Provisional chapter

Biomarkers of Cardiac Ischemia

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Additional information is available at the end of the chapter

1. Introduction

Ischemia (from the Greek ισχαιμία, *ischaimía*; *isch-* root denoting a restriction or thinning or to make or grow thin, *haema* blood) is the restriction of blood supply and thus the inadequate delivery of oxygen and removal of carbon dioxide from cellular tissue. This imbalance may lead to dysfunctional or permanent damage to the affected tissue and organ.

Cardiac ischemia occurs when there is a supply versus demand mismatch in coronary blood flow. In patients who present with unstable angina, ischemia occurs due to partial or total occlusion of a coronary artery due to plaque rupture. In stable angina however, there is progressive vascular occlusion resulting ultimately in a luminal stenosis of greater than 70%, impeding blood flow to the distal tissue. If the ischemia is reversible, no permanent myocardial damage occurs.If however the ischemic episode is prolonged; there will be cellular necrosis which will lead to acute myocardial infarction (AMI).The immediate clinical challenge is to be able to identify acutely impaired myocardial perfusion before the necrotic process starts. Currently, the only strategy for this is to detect ST-segment changes on the electrocardiogram (ECG), however the ECG is non-diagnostic in many cases. The sensitivity of the admission ECG for the diagnosis of AMI is typically around 50%. Reperfusion, be it pharmacological or surgical, is the essential life-saving intervention with the aim of salvaging myocardial tissue localised at the affected site. Many patients however who present with chest pain to the emergency department (ED) do not have a final diagnosis of AMI. There is therefore a need for a strategy which could detect cardiac ischemia before necrosis occurs and result in prompt revascularisation. Blood borne biomarkers for ischemia may be of diagnostic and prognostic value.

To date, a number of candidate biomarkers of ischemia are being researched. However, one, Ischemia modified albumin (IMA®), has been developed into a commercially available cardiac biomarker assay and licensed for routine clinical application both by CE marking in Europe

and Food and Drug Administration (FDA) approval in the United States. This chapter will explore the rational for the necessity of cardiac ischemia biomarker testing and detail the development of the IMA assay with emphasis on its clinical and prognostic utility.

1.1. The cardiovascular disease epidemic

Cardiovascular disease (CVD) accounts for the majority of global deaths. CVD was responsible for 29% of all global deaths in 2004. According to the World Heart Federation, CVD is responsible for 17.1 million deaths globally each year. Surprisingly, 82% of these deaths occur in the developing world. Such numbers are often difficult to comprehend. CVD is responsible for one in every five deaths disease kills one person every 34 seconds in the USA alone. 35 people under the age of 65 die prematurely in the United Kingdom every day due to CVD. It is predicted that by 2030 23 million people will die annually from a cardiovascular related disease. Data from the USA suggests that CVD was responsible for 34% of all deaths in 2006 and over 151,000 Americans who died were under 65 years of age.

1.2. Acute chest pain

Patients with chest pain constitute the largest single category of patients admitted to hospitals in the UK [1]. In the USA, registry data recorded 11.2 million chest pain presentations to the ED in 2008 alone. The presentations are also diagnostically challenging. The majority of admissions have either stable ischemic heart disease (IHD) or no ischemic heart disease [2]. Such admission episodes are often short and clinically inappropriate. Conversely, it has been estimated that between 2 and 7% of patients with AMI are inappropriately discharged from the ED [3, 4] and suffer disproportionate morbidity and mortality. Attempts to improve diagnosis have included risk scoring systems [5], computerised decision support [6, 7] and automated ECG interpretation [8]. Although clinical assessment remains integral to the assessment of patients with chest pain, cardiac biomarker measurement has become an essential component in the diagnostic armamentarium.

2. Pathophysiology of cardiac ischemia

The mechanisms involved in the development of cardiovascular disease are multifactorial and include abnormalities in cholesterol and lipid metabolism, inflammation and oxidative stress processes within the vascular wall, cellular disruption to the endothelium and intra-lumenal platelet activation/aggregation. The ischemia cascade from initiation of local ischemia to the development of symptomatic chest pain is depicted in figure 1.The pathological processes responsible for the development of atherosclerotic lesions and endothelial dysfunction are advanced far earlier than when patients typically become symptomatic and present with chest pain. The disease process does not occur in distinct episodes but rather is a continuum from asymptomatic vascular dysfunction thorough to angina in those with myocardial ischemia, which, without intervention can progress to non-ST segment elevation myocardial infarction (NSTEMI) or cumulate into ST segment elevation myocardial infarction (STEMI). Patients

presenting at any stage in the process may be diagnosed with acute coronary syndrome (ACS). The earlier in the disease continuum the presentation is; the greater the opportunity for successful myocardial tissue preservation. As there is no definitive biomarker for ischemia, current treatment focuses on the need for urgent therapeutic revascularisation in patients with established cardiac necrosis, identified by the cardiac troponins.

