myocardial infarction (MI), are the clinical manifestations of destabilization of coronary atherosclerotic plaques. There is increased recognition that inflammation plays a key role in the pathogenesis and outcome of ACSs (3). Previous studies have constantly shown that high circulating levels of C-reactive protein (CRP) incur an increased risk of long-term cardiovascular mortality in patients with either ST-segment elevation MI (STEMI) (4–7), or non-ST-segment elevation ACSs (NSTE-ACS) (7–11). Elevated concentrations of CRP can also be found in subjects with ACS. Thus, CRP is considered to play a role in risk stratification, both in acute MI (AMI) and in UA.

There is a need for early and sensitive markers of cardiac ischemia as current diagnostic tests fail to identify many chest pain patients presenting with ACS who are at high risk for adverse cardiac events. Furthermore, the lack of sensitivity of available diagnostic tools precludes early discharge of patients without ACS. We evaluated the role of ischemia-

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modified albumin (IMA), a new marker of ischemia, in patients with ACS. IMA is produced during an ischemic attack and is present in blood in easily detectable concentrations (12–14).

In the presence of tissue ischemia, circulating albumin undergoes a conformational change, which renders it unable to bind transition metals. This ischemia-rendered change yields a compound known as IMA, which is rapidly detectable following the onset of coronary ischemia and, importantly, is elevated in states of coronary ischemia not only with, but also without, cardiac injury (15,16). The aim of this study was to compare the clinical performance of IMA and CRP, in patients presenting to the ED with symptoms suggestive of ACSs. If a rapid blood test that could rule out the presence of acute myocardial ischemia, it would dramatically improve the triage process of patients with acute coronary symptoms, eliminate many prolonged patient observation times, and reduce health care costs.

MATERIALS AND METHODS

Patient Population

This study was performed at Peking University Third Hospital, Beijing, China, and was approved by the local ethics committee. We recruited, on a prospective sequential basis, patients who arrived at the ED within 12 hr after onset of chest pain. The enrolment period was between May 2006 and October 2006. A total of 156 patients were recruited for the study. However, 31 patients were excluded from the final analysis as a result of inaccuracy of timing for sample acquisition, and a further 12 because of incomplete biochemical characterization. Therefore, the total study population comprised 113 patients, 71 male and 42 female. Mean age was 64 years (range, 34–85 years). The patients were divided into two groups: the ACS group and nonischemic chest pain (NICP) group. Pregnant women, and patients with symptoms and signs suggestive of acute mesenteric ischemia, acute renal failure, peripheral vascular disease, or brain ischemia were not enrolled in the study.

Discharge Diagnosis

Coronary angiography was carried out on 113 patients. All angiographic images were reviewed by an experienced cardiologist blind to the patient's clinical characteristics and biochemistry results. A positive angiogram was defined as stenosis $\geq 70\%$ diameter reduction in any major epicardial vessels. Final diagnosis for this study was based on ED discharge diagnosis for patients discharged from the ED, and hospital discharge diagnosis for patients admitted to the hospital for further investigation and management. The ED consultant or medical consultant were responsible for assigning a final diagnosis on the basis of history, clinical examination, serial cardiac troponin T (cTnT) results, and data from medical records. These included results of electrocardiograph (ECG), exercise stress testing, and coronary angiography, as available. Results of all investigations were reviewed blind to IMA results. Patients were classified as NICP when: 1) a reported noncardiac mechanism was confirmed as the cause of chest pain; 2) all of the following criteria were met: negative cTnT results on serial sampling (over a 6–9 hr interval), presence of normal ECGs, and absence of any current (lesions $<70\%$ diameter reduction in any major epicardial vessel) or previous evidence of coronary artery disease (CAD); or 3) a diagnosis of ACS was objectively excluded after admission to the coronary care unit (CCU).

Laboratory Methods

All samples was collected when the patients arrived at the ED as soon as possible. Blood was collected in serum separator Vacutainer tubes (INSEPACK, Ji Shui Chuang Ge, Beijing, China) as quickly as possible. Specimens were allowed to clot and then centrifuged for 10 min at 1,000 *g*. Serum was harvested and stored at $-80^{\circ}C$ until testing. Frozen samples were mixed thoroughly after thawing and recentrifuged before analysis. Serum samples were subsequently tested for IMA (Yi An Bioscience, Chang Sha, Hu Nan, China) and CRP (Shen Suo You Fu Diagnostics, Shanghai, China) on the Hitachi analyzer 7170 (Hitachi, Rili, Japan). Serum IMA was measured by albumin cobalt binding (ACB) test. Serum CRP was analyzed by latex nephelometry method.

