Eight million patients with chest pain present annually to emergency departments (ED).\(^1\) Five million of this group are judged to have suspected acute coronary syndromes (ACS) and are admitted to the hospital. Less than half ultimately are found to have a cardiac diagnosis, causing costly, unnecessary admissions. Three million of this group are discharged from the ED each year, and 40,000 of these are inadvertently discharged as myocardial infarctions (MI). Mortality rates of missed diagnoses in patients sent home are 2-fold greater than those patients admitted.

Thus, there is a considerable amount of clinical interest and research to identify markers of myocardial ischemia that could be monitored during the early, reversible stage of ACS to assist in the appropriate triage of patients presenting with symptoms suggestive of ACS.
IMA and the ACB Test

Currently, cardiac troponin, a marker of myocardial cell necrosis, is the biomarker defined by the ESC/ACC/AHA as the “standard” for detection of myocardial injury. Further, in the clinical setting of ischemia, an increased cardiac troponin is the basis of the diagnosis of MI. However, since it may take 2 to 6 hours before cardiac troponin becomes increased above a predetermined reference limit in the circulation, the quest to identify an early and ideally, a cardiac tissue-specific marker of myocardial ischemia, continues. The albumin cobalt binding test, which measures ischemia modified albumin, is the first FDA-cleared assay to attempt to detect myocardial ischemia.

The observation that serum albumin in patients with myocardial ischemia produced a lower metal binding capacity for cobalt than serum albumin in non-ischemic normal controls lead to the development of the recently FDA-cleared ACB test. The ACB test is a quantitative assay that measures IMA in human serum. In principle, in serum of patients with ischemia, cobalt added to serum does not bind to the N-terminus of IMA, leaving more free cobalt to react with dithiothreitol and form a darker color. At present, the assay is configured to be measured on the COBAS Mira Plus instrument (Roche Molecular Systems, Pleasanton, CA) with an absorbance read at 500 nm. Protocols are being developed on additional instruments using the colorimetric assay, as well as investigations into the use of an immunoassay technology. Specific pre-analytical requirements need to be followed, including: avoiding use of collection tubes with chelators, performing assay analysis within 2.5 hours or freezing at <20°C, and avoiding sample dilutions. In addition, ACB test results should be interpreted with caution when serum albumin concentrations are <2.0 g/dL or >5.5 g/dL. Because of specificity issues, increased IMA values may be found in patients with cancer, infections, end-stage renal disease, liver disease, and brain ischemia. The analytical characteristics of the assay appear to be good, with no known drug interferences, acceptable assay total imprecision (<9% CV) at the medical decision cutoff, a lower limit of detection at 14 U/mL, and linearity to 200 U/mL. Expected (normal) values determined from a population of 283 healthy subjects ranged from 52 to 116 U/mL, with a 95th percentile at 85 U/mL.

Clinical Studies

In this paper, we will review several clinical studies that have evaluated the performance of the ACB assay in cardiac patients. Studies were typically performed in small numbers of patients with various study designs. Consequently, published data is preliminary and needs to be confirmed in larger trials. The goals of these studies have been to demonstrate that the ACB assay is a sensitive, early marker of reversible and irreversible cardiac ischemia, and to demonstrate that IMA increases before markers of myocardial necrosis (cardiac troponin). However, many questions remain unanswered. The ACB test needs to be evaluated by incorporating it into decision-making algorithms under emergency department conditions. The highest expected benefit of the test would be to rule out ACS, in low to moderate pre-test probability conditions with negative necrosis markers and a negative ECG. This was the language for which the ACB test was cleared by the FDA for clinical use.

T1 summarizes the clinical studies reviewed. In the first descriptive clinical study published, IMA was measured in 99 patients with cardiac chest pain and 40 patients with non-cardiac chest pain. Samples were collected within 4 hours of presentation to the ED in patients with the primary complaint of chest pain. Ninety-five of 99 patients with cardiac chest pain had increased IMA levels. Thirty-seven out of 40 patients with non-cardiac chest pain had normal values. However, no other biochemical markers or ECG status were reported at the time of presentation for comparison.