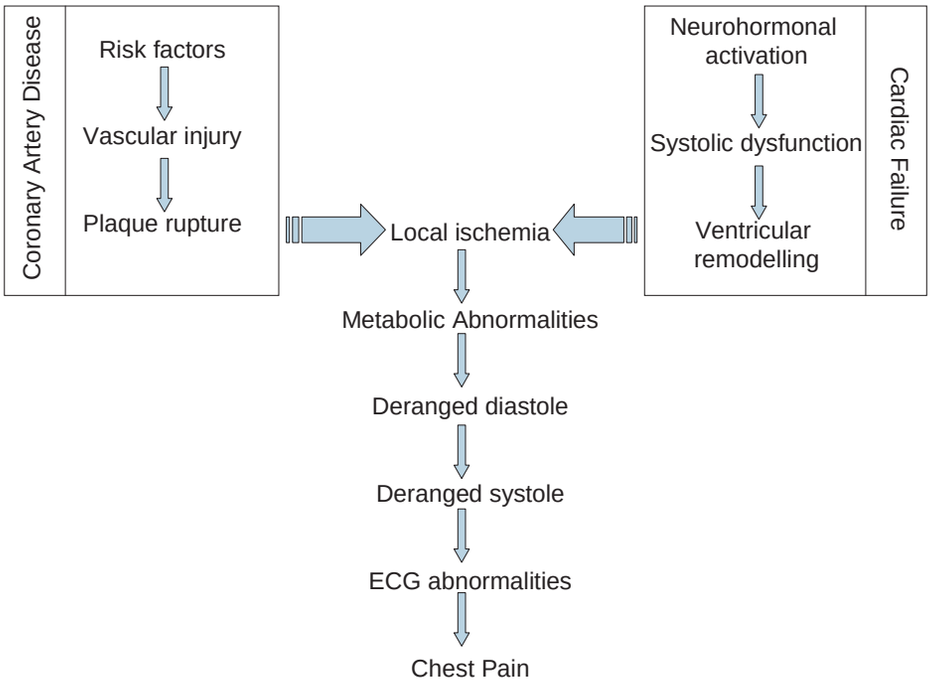

Figure 1. Development of Ischemia in the ischemia cascade.

The pathological process unless disrupted by therapeutic intervention results in the death of cardiac myocytes. Predisposing this terminal event, a vulnerable atherosclerotic plaque becomes disrupted exposing the thrombogenic lipid core and sub endothelium to the luminal milieu. Exposure results in platelet activation and aggregation and along with the coagulation cascade, an intracoronary thrombus forms. The thrombus may not obstruct the lumen and the patient is asymptomatic, however if the lumen is totally occluded, AMI will ensue. A partially occluded lumen and reduced oxygen supply both contribute to the development of ischemic myocardium.

3. Clinical detection of cardiac ischemia

The clinical presentation of cardiac ischemia is difficult to definitively diagnose. Currently there is no gold standard test to detect ischemia however a number of reliable tests exist.

Historically patients were admitted for monitoring or discharged on the basis of clinical interpretation by the ED physician. It is accepted that this is no longer acceptable clinical practice.

The typical presentation is exertional or stress induced central chest pain. These episodes usually last from a few minutes to hours and can resolve upon rest. Common descriptions by the patient include tightness, crushing stabbing or burning pain. Patients may also have nausea and vomiting, dyspnoea, palpitations. Typical symptoms increase the likelihood of an AMI however atypical presentations cannot be used to exclude AMI. Women, the elderly and those with diabetes mellitus often present with atypical chest pain.

The clinical history and physical examination will assess the presence of risk factors for AMI, however alone; the initial clinical examination is insensitive and unspecific for diagnosis. It may however give insight to differential or alternative diagnoses in those patients who, upon further investigation do not have an AMI. The 12 lead ECG is additive to the physical examination. The majority of ECG traces performed at admission are non-diagnostic with approximately 5% of suspected AMI patients having a diagnostic trace indicative of AMI. Although the ECG is relatively insensitive, the presence of ST segment elevation however is 100% diagnostic for AMI and serves as the criterion for immediate induction of fibrinolytic therapy or emergency interventional revascularization.

3.1. Cardiac imaging

Recently cardiac imaging has played an important role in the detection of ischemia. Perfusion abnormalities can be detected by single-photon emission computer tomography (SPECT) myocardial perfusion imaging (MPI) and mechanical dysfunction can be detected by echocardiography or gated MPI. Gated SPECT MPI can identify regional and global dysfunction of the left ventricle as ischemia impairs myocellular contractility. SPECT requires uptake of an isotope by active membrane transport mechanisms and caution should be advised in those patients with impaired renal clearance. Both echocardiography and SPECT are sensitive and specific and have a high negative predictive value for the diagnosis and prognosis of patients with suspected ACS. These diagnostic modalities however are grossly expensive, time consuming, technically more challenging and are not as widely available as compared to the simple ECG or a blood borne biomarker. The use cardiac imaging in the ED on a 24 hour, 7 day a week basis is therefore compromised.

