Recrudescence

Ischemia Modified Albumin: A Novel Biomarker for the Detection of Cardiac Ischemia

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Summary: The diagnosis of cardiac ischemia remains a challenge in contemporary emergency medicine. A blood-borne biomarker is an attractive alternative to cardiac imaging or stress testing as it would be cheaper and logistically faster to obtain. A number of candidate biomarkers have been proposed for the detection of cardiac ischemia; however, only Ischemia Modified Albumin (IMA) has been released for clinical use. IMA is a good discriminator between ischemic and non-ischemic patients. Changes in IMA concentration have shown to occur during coronary angioplasty-induced ischemia. Clinical studies indicate that IMA appears to offer on admission an early test which can be combined with electrocardiographic findings and cardiac troponin measurements for the early exclusion of acute coronary syndrome. IMA is an independent predictor of short and long term adverse outcomes in patients with acute chest pain. However, this test is relatively new and uncertainties remain. Elevations of IMA occur in conditions other than chest pain, thus questioning its specificity. The mechanism of IMA formation and the precise entity being measured are not fully known. Nevertheless, IMA measurement remains the only current clinical biomarker which may be used for the diagnosis of patients suspected of cardiac ischemia.

Keywords: Ischemia; Albumin; Biomarker; Ischemia modified albumin; Cardiac ischemia; Clinical study; Oxidative stress; Albumin cobalt binding assay

Introduction

Ischemia occurs when there is a supply demand mismatch in cardiac blood flow. In unstable angina, this occurs due to partial or total occlusion of a coronary artery due to plaque rupture. In stable angina, there is progressive vascular occlusion resulting ultimately in a stenosis of more than 70%, impairing blood flow. If the ischemia is reversible, no myocardial damage occurs. If the ischemia is prolonged, there will be cellular necrosis and myocardial infarction. The interventional challenge for medicine is to be able to identify acutely impaired myocardial perfusion before necrosis has occurred. Currently, the only strategy for this is to detect ST segment changes on the electrocardiogram (ECG). Reperfusion therapy can then be initiated and is life-saving and results in myocardial salvage. Many patients who present with chest pain do not have acute myocardial infarction (AMI). The sensitivity of admission ECG is typically around 50%. There is therefore a need for a strategy to detect ischemia before necrosis occurs and conduct prompt revascularisation. The challenge is to use additional quantitative risk stratification tools as potential biomarkers for ischemia. Currently, three markers have been proposed: choline, free fatty acids and ischemia modified albumin (IMA®). 1) Of these, only ischemia modified albumin is currently available as a licensed test for routine clinical application measured using the Albumin Cobalt Binding (ACB®) assay.

Mechanism of IMA generation

The N-terminal portion of human serum albumin (HSA) is a binding site for transition metal ions such as cobalt, copper and nickel. 2) It is currently not known if there are significant changes in total human serum albumin between ischemic and non ischemic patients in the general chest pain population. Many divalent metals bind HSA in the circulation but in concentrations far lower than that required to impact albumin directly. The N-terminal portion of HSA is susceptible to biochemical degradation and is less stable than the albumin of other species. 3) IMA is a form of HSA in which the N-terminal ami-
Reduced blood flow due to ruptured atherosclerotic plaque results in insufficient oxygen to tissue (1) causing a lower pH (2). Copper (Cu\textsuperscript{2+}) is released from weak binding sites on plasma proteins (3). Metal bound at N-terminus. In the presence of ascorbic acid, Cu\textsuperscript{2+} is converted to Cu\textsuperscript{+} (4). Cu\textsuperscript{+} reacts with oxygen to form superoxide radicals (O\textsubscript{2}\textsuperscript{-}) (5). The enzyme superoxide dismutase (SOD) dismutates the O\textsubscript{2}\textsuperscript{-}, forming hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) (6). Normally, hydrogen peroxide is harmlessly degraded into water and oxygen by the action of a second enzyme, catalase. However, in the presence of metals such as copper or iron, H\textsubscript{2}O\textsubscript{2} undergoes a Fenton reaction, forming hydroxyl free radicals (OH\textsuperscript{-}) (7). Free copper (Cu\textsuperscript{2+}) is scavenged by HSA, on which it binds tightly to the N-terminus. OH\textsuperscript{-} is highly reactive and capable of damaging nucleic acids, lipids and proteins, including albumin. One site of damage is the N-terminus, where OH\textsuperscript{-} alters amino acids (8). Altered albumin is incapable of binding to Cu\textsuperscript{2+}. Bound copper is released from the albumin (9), where it may be taken up again by the N-terminus of another albumin in a chain reaction so that the process of albumin binding and OH\textsuperscript{-} formation is repeated.