The mechanism whereby IMA represents a marker of ischemia is based upon the fact that human serum albumin (HSA) has the ability to bind certain transition metal ions, particularly cobalt and copper, at the N-terminus. Myocardial ischemia produced a lower metal-binding capacity for cobalt to albumin, which led to the development of the recently U.S. Food and Drug Administration (FDA)-cleared ACB test. In principle, cobalt added to serum does not bind to the NH_2 terminus of IMA, leaving more free cobalt to react with dithiothreitol and form a darker color in samples from patients with ischemia (16). According to the capacity of ACB, it reflected the IMA concentrations. Results of IMA was measured by ACB test.

Statistical Analysis

Diagnostic assay data for normally distributed continuous variables are expressed as mean $\pm$ standard deviation (SD) and continuous variables with nonnormal distribution are presented as median and 25–75%

percentile. Categorical data are shown as number (percentage). Differences between the two groups were compared with independent sample tests. Statistical significance was established at the 0.05 confidence level. The cutoffs for IMA and CRP were experimentally determined by receiver operator characteristic (ROC) analysis. The sensitivity and specificity of serum IMA and CRP were assessed by the ROC curve. The areas under the curves were calculated and compared (significance was established at the 0.05 confidence level). Positive predictive value (PPV) and negative predictive value (NPV) were calculated. The SPSS 10.0 (SPSS, Chicago, IL) and MedCalc version 6.0 (MedCalc, Mariakerke, Belgium) statistical software package were used for all calculations.

RESULTS

Of the 113 enrolled patients, 90 were discharged with a final diagnosis of ACS and 23 with the diagnosis of NICP. Table 1 shows demographic and baseline clinical characteristics of the study patients. Among the ACS patients, 37 had AMI and 53 had UA. In the AMI group, 33 patients had $\geq 70\%$ stenosis on angiography and four patients had 50% stenosis. In the UA group, a clinical diagnosis was made in nine cases and 44 diagnosis were based upon objective testing (37 with $\geq 70\%$ stenosis on angiography and seven with a positive exercise stress test). A clear noncardiac cause of chest pain was identified in 15 NICP patients (six had a final diagnosis of gastroesophageal reflux disease, five musculoskeletal chest pain, one pulmonary embolism, two neuralgia, and one had a suspected esophageal rupture). Among the remaining eight NICP patients, five underwent angiography (normal coronary arteries) and three patients had a negative exercise stress test.

At the basal levels the two groups were compared in terms of age, sex, smoking, hypertension, hypercholesterolemia, family history of CAD, and diabetes mellitus ($P > 0.05$). Statistically, there was no significant difference in the case choice between the two groups. However serum samples from the ACS group (n = 90) had decreased capacity of ACB (60.944 ± 6.605 U/mL) compared with the NICP group (n = 23) samples (72 [71–74] U/mL) ($P < 0.05$) . CRP levels were significantly higher in patients with ACS (5.595 [2.23–17.035] mg/L) compared to NICP patients (1.44 [0.73–3.52] mg/L) ($P < 0.05$).

The optimum diagnostic cutoff point for ACB levels in this study population was found to be 70.0 U/mL by ROC analysis (Fig. 1). ACB levels < 70.0 U/mL demonstrated a sensitivity of 94.4% and a specificity of 82.6% (area under ROC curve = 0.948 [95% CI, 0.889–0.981]) for the diagnosis of ACS. The NPV was 79.2%. ACB levels were under the optimum cutoff point on admission in 85 (94.4%) ACS patients vs. 4 (17.4%) NICP patients; $P < 0.05$.)

The optimum diagnostic cutoff point for CRP levels in this study population was found to be 3.16 mg/L by ROC analysis (Fig. 1). CRP levels ≥ 3.16 mg/L demonstrated a sensitivity of 70.0% and a specificity of 73.9% (area under ROC curve = 0.746 [95% CI, 0.655–0.823]) for the diagnosis of CRP. The NPV was 38.6%. CRP levels were above the optimum cutoff point on admission in 63 (70.0%) ACS patients vs. 6 (26.1%) NICP patients ($P < 0.05$.)

DISCUSSION

The present study has shown for the first time that IMA is a sensitive biomarker for the identification of ACS in patients presenting to the ED with acute chest pain. The sensitivity and specificity of IMA for the diagnosis of acute ischemia chest pain are significantly greater than that of CRP. This early prediction by a biochemical marker of ischemia is

TABLE 1. Clinical characteristics of the study group*

	Nonischemia chest pain (n = 23)	Acute coronary syndrome (n = 90)	P
Age (years)	64 ± 11	62 ± 14	NS
Male (%)	14 (60.9)	57 (63.3)	NS
Smoking (%)	4 (17.4)	18 (20.0)	NS
Hypertension (%)	7 (26.1)	45 (56.7)	NS
Hypercholesterolemia (%)	6 (26.1)	43 (47.8)	NS
Family history of CAD (%)	7 (30.4)	32 (35.6)	NS
Diabetes mellitus (%)	1 (4.3)	4 (4.4)	NS
ACB (U/mL)[a]	72 (71–74)	60.9 ± 6.6	0.003
CRP (mg/L)[a]	1.44 (0.73–3.52)	5.60 (2.23–17.04)	< 0.0001

*Data are expressed as mean $\pm$ SD or number (%).