3.2. Cardiac biomarkers of ischemia

There have been progressive developments within basic and clinical research to identify candidate biomarkers of ischemia and to develop simple to use assays. Any such assay needs to have similar analytical (limit of detection, precision, reference intervals) and clinical performance (sensitivity, specificity, risk stratification and predictive value) compared to that of markers of necrosis, such as high sensitivity cardiac troponin assays. A number of candidate biomarkers have been identified. However very few make it from a research grade assay to a fully licenced automated assay for clinical use. The most promising biomarkers to date are

reviewed below. Of these, Ischemia Modified Albumin has been the most successful biomarker and greater attention is given to this marker.

4. Malondialdehyde low density lipoprotein

Malondialdehyde low density lipoprotein (MDA-LDL) is a sensitive biomarker for ACS patients with unstable angina and AMI. MDA is a candidate compound which causes oxidative modification of LDL. MDA (propanedial, $C_3H_4O_2$) is a reactive aldehyde produced by degradation of polyunsaturated lipids or released during prostanoid metabolism. This reactive oxygen species causes oxidative modification to LDL. MDA-LDL reacts with the charged amino group of B-100 protein lysyl residues. Plasma concentrations of MDA-LDL identify patients with coronary artery disease. Modified LDL may also instigate an immune response leading to autoantibody and LDL immune complex production. MDA-LDL not only serves as an oxidative stress marker but as a marker of plaque destabilisation.

5. Myeloperoxidase

Myeloperoxidase (MPO, EC 1.11.2.2, 1.11.2.2) is a 150 KDa protein dimer consisting of two 15 KDa light chains and two variable weight glycosylated heavy chains bound by a heme group responsible for the green colour when secreted in pus and mucus. There are three known isoforms which differ in the size of the heavy chain [9]. It is encoded by the MPO gene located on chromosome 17 [10]. MPO is most abundant in neutrophil granulocytes. It is a lysosomal enzyme stored in azurophilic granules of polymorphonucleocytes and macrophages. MPO catalyses the conversion of chloride and hydrogen peroxide into hypochlorite (hypochlorous acid). Furthermore, MPO oxidises tyrosine to the tyrosyl radical using hydrogen peroxide as an oxidising agent. Both hypochlorite and the tyrosyl radical are cytotoxic and are produce to kill pathogens in response to infection. Elevation in MPO is therefore not indicative of cardiac ischemia, as increases occur in infection, inflammation and infiltrative disease processes, thus reducing the specificity for cardiac ischemia.

MPO may contribute to the pathophysiology of ACS, as the hypochlorite end product is an oxidizing agent of low density lipoprotein (LDL) and may play a key role in the degradation of collagen and contributing to the destabilisation of the plaque. Patients with ACS who have elevated MPO are at risk of short and long-term adverse outcomes. In a case-control study from the USA, Zhang and colleagues demonstrate that MPO concentrations are significantly greater in patients (n=158) with coronary artery disease compared to controls (n=175) who do not demonstrate angiographically significant coronary disease [11]. Plasma MPO concentrations identify patients at risk of major adverse cardiac events in the absence of necrosis. In 604 sequential chest pain admissions, MPO predicted adverse cardiac events (AMI, need for revascularisation or death)at 30 days (odds ratio 2.2, 95%CI 1.1-4.6) and 6 months (odds ratio 4.1, 95%CI 2.0-8.4) [12].

6. Whole blood choline

Choline (2-hydroxy-N,N,N-trimethylethanaminium, $C_5H_{11}NO$) is a water soluble essential nutrient. It is a product of phosphodiesteric cleavage of membrane phospholipids such as phosphatidylcholine and sphingomyelin; catalysed by phospholipase D (EC 3.1.4.4). Choline is the precursor to actylcholine production.

Physiologically choline provides cell structural integrity, is the precursor for acetylcholine production and a source of methyl groups that participate in the S-adenosylmethionine synthesis pathway.

Whole blood (WBCHO) and plasma choline concentrations increase after stimulation of phospholipase D and the activation of coronary plaque cell surface receptors or ischemia. Phospholipase D activation in coronary plaques causes stimulation of macrophage by oxidised LDL, secretion of matrix metalloproteinase enzymes and activation of platelets. WBCHO can be measured by high performance liquid chromatography coupled to mass spectrometry (HPLC-MS). In a study of over 300 patients with suspected ACS, WBCHO measured at admission was a significant predictor of cardiac death, cardiac arrest, arrhythmia, heart failure or the need for percutaneous coronary intervention (PCI) at 30 day follow up [13]. The predictive power was enhanced by the addition of either cTnT or cTnI and served not as a marker of myocardial cell necrosis but identified patients at high risk with unstable angina. WBCHO is therefore a better predictive tool than plasma choline for early risk stratification in patients who are cardiac troponin negative on admission. The current detection methodology using HPLC-MS is not suitable for urgent clinical use.

7. Free fatty acids

Fatty acids are carboxylic acid molecules with a long aliphatic tail known as a chain, which are either saturated or unsaturated. Most naturally occurring fatty acids have an even number of carbon atoms (4 to 28) in the tail region. Fatty acids are produced from the breakdown of triglyceride or phospholipid. The majority fatty acids circulate bound to albumin with a very small percentage appearing as the unbound free fatty acid (FFAu) form [14]. The circulating level of FFA is limited to the availability of the albumin binding sites.