Myocardial ischemia to generates free radicals; acidosis develops\textsuperscript{5} and release of free iron and copper ions occurs.\textsuperscript{7,8} HSA is a scavenger for divalent metal ions. It may thus be postulated that in ischemia, these processes may result in change in the ability of the N-terminus to bind to transition metal ions (Fig. 1). The release of these ions likely initiates one potential pathway for IMA generation and thus need not be considered an interference to negatively affect IMA. In support of this suggestion, decreased albumin cobalt binding was reported in patients with chest pain and myocardial ischemia\textsuperscript{9} and following coronary artery angioplasty the baseline was resumed after six hours.\textsuperscript{10} Using nuclear magnetic resonance spectroscopy, high-performance and liquid chromatography combined with mass spectrometry, it was confirmed that the N-terminal aspartate-alanine-histidine-lysine sequence binds to cobalt. Modification of this site by N-terminal acetylation or deletion of one or more residues abolishes this cobalt binding.\textsuperscript{11}

The postulated mechanism is that localised ischemia results in acidosis and the release of copper II from weak binding sites on circulating proteins. In the presence of a reducing agent such as ascorbic acid, free copper II is converted to copper I which reacts with oxygen to form copper II and generate superoxide free radicals. Superoxide dismutase converts the superoxide free radicals to hydrogen peroxide which is then degraded by catalase. Copper II ions released are immediately scavenged by albumin but are tightly bound to the N terminus. Copper bound albumin is then damaged by hydroxyl free radicals, causing removal of the three N terminal amino acids and release of copper II ions to repeat the process in a chain reaction.\textsuperscript{12} This postulated mechanism, although theoretically attractive, has not been borne out in practice. In a study of patients with increased IMA, the N-terminal portion of albumin was sequenced in 8 cases. No evidence of N-terminal degradation or truncation was found.\textsuperscript{13} Recent physicochemical studies of cobalt binding to HSA suggest a different explanation. Three binding sites for cobalt were identified, two of which showed greater avidity than the N-terminal binding site.\textsuperscript{14} Fatty acid binding to albumin occurs at one of the additional cobalt binding sites with negative allosteric interaction. Possibly, in myocardial ischemia the release of fatty acids results in binding of fatty acids to albumin. This should
reduce the ability of albumin to take up cobalt and thus account for the presence of IMA. If this produces a conformational change in the albumin affecting the N terminal site, reduced cobalt binding should occur.

**IMA - A Marker, a Cause of Oxidative Stress?**

There is a wealth of candidate biomarkers used to determine the extent of oxidative stress, including lactate, myeloperoxidase, nitric oxide, free fatty acids, glutathione and thiobarbituric acid reactive substances (TBARS). There is a lack of direct comparison between IMA and oxidative stress markers. Senes and colleagues have demonstrated a positive correlation between IMA and TBARS \((r = 0.276, p = 0.029)\) in patients with acute ischaemic stroke. In the percutaneous coronary artery angioplasty model, IMA is not correlated to lactate concentrations.

Advanced oxidation protein products (AOPP) are generated by the oxidation of plasma proteins and along with carbonyl residues are markers of oxidative protein damage. Correlation of IMA to AOPP and carbonyl residues has been performed in patients with scleroderma. In patients with scleroderma, oxidative stress markers were elevated, but carbonyl residues and not AOPP were correlated with IMA. AOPP is generated via a pro-inflammatory mechanism in neurodegenerative diseases, vascular calcification and renal failure. IMA, however, is not a signal protein and not generated in a pro-inflammatory state alone but is rather an end product of oxidative stress. The association of AOPP and carbonyl residues with respect to cardiovascular disease has not been investigated.

In normal human serum, neither H\(_2\)O\(_2\) nor superoxide anions produced change in IMA *in vitro*. Conversely hydroxyl radicals generated by the Fenton reaction were associated with a rapid rise in IMA. The addition of mercaptopropionylglycine, a hydroxyl radical scavenger resulted in attenuation of IMA. Furthermore, as Co\(^{2+}\) may interfere in ACB assay; Co\(^{2+}\) in the absence of hydroxyl radicals does not alter the concentration of IMA.

Like IMA, oxidative stress markers lack tissue specificity and therefore their utility as diagnostic markers in the general chest pain population is questionable. Although IMA is implicated in oxidative stress mechanisms, it is further downstream in the pathological process than traditional markers such as those mentioned above and may confer greater cardiac specificity.

