Albumin Cobalt Binding (ACB) Test: Its Role as a Novel Marker of Acute Coronary Syndrome

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ABSTRACT

The biochemical marker of myocardial ischemia is detected prior to the development of myocardial necrosis, i.e. a novel biochemical evaluation based on human serum albumin binding to cobalt, a transitional metal. The evaluation is known as Albumin Cobalt Binding (ACB) Test. ACB Test is applied to detect the presence of Ischemia Modified Albumin (IMA), an albumin which has altered binding capacity to bind metal ion such as cobalt (Co), copper (Cu) and nickel (Ni) in N-terminus region. It is produced when the serum albumin convenes with ischemic heart tissues.

ACB Test detecting the presence of myocardial ischemia that occurs prior to myocardial necrosis has been studied by some researchers and they found an ACB increase prior to troponin increase. The cut off point of ACB evaluation was 85 U/ml. Provided that the value was greater than 85 U/ml then there was positive myocardial ischemia. But it should be noticed that IMA increase in the plasma may be due to other tissues such as gastrointestinal tissues or skeletal muscles tissues. We should also consider other factors which may affect the evaluation result such as severe hypoalbuminemia that will cause a false-high result.

ACB Test may be used as an early marker of myocardial ischemia that occurs prior to myocardial necrosis.

Key words: albumin cobalt binding (ACB) test, ischemia modified albumin.

INTRODUCTION

Present and future biochemical markers have an important role, i.e. either for determination or therapy of acute coronary syndrome (ACS) patients. The term of acute coronary syndrome is used for a series of myocardial ischemia, from angina to myocardial infarction. It reflects a physiologic process of acute myocardial ischemia; based on the clinical point of view, it includes a series of risks for patient with a chest pain.1 Based on WHO criteria, during three last decades, acute myocardial ischemia has been determined as myocardial infarction (MI) patient or non-MI patient. The diagnosis of MI is established based on two criteria of these three criteria, i.e. clinical symptoms leading to MI, electrocardiography changes (ECG) and biochemical measures. Clinical symptoms are very important, but there are unspecific symptoms in one-third of cases, especially in diabetic patient and elderly patient, who usually demonstrate atypical symptoms. ECG changes has a low sensitivity which is only about 50%. In the past, biochemical measure had been used as a gold standard, i.e. the activity of CK-MB enzymes. Because of sensitivity limitation, other biochemical marker has been attempted such as myoglobin, cardiac troponin (cTn) T and cTn I. Combination of several biochemical test will increase diagnostic accuracy.1,2,3

Recently, a novel biochemical evaluation has been developed based on human serum albumin binding to a transitional metal, cobalt. This evaluation is called Albumin Cobalt Binding (ACB) test. This test is used to detect the presence of Ischemia Modified Albumin (IMA), an extremely early indicator of myocardial ischemia before necrosis occurred.4,5,6 This article is aimed to discuss about ACB test and its correlation with acute coronary syndrome.

BIOCHEMISTRY ASPECT

Albumin

Albumin is the most abundant protein in serum and it is produced in the liver. Serum albumin level in neonates is approximately 3.9 g/dL. It decreases to 2.8 g/dL at 9 months of age and it is slowly increased until the adult age, i.e. about 3.5 g/dL to 5.5 g/dL.7

As we all know, albumin has two main functions, i.e. to maintain the coloid osmotic pressure of intravascular fluid, and to bind various substance in the blood plasma such as bilirubin, fatty acid, calcium ion, magnesium ion and various drugs.7...
A decrease in albumin serum levels may be caused by several conditions such as lack of amino acid sources in malnourished patients, in liver disease which may inhibit the hepatocyte ability to produce albumin, urinary albumin release in renal disease, albumin release in the interstitial fluid because of inflammation processes and intestinal mucosa disorder.  

Human normal albumin in N-terminus region consists of a sequence of amino acids N-Asp-Ala-His-Lys, which has high ability to bind metallic ion such as cobalt (Co), copper (Cu) and nickel (Ni) as seen on figure 1.  

Ischemia Modified Albumin  
Ischemia-modified albumin is a serum biomarker that can be used to evaluate the presence of ACS rapidly in patients with chest pain, which is assumed as cardiac chest pain. It was found for the first time in 1990 by an emergency doctor who had specially studied a rapid blood test in order to recognize myocardial ischemia. IMA is an albumin which has altered binding capacity to bind metal ion such as cobalt (Co), copper (Cu) and nickel (Ni) in N-terminus region. It is produced when the serum albumin convenes with ischemic heart tissues. During ischemia, there are several chemical reactions that alter albumin into IMA. IMA is produced continuously during ischemia, which means that IMA blood level will change rapidly.  

In patients with low-risk ACS the IMA level is low, while in the high-risk patient the IMA level is high. Hence, ischemic patient will have a higher IMA level than the non-ischemic patient.  