A multicenter study involving 224 ED patients with signs and symptoms...
Summary of Clinical Studies Measuring IMA by the ACB Assay

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Setting</th>
<th>Number of Patients</th>
<th>NPV for Ischemia</th>
<th>PPV for Ischemia</th>
<th>Outcome Measure</th>
<th>Type of Study</th>
</tr>
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<tbody>
<tr>
<td>Bar-Or, 2000&lt;sup&gt;2&lt;/sup&gt;</td>
<td>ACS</td>
<td>139</td>
<td>NA</td>
<td>NA</td>
<td>Final diagnosis of ischemia</td>
<td>Prospective enrollment</td>
</tr>
<tr>
<td>Christenson, 2001&lt;sup&gt;7&lt;/sup&gt;</td>
<td>ACS</td>
<td>224</td>
<td>96%</td>
<td>33%</td>
<td>cTnI at 6-24 hours</td>
<td>Prospective enrollment</td>
</tr>
<tr>
<td>Bar-Or, 2001&lt;sup&gt;9&lt;/sup&gt;</td>
<td>PTCA</td>
<td>54</td>
<td>NA</td>
<td>NA</td>
<td>Change in ACB assay value after procedure</td>
<td>Prospective enrollment</td>
</tr>
<tr>
<td>Bhagavan, 2003&lt;sup&gt;6&lt;/sup&gt;</td>
<td>ACS</td>
<td>167</td>
<td>91%</td>
<td>92%</td>
<td>Final diagnosis of ischemia</td>
<td>Retrospective enrollment</td>
</tr>
<tr>
<td>Sinha, 2003&lt;sup&gt;10&lt;/sup&gt;</td>
<td>PTCA</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>Change in ACB assay value after procedure</td>
<td>Prospective enrollment</td>
</tr>
</tbody>
</table>

The inflation of a balloon during angioplasty causes transient myocardial ischemia in humans. Based on previous reports demonstrating that percutaneous transluminal coronary angioplasty (PTCA) can be used as an in vivo model of mild transient myocardial ischemia in humans, a fourth study examined 41 patients undergoing elective PTCA. The ACB test, creatine kinase MB (CK-MB), myoglobin, and cTnI were monitored before, immediately after, and 6 and 24 hours after PTCA. During the procedure, 37 of 41 patients had stents placed, and 33 had signs, symptoms, and/or ECG signs of ischemia. Thirty-four of 41 patients had increased IMA values immediately after the procedure, representing a 10.1% mean difference for IMA concentrations between those with and without a change. There was no significant mean percent change of IMA from baseline at either 6 or 24 hours, however. There also was no significant difference between the patients who did and who did not have signs and symptoms of ischemia during the procedure. Myoglobin, CK-MB, and cTnI showed no changes immediately after the procedure, but at 6 and 24 hours there were significant IMA increases. However, no patients showed an increase above their respective upper reference limits. Further, there was no correlation between ACB test results and the other biomarkers. In addition, a control group of 13 patients were also tested. These patients had coronary angiography without angioplasty and stenting. None had symptoms and signs of ischemia during the procedure and there were no significant mean percent changes from baseline for any biochemical markers. This study demonstrated that changes in IMA occurred minutes after the onset of transient ischemia and returned to baseline at 6 hours in the PTCA setting. No samples were taken, however, between the procedure and 6 hours post-procedure. Thus, kinetic data, which were not demonstrated in this study, are needed for a better understanding of the mechanisms of IMA formation, release, and clearance.

In a study by Sinha,<sup>10</sup> IMA and cTnT were compared before, immediately after, 30 minutes after, and 12 hours after elective PTCA. The study group consisted of 19 patients who had >70% single vessel disease, and all of whom had chest pain and/or ischemic ECG changes during the procedure. Stents were deployed as required. Ischemia modified albumin levels were elevated from baseline (72 U/ml) in 18 of 19 patients immediately after (101 U/ml) and 30 minutes after (87 U/ml) the procedure, and returned to below baseline at 12 hours. None of the patients had cTnT levels above the upper limit of normal. A control group of 11 patients undergoing diagnostic angiography were also included who did not have significant changes in IMA levels. While this study suggests the concept that IMA is an early marker of transient ischemia in the PTCA setting, larger trials are necessary to validate this hypothesis.