**Kinetic Release of Ischemia Modified Albumin**

Studies on patients receiving angioplasty where ischemia is induced in a controlled manner, have indicated the kinetics of IMA production. There is rapid rise in IMA after balloon inflation with subsequent fall at 6 hours and return to normal values by 24 hours. The rise in IMA occurs earlier than rise in cardiac troponin and natriuretic peptides (Fig. 2) The magnitude of IMA elevation has been found correlated with the number and frequency of balloon inflations during PCI, the number of collaterals present, and the need for subsequent revascularisation and to parallel the transmyocardial lactate gradient. Elevation has been recorded following coronary vasospasm. Other studies involving invasive cardiac procedures show rise in IMA where ischemia might occur, occurring concurrently to ECG changes in cardiomegaly, but show variation when there is non-ischaemic myocardial damage such as cardiac ablation.

**Half-life and Clearance of Ischemia Modified Albumin**

The half life of human serum albumin is 19–20 days. If a slightly truncated form is responsible for generating IMA, it would presumably have similar stability properties. IMA however returns to the baseline rapidly after an ischaemic cardiac event. This indicates that alteration in albumin is possibly transient and reversible, rather than a definitive chemical alteration. However IMA may possibly undergo preferential proteolytic degradation. There are no data that focus specifically on IMA clearance apart from in vivo kinetic studies in models of ischemia. This warrants further investigation.

**Clinical Studies using Ischemia Modified Albumin**

Clinical validation of any test for ischemia is difficult as there is no accepted diagnostic gold standard. In addition, there is no predicate test which can be used against which initial validations can be performed. Initial studies on IMA were based on the ability of early measurements to predict a final diagnosis of AMI as defined by cardiac troponin. Two studies utilized the pre-release ACB test, the third an in-house method. The first study examined acute coronary syndrome (ACS) patients and utilized serial sampling on admission and two subsequent samples. Diagnostic sensitivity of the admission sample for a final diagnosis of AMI was 23.9% for cardiac troponin I (cTnI) alone, 39.1% for IMA alone and 55.9% for the two combined. The second study examined 256 ACS patients. The area under the curve (AUC) of the receiver operator characteristic (ROC) curve for the ACB test was 0.78 with a sensitivity and specificity of 83% and 69% respectively at the optimised decision threshold for AMI. The third study was conducted on 75 patients with ischemia and 92 non-ischaemic patients. IMA had poor predictive power in discriminating between AMI and non-AMI in patients with underlying ischaemic heart disease (AUC of 0.66). However, the test gave good discrimination between patients with or without ischemia. AUC for the ROC curve for diagnosis of ischemia was 0.95 with a sensitivity of 94% and specificity of 88%. In these initial studies, there were significant problems with sample stabili-
ty and the assay involved the use of calcium chloride and centrifugation as part of the routine. This made the method unsuitable for routine analysis and the assay was reformulated.

Most patients brought to the hospital with chest pain and suspected of ACS are eventually ruled out for acute myocardial infarction and active unstable coronary disease. The ideal role of an ischemia marker would thus be as a rule out test. The most logical place to use such a test is in the Emergency Department (ED). A study on ED presentations examined 208 patients and the diagnostic sensitivity of IMA measurement alone was 82% at 46% specificity in samples taken within the first 3 hours. A combination of ECG, cardiac troponin T (cTnT) and IMA showed 95% sensitivity for diagnosis of ACS at presentation.28) One year follow up on this population demonstrated a survival disadvantage in patients with IMA greater than the median concentration of the study group (Fig. 3).29) In a subsequent study on 538 patients admitted for chest pain evaluation admission measurement of IMA plus cTnT indicated 100% sensitivity for prediction of a final diagnosis of AMI.30) The presence of elevated IMA and elevated cTnT on admission predicted 21% risk of major adverse cardiac events (MACE) compared to patients for whom both were not elevated, even in patients where the final diagnosis excluded AMI by troponin based criteria. IMA measurement appears to work best as part of other tests or a test sequence.31) Admission measurement of IMA has been found superior to biomarkers of necrosis and to show 97% sensitivity when combined with them. Not all investigators consider the diagnostic performance of IMA either alone or in combination with cardiac troponin, or other biomarkers of necrosis, to be adequate. A prospective ED study on 277 patients using a positive IMA or troponin as the index test and 8 hour troponin as the definitive test found only a 97.6% sensitivity with 97% negative predictive value. This was not considered adequate compared to troponin but no follow-up data were provided.32) A second large study prospectively on 189 patients presenting ED with chest pain indicated elevated IMA to be a poor predictor of cardiac events within the next 72 hours.33) Conversely, another study found elevated IMA to predict long-term cardiac events.34) The most consistent finding in all studies of IMA was a high negative predictive value. This has been highlighted in a recently published meta-analysis specifically examining the role of IMA as a rule out test.35)

**Measuring Ischemia Modified Albumin**

Measurement of IMA is done by albumin cobalt binding (ACB®) assay (Inverness Medical, Stockport, UK). The assay measures cobalt binding capacity of albumin in a sample. A known amount of cobalt is added to a patient serum sample. Dithiothreitol (DTT) is added which binds to any remaining unbound cobalt and colorimetric
change is measured spectrophotometrically. In serum from non-ischaemic patients, cobalt binds to the N-terminus of HSA, leaving little cobalt to react with DTT to form a colored product. Conversely, in serum of patients with ischemia, cobalt does not bind to the N-terminus of modified HSA, leaving more free cobalt to react with DTT to produce a darker colour. As normal albumin binds to cobalt, the amount of free cobalt and hence absorbance will be proportional to IMA present.