Iskemia modified albumin may be used as an early marker of myocardial ischemia that occurs before myocardial necrosis or myocardial infarction. Until now, to detect the presence of IMA, we use the cobalt metal ion through the ACB test. This was reported by Bar-or, et al. who demonstrated that IMA was rapidly increased as a response against transient ischemia caused by balloon angioplasty.  

Negative or normal IMA level might be used to predict a negative result of troponin test. In other word, negative IMA level may be applied to rule out acute myocardial infarction (AMI).  

Biochemical changes in ACS  
There is a series of biochemical changes after coronary artery obstruction. It is caused by decreased oxygen supply to myocardial tissues and inability to eliminate metabolic waste. These changes depend on the severity of ischemia. The time needed by the tissues to suffer an irreversible damage or necrotic tissues is indeterminate and it varies in every person.  

Lack of oxygen (anoxia) will rapidly decrease the energy supply, reducing unnecessary energy expenditure process, and promote the cell to find alternative energy (anaerobic metabolism, glycogenolysis, gluconeogenesis). Failure of the ATP-ase ion pump causes ion leakage, especially potassium ion.  

Negative blood flow will also disturb the metabolite clearance produced in normal cell. Therefore, there is metabolite accumulation including organic phosphor, lactate, adenosine and hydrogen ion.  

Changes that occur in the first few minutes are still reversible, for instance, obstruction clearance and blood flow reperfusion will bring back the normal cell function. Therefore, small sized ion and metabolites will rapidly pass from intracellular fluid to interstitial fluid. But a prolonged obstruction will cause irreversible damage. It will be noticed by macromolecule release such as enzyme and protein. In contrast, macromolecule clearance will be slower because larger molecules need lymphatic drainage and it will be released only in irreversible damage. (Figure 2)  

Biochemical Marker for ACS  
The history of cardiac markers usage for acute coronary syndrome can be seen on figure 3.  

The first biochemical marker for IM diagnosis is aspartate aminotransferase (AST), which was initially used in 1954. This measure was substituted by creatine kinase (CK) in 1965. After development of electrophoresis, CK isoenzyme and Lactate dehydrogenase (LDH) was
approved with higher specificity than CK. It was in 1975 that CK-MB immunoinhibition method and myoglobin radioimmunoassay (RIA) method were developed. IM diagnosis criteria of WHO were determined in 1979. This criteria included enzyme evaluation i.e. CK-MB as one of three IM diagnosis criteria. In 1985, the first CK-MB antibody was primarily used, and recently almost all of commercial CK-MB immunoassay kit uses this kind of antibody. Troponin T (cTn T) for IM marker was introduced in 1989, while Troponin I (cTn I) was introduced 1992. The application of cTn T for UA was initially introduced in 1992, cTn I was initially applied in 1994. Both of them are useful for risk stratification through a large study reported in 1996.13

Prerequisites for the ideal cardiac marker include: evenly distributed in all of cardiac tissues, high cardiac specificity, high sensitivity and high precision, rapidly found after a heart attack and well-developed in clinical trial.14

As we have known, ACS and development of thrombus are the basic pathophysiology of Acute Myocardial Infarction (AMI). Elderly patient or patient with endocrine and metabolic disorder who has asymptomatic ACS may be classified as silent myocardial ischemia. Symptomatic ACS patient with chest pain may be classified as angina pectoris. Patient with chest pain at rest may be classified as UA, which is an acute syndrome correlated to plaque tearing, platelet aggregation, white thrombus, and the development of red thrombus. Unstable angina correlated with total obstruction of coronary artery will develop MI.12

An accurate diagnosis and therapy of UA patient will decrease the risk of MI development or will lessen the morbidity and mortality rate. Diagnosis strategy and risk stratification to predict prognosis require an adequate measurement (in this case is biochemical marker). Recently, biochemical marker of ACS is applied in UA and MI diagnosis, in risk stratification, as well as in the follow up of post-therapy reperfusion. Furthermore, it also has an important role in ACS series, i.e. from detection of tearing plaque, presence of vascular inflammation, myocardial ischemia to acute condition such as UA or MI.12

**IMA TEST**

The method of IMA test was primarily introduced by an emergency doctor in early 1990’s using ACB test with colorimetric method. This test was simple enough and has a relatively fast result.5,6

**Albumin Cobalt Binding (ACB) Test**

Albumin Cobalt Binding (ACB) test is a quantitative diagnostic evaluation in vitro on human serum used to detect the presence of IMA in plasma by measuring the albumin binding ability to bind Cobalt metal. This test has been developed by Ischemia Technologies Incorporate, Denver, Colorado. According to the American Food and Drug Administration (FDA), this test is the newest laboratory test on human plasma to evaluate the heart attack since troponin test has been introduced, i.e. a protein which appear in blood plasma after heart attack in 1994.5,6