The mechanism of FFAu release is not fully understood however increased catecholamines following cardiac ischemia may activate FFAu release following lipolysis in adipocytes. FFAu are 14-fold higher post-PCI, compared to pre procedural concentrations and were higher in those with associated ischemic ST segment changes [15]. A recombinant fatty acid binding protein bound to a fluorescent tag (ADIFAB) [16;17] has been developed and a second generation assay using a fluorescent molecular probe (ADIFAB2] and a portable reader makes this a potential early marker for the point of care setting. Whilst this marker shows promise in the early phase of ischemia induced ACS, further trials are required to evaluate the diagnosis and prognostic value of FFAu in the chest pain population.

8. Ischemia modified albumin

The NH_2-terminal of human serum albumin (HSA, 66.5 kDa, 585 amino acids) is known to be a binding site for transition metal ions such as cobalt, copper and nickel [18]. Using one and two dimensional 1H-NMR studies, Sadler and colleagues demonstrated binding of Ni^{++}, Cu^{++}, Co^{++}, Cd^{++} and Al^{+++} to bovine and human serum albumin. Strong binding was associated with three N-terminal amino acid residues (Asp-Thr-His in bovine albumin and Asp-Ala-His in human albumin). A Lysine residue designated Lys4 is also involved in the binding site. The authors demonstrated for the fist time selective reduction in the intensities of resonances to the εCH_2 resonance of Lys4 on the addition of Co^{++} to HSA. There are in fact, four metal-binding sites with different specificities in HSA. In addition to the NH_2-terminal, three other sites occur at (i) reduced cysteine at residue Cys34, (ii) site A, including histidine at His67 as a ligand and (iii) the non-localized site B. Cu^{++} and Ni^{++} preferentially bind the NH_2-terminus site. Cd^{++} bind sites A and B, Zn^{++} binds site A and Au^{+} and Pt^{++} bind at residue Cys34.

A reduction in oxygen supply causes localized acidosis and the generation of free radicals. Copper and zinc ions, normally bound to proteins in the plasma are released from protein binding sites to circulate in the free form [19-21]. The N-terminus of albumin binds transition metals. The N-terminus however, is susceptible to biochemical alteration [22]. The altered form is referred to as ischemia modified albumin (IMA). Following a period of ischemia, a reduction in the ability of albumin to bind cobalt is apparent. This is the basis of the albumin cobalt-binding test (ACB® test) for IMA. IMA has been extensively studied in the basic science and clinical research settings and is an FDA cleared CE marked clinical assay for the detection of cardiac ischemia.

It is currently not known if there are any 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 [22] including bovine, dog, goat, horse, pig rabbit, rat and sheep but not chicken. Using electrospray-mass spectrometry and N-terminal sequencing, Chan and colleagues have demonstrated degradation corresponding to the first two resides (Asp-Ala) which is dependent both on temperature and the N-terminal alpha-amino group.

IMA however is a form of HSA where the N-terminal amino acids are unable to bind transition metal ions. Myocardial ischemia is known to generate free radicals [21;23], induce localised acidosis [20] and the release of free iron and copper ions bound to enzymes and proteins. [19;24]. Direct evidence of Cu/Fe mobilization in the coronary flow following prolonged (25-60 minute) ischemia but not short (15-21 minute) ischemia has been demonstrated [24]. Both copper and iron concentrations in the first coronary flow fraction were 50-fold and 15-fold higher respectively following prolonged ischemia, compared to pre-ischemic concentrations. This suggests that both copper and iron play a causative role in ischemic cardiac injury by their ability to catalyse the production of free radicals and could be the target of therapeutic intervention to salvage tissue damage [19]. It was therefore postulated that following a period

of cardiac ischemia, these processes would result in a change in the ability of the N-terminus of HSA to bind transition metal ions. The release of these ions likely initiates one potential pathway for IMA generation, rather than be considered an interference that may negatively affect IMA. In support of this suggestion, decreased albumin cobalt binding was reported in 99 acute chest pain patients with myocardial ischemia [25] compared to 44 chest pain patients with no evidence of myocardial ischemia. Albumin cobalt binding was also assessed in 41 patients undergoing elective coronary artery angioplasty. Samples were tested using the Albumin Cobalt Binding (ACB) assay before, immediately after, 6 and 24 hours post procedure and compared to results from 13 patients undergoing cardiac catheterization without balloon angioplasty, thus serving as the control group. ACB concentrations were significantly elevated immediately post procedure, compared to the control population and ACB concentrations returned to baseline after six hours [26]; suggesting that HSA undergoes a significant reduction in the capacity to bind exogenous Co^{++} immediately after coronary artery occlusion induced during elective angioplasty. Modification of the Asp-Ala-His-Lys site by N-terminal acetylation or deletion of one or more residues abolishes this cobalt binding [27].

The postulated mechanism (figure 2) of IMA generation is that localised ischemia results in acidosis. The localised acidotic environment stimulates the release of Cu^{++} ions from weak binding sites on circulating proteins such as caeruloplasmin. Caeruloplasmin (EC 1.16.3.1, 151kDa) is a ferroxidase enzyme encoded by the *CP* gene located on Chromosome 3. The enzyme is synthesised in the liver and carries approximately 70% of the total copper in human plasma (a further 15% carried by HSA and the remainder by macroglobulins). Each enzyme molecule contains 6 atoms of copper within its structure.