The stability of IMA has been shown to be two hours at 4°C or 20°C, but values increase significantly after four hours irrespective of storage temperature. It is likely that change is due to in vitro pH change that would alter the metal binding capacity of albumin. Samples frozen at −20°C are stable although values have been reported slightly higher compared to freshly analysed samples.

A reference range study on 109 subjects (55 men and 54 women; age range, 20–85 years) to determine the 95th percentile reference range for IMA has been performed based on the first generation of the assay. Further studies on healthy subjects report higher IMA ranges. The biological variation of IMA has been studied. Gender differences in IMA amongst healthy individuals do not occur but IMA is statistically different between Caucasian and Black populations.

Albumin values may be expected to affect IMA measurement. There is a relationship between IMA and serum albumin levels although this is much less marked across the reference interval for albumin. The use of an albumin adjusted correction has been proposed although a reference interval study found albumin correction to have little impact compared to other analytical factors.

Changes in IMA observed in patients with chest pain may be attributable only to changes in the albumin concentration.

Assessment of the Cardiospecificity of Ischemia Modified Albumin

The specificity of IMA has been studied in clinical situations (Table 1). Studies of patients with skeletal muscle ischemia have produced contradictory results. In healthy subjects undergoing arduous physical exertion, IMA has been reported to fall immediately post exercise and then subsequently rise or return to normal. Subjects undergoing a forearm ischemia test (forearm muscles are exercised for 1 minute with blood supply interrupted by external compression) showed a fall in IMA, maximal at 3 minutes from the test, returning to baseline by 30 minutes. Similar rise in serum lactate occurred. Conversely, during calf muscle ischemia, a rise in IMA, peaking at 30 minutes occurred. Peri-operative skeletal muscle ischemia induced by a tourniquet was followed by rise in IMA. Patients with peripheral vascular disease (PVD) undergoing a treadmill walk test demonstrate decrease in IMA immediately post test, as do patients undergoing exercise electrocardiography with a fall at peak exercise and then subsequent rise. However, revascularisation for PVD is accompanied by post procedural rise in IMA. In skeletal muscle ischemia, an initial fall with subsequent rise appears a consistent finding without adequate explanation. Smooth muscle ischemia does not appear associated with rise in IMA.
the application of IMA after cardiac stress testing for detection of myocardial ischemia and may explain the inconsistent findings.42,52,55,56)

There have been reports of elevated IMA in under other conditions where cardiac ischemia and troponin elevation may also occur, including acute stroke,5,57,58) pulmonary embolus,59) polytrauma,60) end stage renal disease,61–63) vascular53) and non-vascular surgery.64) IMA may also be of benefit in hypercholesterolaemic patients where IMA is correlated to cholesterol, low density lipoprotein (LDL) and antibodies to oxidised LDL.7) IMA may offer diagnostic potential for nomolipidaemic AMI patients.65)

Patients with type 2 diabetes mellitus who demonstrate poor glycaemic control have higher IMA concentrations than those with good glycaemic control.66) More intriguingly, IMA elevation occurs in obstetric conditions associated with placental ischemia.67–69) IMA elevation may not be specific for cardiac ischemia, but there are also a large and ever increasing number of clinical conditions apart from ACS where cardiac troponin is elevated.70)

Ischemia Modified Albumin - The Way Forward

The mechanism of IMA generation remains unexplained. It has been suggested that IMA is in fact a marker of oxidative stress as conditions associated with raised IMA may be associated with other markers of oxidative stress.15,17) Unfortunately such markers may also be associated with elevated markers of cardiac damage and dysfunction.64)

Despite this, IMA currently remains the only ischemia assay to have reached the clinical validation stage that is CE marked and FDA approved for the diagnosis of cardiac ischemia. There are a number of remaining issues which should be researched. The mechanism for generation should be the subject of future research. The assay needs to be studied combined with recently developed ultra-sensitivity cardiac troponins but the lack of cardiосpecificity remains the biggest barrier for clinical use. IMA may be enormously valuable to the emergency physician assessing chest pain patients but we require a better understanding on this marker before it is ready for prime time use.

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