[a]Data are expressed as median (interquartile range).

NS, not significant; CAD, coronary artery disease; ACB, albumin cobalt binding; IMA, ischemia-modified albumin; CRP, C-reactive protein.

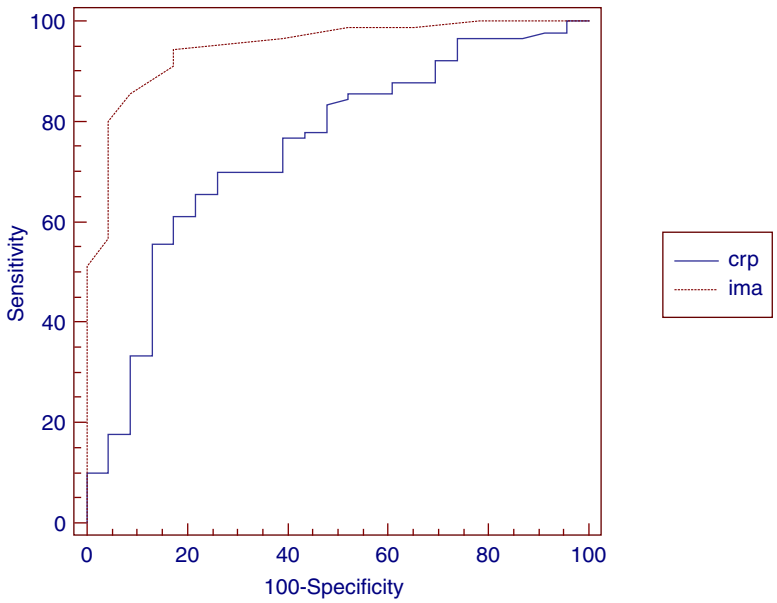

Fig. 1. ROC curves of IMA and CRP for diagnosis of ACS.

important as it may improve our ability to recognize stratify acute chest pain patients and guide therapeutic decisions (12,16).

In this study, both IMA and CRP values were significantly higher in patients with ACS compared with NICP. Our observation also supports the study by others (12,16,17), because an ischemic event may cause as much or more damage to serum albumin and the surrounding tissue as ischemia itself. Biochemical mechanisms involved in the in vivo alterations to metal-albumin binding during either ischemia or reperfusion may include hypoxia, acidosis, free radical damage, membrane energy-dependent sodium and calcium pump disruptions, and free iron and copper ion exposure (18,19). Most of these conditions occur in vivo within minutes after the onset of acute myocardial ischemia.

We found that the ROC plot area of serum IMA was greater than that of serum CRP and the areas under ROC curve of serum IMA and CRP were significantly different ($P < 0.05$). At any cutoff value, serum IMA had higher sensitivity and specificity than serum CRP. These results prove that serum IMA is a better marker than serum CRP for diagnosis of ACS.

This study shows that IMA has high sensitivity for prediction of a discharge diagnosis of ACS, but comparatively low specificity. One of the reasons for this may well be that IMA is detecting ischemia that is subclinical and beyond the ability of conventional diagnostic methods to identify. In addition, we used stringent criteria for a positive angiogram (that is, $\geq 70\%$ stenosis). Therefore a number of patients with lesser stenoses but capable of causing myocardial ischemia were classified as NICP.

These initial results are promising but the study has several important limitations. First, the patient selection probably had a bias in the study group. Second, in our study, IMA results were compared with the final hospital diagnosis, which represented the "gold standard" for the purpose of the study. Final hospital diagnosis resulted from the analysis of all available clinical data—that is, the clinical history, 12-lead ECG, exercise stress test, and coronary angiography. There was no consistent "gold standard" test for myocardial ischemia. Additionally, including both UA and AMI, patients into the myocardial ischemia group may have affected the overall results and precluded any final distinction between transient ischemia and myocyte necrosis. Sampling patient serum for the cobalt-albumin assay only upon arrival in the ED does not take into account the specific elapsed time from the onset of symptoms or kinetics of quantitative assay changes over time. This study did not determine how long after the ischemic event any cobalt-binding changes are detected by the assay. Although the study included only ED patients with chest pain, other noncardiac sources of ischemia could not be conclusively excluded and, theoretically, the assay may not be specific for cardiac ischemia alone.

Despite the limitations of this study, we have shown that IMA has potential as a diagnostic biomarker for ACS. Significantly more ACS patients were recognized at presentation by IMA than by CRP. If present results

are confirmed by further studies, the use of IMA may improve current diagnostic strategies for ACS.

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