In the presence of a reducing agent such as ascorbic acid, free copper II is converted to copper I which can react with oxygen to form copper II and generate superoxide free radicals ($O_2^{\bullet -}$). Superoxide dismutase (EC 1.15.1.1) converts the superoxide free radical to hydrogen peroxide which is then degraded by catalase. The copper II ions released are immediately scavenged by human serum albumin but they are tightly bound to the N-terminus. Copper bound albumin is then damaged by hydroxyl free radicals ($OH^{\bullet}$), causing removal of the three N terminal amino acids and release of the copper II ion to repeat the process in a chain reaction [28].Marx and Chevion demonstrated by SDS/polyacrylaminde gel electrophoresis the site specific alteration of HSA in the presence of 50 μM Cu^{++} and increasing portions of 0.2 m Mascorbate; where after the addition of 5 portions, bands at 3, 18, 22, 47 and 50 kDa were observed. The authors also demonstrated that degradation does not occur in the absence of Cu^{++} or in the addition of 1 m Methylenediaminetetraacetic acid (EDTA) or citrate chelating agents [28].

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 [29] by cleavage of the 11 amino acid resides at the NH_2 terminus, by rapid liquid-phase Edman degradation. The N-terminal amino acid sequence showed normal residues for 6 of 7 patient samples with elevated IMA and one non-ischemic sample (table 1). The remaining patient sample with high IMA demonstrated two missing amino acids at the N-terminus. Clinically this patient did not have an ischemic cardiac event.

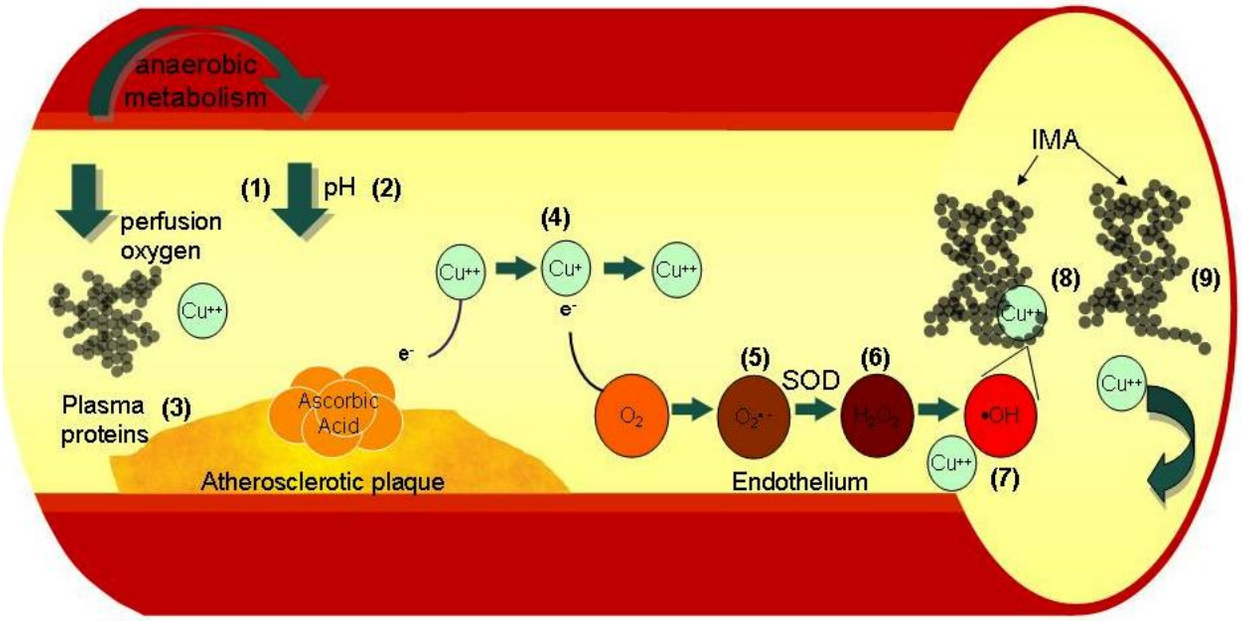

Figure 2. Mechanism of Ischemia Modified Albumin generation. [1]Tissue hypoxia from anaerobic metabolism reduces ATP and causes a [2]lower localized pH inducing acidosis. [3]Cu^{++} ions are released from plasma proteins such as caeruloplasmin. In the presence of ascorbic acid, [4]Cu^{++} is converted to Cu^{+}. Cu^{+} reacts with O_2 to form [5]$O_2^{\bullet-}$. Superoxide dismutase dismutates the $O_2^{\bullet-}$ to [6]H_2O_2, which in presence of Cu^{++} or Fe^{+}, undergoes the Fenton reaction forming [7]OH •hydroxyl radicals. Free Cu^{++} is scavenged by [8]HSA, where it binds tightly to the N-terminus. OH • radicals alter the amino acid N-terminus of [9]HSA rendering it incapable of binding Cu^{++}. These two altered forms are known as IMA.