In a recent abstract presented at the 2003 meeting of the Society of Academic Emergency Medicine, the utility of IMA in making risk stratification decisions at ED presentation was projected by reviewing 251 records in a prospective design.<sup>11</sup> A risk level was assigned to each patient based on the reviewer’s clinical practice, using demographics, cardiac risk profile, signs and symptoms, ECG, and biochemical marker status. After 2 weeks, risk levels were reassigned, adding the IMA...
concentration measured at presentation into the assessment. The study population was at low risk, with a 10% frequency of ACS. Without knowledge of the IMA result, 66 very-low-risk risk assessments (consistent with expeditious discharge home) were made. With the knowledge of IMA, 236 cases were identified as very low risk. No patients with negative IMA results were found to have ACS demonstrating a NPV of 100%.

The package insert of the ACB assay shows that a combination of the ACB test with a negative cardiac troponin, a non-diagnostic ECG, and appropriate risk stratification may bring about a near 100% negative predictive value. The test seems to have limited specificity, however, with many false positives. There also seems to be considerable overlap between normal and ischemic IMA levels. A positive ACB test result has not been shown to discriminate between unstable angina and early myocardial necrosis, where necrosis markers are not yet elevated. A negative ACB test differentiates effectively these 2 groups from non-cardiac patients with angina-like symptoms. However, additional clinical evidence will need to evolve to support the intended claims.

What We Do Not Know

Ischemia modified albumin, which appears to be an indicator of oxidative stress, may not be specific for cardiac ischemia. There is limited data about IMA levels in non-cardiac ischemia. There is anecdotal evidence suggesting that IMA increases in stroke, end-stage renal disease, and some neoplasms. A group of marathon runners, IMA did not increase immediately after a marathon run, indicating that skeletal muscle ischemia during exercise does not change IMA levels. However, there were significant increases 24 to 48 hours after the run, attributed to exercise-induced latent gastrointestinal ischemia. This latent increase is an issue that may potentially complicate the use of the test in clinical practice. Resolving the influence of fluid shifts and albumin concentration changes that occur following strenuous exercise and other pathologies need to be more fully understood.

Additional studies that are needed for the evolution of this new assay include: normal population distributions by gender and ethnicity; an optimum cut-off value for a high versus normal concentration in ACS patients; comparing IMA levels in common disease states with or without accompanying cardiac disease; and added information in common diseases that coexist with cardiac ischemia, such as congestive heart failure, diabetes mellitus, chronic renal disease, and hypertension. In our opinion, a better understanding of IMA kinetics over the early hours after the onset of an ACS is essential.

Future

Numerous questions remain; specifically, how will clinicians interpret a positive IMA finding. The positive predictive value of the ACB assay seems to be too low for use in ruling in ischemia, a use that clinicians hope the laboratory could provide. It is not known if patients with negative ECG and necrosis markers (cardiac troponin) and a positive IMA result might benefit from early triage and intervention according to stratified pre-test probabilities. In clinical practice, this lack of information can potentially lead to over-treatment of low-risk patients with a positive result. Whether positive results in non-cardiac patients may be associated with significant clinical conditions justifying admission for a more detailed examination also need to be explored.

In summary, IMA measured by the ACB test is proposed as a novel marker of oxidative stress, and sensitive to cardiac ischemia. It has the potential to become a triage tool in suspected ACS patients, especially to rule out ACS, and might also find utility in stroke, stress testing, nuclear imaging, states of non-cardiac ischemia, and oxidative stress. However, it is not highly specific. There is a continued need to improve the evidence base of IMA to substantiate its clinical use, as well as continue the search for a marker that would effectively rule-in as well as rule-out early cardiac ischemia.

The ACB test has only scratched the surface of what exciting challenges and discoveries lie ahead for assisting clinicians in the early detection of myocardial ischemia, to assist and improve patient triage, therapy, and management.