This test is based on observation of decreased affinity of cobalt ion in N-terminus region of human albumin. Changes in ACB test may be found in a few minutes after transient occlusion and coronary artery reperfusion during angioplasty and it will become normal in 6 hours period.5,6,10,12
Principles of The ACB Test

Human serum albumin, the main protein in blood circulation, will become an Ischemia Modified Albumin (IMA) when it is convened with ischemic heart tissues. This kind of albumin is unable to bind metal Cobalt on its N-terminus region. Hence, when metal cobalt is added to a plasma containing IMA, metal cobalt is not bound to IMA. The level of cobalt which is not bound to albumin describes the level of IMA in blood plasma.\(^5,6\)

Material Collection and Stability

Blood sample is collected into a vacuum tube with or without lithium heparin anticoagulant. In 2 hours, a centrifuge with the speed of 1000 G for 10 minute is performed. After that, the heparin serum or plasma is withdrawn from the blood cells. The heparin serum/plasma heparin is stored in 2-8\(^\circ\)C for 2 weeks maximum. If there is any delay of evaluation, the sample may be frozen in –20\(^\circ\)C for years. The frozen sample which has been thawed should not be re-frozen.\(^4\)

The Normal Value of IMA

The suggested normal value of IMA by using ACB test is < 85 U/mL. Any result greater than that value is claimed as myocardial ischemia.\(^4,11\)

The Test Limitation

Although circulated IMA is derived from heart tissues, there are other causes that may increase the IMA blood level such as any disorder that may cause ischemia in other tissues, i.e. gastrointestinal or skeletal muscles. This is revealed by increased IMA levels 24-48 hours after a marathon race. Another factor that may affect the result of ACB test is severe hypoalbuminemia condition, which will cause a false-high result. Those conditions should be consider when we read the blood level of IMA between lines.\(^10\)

THE ROLE OF ACB TEST

Determination of myocardial ischemia prior to cell necrosis is an important measure to conclude any decision of further management. So far, the diagnosis of myocardial ischemia difficults and there is no standard criteria of verification. Therefore, the diagnosis of myocardial ischemia is depend on individual competence of the examining physician, who needs clinical judgment and additional aid such as ECG, echocardiography and laboratory evaluation.\(^14\)

Without any ECG abnormality, it will be difficult to have an early diagnosis of myocardial ischemia shortly after clinical symptoms occurred, especially because there is no reliable blood evaluation for it.

Increase of ACB level in ischemia prior to cell necrosis can be verified in patient with transient occlusion of coronary artery after an elective PTCA.\(^11\) Bagavan, et al.\(^15\) demonstrates that there is an increase of ACB level before the troponin level increased in patients with myocardial ischemia.

According to Cristensen, et al.\(^4\) The ACB test may be applied to detect any myocardial ischemia prior to necrosis. The normal value of present study is 80.2 unit/mL, with 83% sensitivity and 69% specificity in 256 emergency patients who had been included in ACS category. The present study was conducted in four different sites using the Cobas mira plus and Cobas fara. We found an average CV value of 7.3%, with a range CV value of each institution about 6.0-8.7%.\(^4\) (Table 1)

For patient with a chest-pain complaint in the emergency room, who has a low ACB level, negative troponin level and normal ECG, it can be ascertained that there is no myocardial infarction so that the patient requires no longer treatment.

CONCLUSION

Acute coronary syndrome is a manifestation of ischemic heart disease, including stable angina, unstable angina, non-Q-wave MI and Q-wave MI. The diagnosis of MI is established based on two criteria of these three criteria, i.e. clinical symptoms leading to myocardial infarction; electrocardiography changes (ECG) and

### Table 1. ACB Test in Several Institutions\(^4\)

<table>
<thead>
<tr>
<th>Institution</th>
<th>The number of ACS patient</th>
<th>The number of control patient</th>
<th>CV (%) ACB test</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hennepin country medical center</td>
<td>63</td>
<td>27</td>
<td>6.0</td>
<td>Cobas fara</td>
</tr>
<tr>
<td>Minneapolis, MN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hartford hospital</td>
<td>84</td>
<td>47</td>
<td>8.7</td>
<td>Cobas fara</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University of Tennessee</td>
<td>65</td>
<td>32</td>
<td>7.1</td>
<td>Cobas mira plus</td>
</tr>
<tr>
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<tr>
<td>University of Maryland</td>
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<td>50</td>
<td>7.4</td>
<td>Cobas mira plus</td>
</tr>
<tr>
<td>Baltimore, MD</td>
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biochemical measures. To date, the biochemical measures of myocardial infarction include utilization of enzyme activity CK-MB, cardiac troponin (cTn) T and cTn I.

ACB test can be used as a marker of myocardial ischemia early diagnosis prior to the necrosis.

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