Subject	NH_2-terminal HSA Sequence
Control (wild type)	DAHKSEVAHRF
Non-ischemic patient, high serum IMA	----HKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF

Table 1. Amino (NH_2) terminal sequence analysis of human serum albumin (HAS) from 6 ischemic patients, a control (wild type) and one non ischemic patient with a high serum IMA concentration. (Source: Adapted from Bhagavan et al, ClinChem 2003;49:581-585)

The *in vivo* half-life of HSA is 19-20 days. HSA with a truncated NH_2-terminus would presumably have similar *in vivo* half life properties and yet IMA returns to baseline rapidly after an ischaemic cardiac event. This indicates that the alteration to albumin to create IMA is transient and reversible, rather than a finite chemical alteration. Recent physicochemical

studies using electronic absorption EPR and NMR spectroscopy of Co-binding to HSA under anaerobic conditions to prevent Co^{++} oxidation have suggested a different explanation. Using competition experiments with cadmium (Cd^{++}) which binds sites A and B and Cu^{++} which binds the NH_2-terminus, three binding sites for Co^{++} were identified on HSA. Sites A and B showed greater avidity for Co^{++} binding than the NH_2-terminal binding site [30]. Fatty acid binding to albumin occurs at one of the additional cobalt binding sites with a negative allosteric interaction. It is hypothesised, that in myocardial ischemia the release of fatty acids results in binding of fatty acids to albumin. This would then reduce the ability of albumin to take up cobalt hence account for the presence of IMA [30]. If this also produced a conformational change in the albumin affecting the N terminal site, this would also reduce cobalt binding.

8.1. Kinetic release of ischemia modified albumin

Studies in patients receiving angioplasty where ischemia is induced in a controlled manner, have defined the kinetics of IMA production. There is a rapid rise in IMA values after balloon inflation with a subsequent fall at 6 hours and return to normal by 24 hours [31;32]. The rise in IMA occurs earlier than the rise in cardiac troponin and natriuretic peptides (figure 3) and occurs early after the onset of plaque rupture.

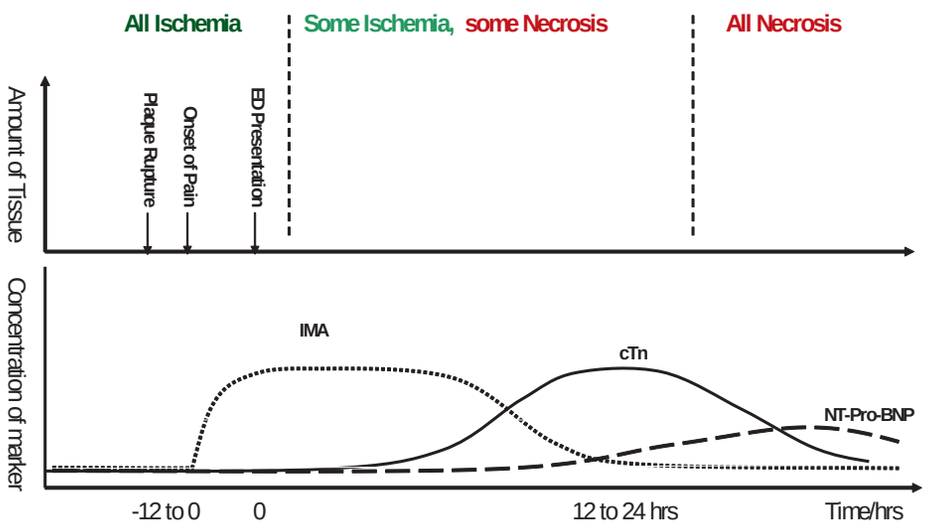

Figure 3. Kinetic release of Ischemia modified albumin (IMA, dotted line) and other cardiac markers, cardiac troponin (cTn, solid line) and natriuretic peptide (NTproBNP, dashed line) [bottom panel], in relation to extent and timing of tissue damage [top panel]

The magnitude of IMA elevation has been found to correlate with the number and frequency of transluminal balloon inflations during the PCI procedure [33]. 34 patients received standard

routine care for elective single vessel PCI for the management of stable angina pectoris. 44% of patients received 1-4 balloon inflations whilst, 56% received >5 inflations. IMA concentrations were higher in those with more balloon inflations, higher pressure load of the balloon and the longer the duration of the inflation. IMA is thus not only a marker of the occurrence of ischemia but is also an indicator of the severity of the ischemic episode.

IMA concentrations are lower in patients who demonstrate angiographic evidence of collateral vessels present in the coronary circulation, according to Rentrop's classification [34]. IMA levels post-PCI are higher than baseline, however post-PCI values are lower compared to post-PCI values in those patients without a collateral circulation; irrespective of the extent of coronary artery disease or those who underwent a large number of balloon inflations for longer duration [35]. The lower IMA concentrations in patients with a collateral circulation likely represent a cardioprotective effect against PCI-induced ischemia. IMA elevation is also correlated to the need for subsequent revascularization [36]. Elevated IMA greater than 130 KU/L was associated with a higher frequency of target lesion revascularization at 4-years follow-up in 60 patients who underwent a successful elective single vessel PCI for stable angina pectoris at baseline. The accepted gold standard blood marker for myocardial ischemia is myocardial lactate extraction. Simultaneous IMA and lactate was measured in 10 patients undergoing PCI for chronic stable angina. Post-PCI IMA concentrations paralleled that of transmyocardial lactate [32].

Elevation in serum IMA has been recorded following coronary vasospasm [37]. Twenty six patients with variant angina underwent intracoronary ergonovine spasm provocation testing. Arterial IMA concentrations were measured pre and post procedure and compared to 18 patients undergoing elective PCI and 10 patients with normal coronary angiography. IMA was significantly elevated following drug induced coronary vasospasm compared to baseline and elevated values detected coronary vasospasm with an area under the curve (AUC) of the receiver operating characteristic (ROC) curve of 0.98 (95%CI 0.92-1.00). Other studies involving invasive cardiac procedures have shown rises in IMA where ischemia might occur, occurring concurrently with ECG changes in cardioversion[38], but show a variable picture when there is non-ischemic myocardial damage as in cardiac ablation [39;40].

8.2. Measurement of ischemia modified albumin

The original biochemical test for IMA was known as the albumin cobalt binding (ACB®) assay. This was developed by Ischemia Technologies Inc, Colorado, USA). The assay measures the cobalt binding capacity of albumin in a sample of serum. A known amount of cobalt is added to the patient serum sample. Dithiothreitol (DTT) is added which binds any remaining unbound cobalt and the colorimetric change is measured spectrophotometrically. In serum from non-ischaemic patients, cobalt binds to the N-terminus of HSA, leaving little free cobalt to react with DTT and form a coloured 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 and form a darker colour. As normal albumin will bind cobalt, the amount of free cobalt, hence the absorbance will be proportional to the amount of IMA present (figure 4).

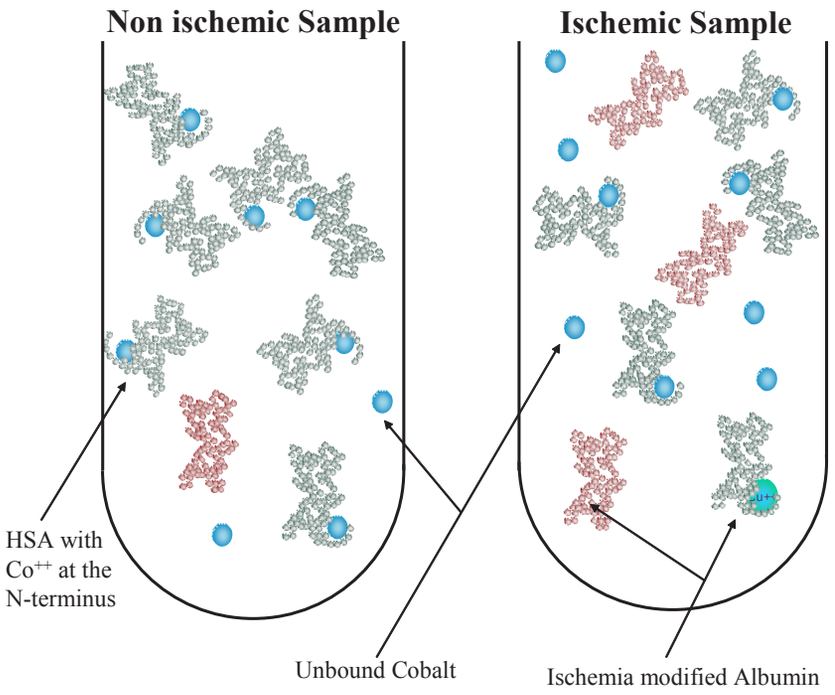

Figure 4. Measurement of Ischemia Modified Albumin by the Albumin Cobalt Binding (ACB) assay. A known amount of $CoCl_2$ is added to a serum sample. DTT is added which binds unbound Co^{++} causing a colorimetric change read spectrophotometrically.

The first generation assay was semi-automated and required a sample pre-treatment step where 500μL of serum sample was added to an Eppendorf tube containing 0.45g $CaCl_2$. The sample was inverted twice and centrifuged at 1200g for 10 minutes. 300μL of supernatant was removed for assay of IMA. For the assay, powdered DTT was provided which required reconstitution in the ratio 15mg DTT: 10 mL diluent. The reconstituted reagent and power required storage at 2-8°C and the working solution had a shelf life of 3-5 days. The second generation of the assay used 7.5mg DTT to 10mL diluent. The third generation assay which became commercially available did not contain the sample pre treatment step and the assay kit contained a concentrated liquid form of DTT which was reconstituted in buffer with a fixed volume 200 μL pipette.

The assay can be performed manually with a spectrophotometer, however it was also initially automated on the Cobas MIRA Plus (Roche Diagnostics) automated spectrophotometer [41]. The assay has since been adapted for other automated clinical chemistry platforms including the LX-20 (Beckman Coulter, Brea, CO, USA) [42], Hitachi 911 (Hitachi, Japan) [43] Hitachi 7600 (Hitachi, Japan) [44] and the Konelab 20 (Thermo Scientific, United Kingdom) [45]. To date a commercialised point of care device for IMA remains to be developed however a pre-commercial portable spectrophotometer and IMA assay has been developed by (Microwells Biotechnology Co. Ltd, Shanghai, China). In the Microwells assay, the DTT has been replaced with a stable azo dye chromogen. An automated method to measure ischemia induced alterations of the binding capacity of HSA for nickel [27] has been described [46].

The nickel binding assay correlated well to the ACB assay (r=0.5387, p<0.001) however the AUC of the ROC curve was higher for the nickel binding assay (0.7582) compared to the ACB assay (0.7289) suggesting nickel binding has a superior ability to discriminate between ACS and non-ACS compared to the ACB assay. There are rumours of an ELISA assay for IMA. This assay is not validated for clinical diagnostic or therapeutic use and an independent performance validation and comparison to the ACB assay does not appear in the current literature. The development of an ELISA however is probably not valid given the rapid alteration and return to baseline of IMA following ischemia, suggesting the alteration is transient and not a permanent change to a specific epitope which could be detected by an antibody.

The *in vitro* stability of the IMA has been shown to be two hours at either 4°C or 20°C, but values increase significantly after four hours irrespective of the storage temperature [33].It is likely that the changes are due to *in vitro* pH changes altering the metal binding capacity of human serum albumin. Samples frozen at -20°C are stable although values have been reported to be slightly higher once thawed, compared to freshly analysed samples [47]. The assay incubation temperature can also affect cobalt binding to HSA and thus influence the IMA concentration [48].

A study of 109 subjects (55 men and 54 women; age range, 20 to 85 years) to determine the 95th percentile reference range for IMA has been performed [49]. The concentrations ranged from 25.7 to 84.5 KU/L with an upper 95th percentile of 80.2 KU/L. This study used the first generation of the assay utilising the pre-handling sample preparation step. Further studies of healthy subjects have reported higher IMA ranges. Abadie and colleagues demonstrated a mean IMA value of 89 KU/L from 69 subjects with a mean age of 49 years [50] whilst Maguire and colleagues demonstrated a 97.5th percentile of 110 KU/L [42] from a population of 81 healthy volunteers (28 men and 53 women aged 22-86 years). Values ranged from 82.0 to 110 KU/L and values were similar between males and females (99.1 vs 100.7 KU/L, p=0.12). The biological variation of IMA has been studied [51].In a population of 17 apparently healthy individuals (7 male, 10 female, aged 26-61 years), the within subject coefficient of variation was 2.89% and the between subject coefficient of variation was 6.76%, calculated from weekly blood draws performed at the same time by the same phlebotomist for 5 consecutive weeks. Again there was no specific gender difference in IMA concentrations however the authors reported statistically different IMA concentrations between Caucasian and Black populations, with higher IMA concentrations in Black males and females compared to Caucasian counterparts.

Total serum albumin concentrations might be expected to affect the performance of IMA measurement. There is a relationship between IMA values and serum albumin concentration, although this is much less marked across the reference interval for albumin [52]. The use of an albumin adjusted correction has been proposed [53] although a reference interval study found albumin correction to have little impact compared to other analytical factors [42]. It has been reported that the changes in IMA observed in patients with chest pain was attributable only to changes in the serum albumin concentration [54].

8.3. Clinical utility of ischemia modified albumin in chest pain patients

Clinical validation of any test for ischemia is difficult as there is currently no accepted diagnostic gold standard, although blood lactate has been used previously.In addition, there is no predicate test which can be used against which to perform an initial validation. The initial studies using IMA were based on the ability of an early measurement to predict the final diagnosis of AMI as defined by the elevation of cardiac troponin at 6-12 hours post chest pain. Two studies utilised the first generation pre-release ACB test and a third study manufactured an in-house method. The first study examined acute coronary syndrome (ACS) patients and utilised serial sampling on admission and two subsequent samples [55]. 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 enrolled 256 ACS patients [49]. AUC of the 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 enrolled 75 patients with ischemia and 92 non-ischemic patients [29]. IMA had poor predictive power in discriminating between AMI and non-AMI in patients with underlying ischemic heart disease (AUC of 0.66). However, the test gave good discrimination between patients with or without ischemia. The AUC for the ROC curve for diagnosis of ischemia was 0.95 with sensitivity of 94% and specificity of 88%. In these initial studies there were significant problems with sample stability and the assay involved in addition of calcium chloride and centrifugation as part of the routine method. This made the method unsuitable from routine analysis and the assay was reformulated.

The majority of patients who present to hospital with chest pain and suspected ACS are eventually ruled out for acute myocardial infarction and active unstable coronary disease. The ideal role of an ischemia marker would therefore be as rule out test.The most logical place to use such a test is therefore in the ED.A study of ED presentations examined 208 patients the diagnostic sensitivity of IMA measurement alone was 82% at 46% specificity in samples taken within the first 3 hours. The combination of ECG, cardiac troponin T (cTnT) and IMA showed 95% sensitivity for diagnosis of ACS at presentation [56]. One year follow up performed on this population demonstrated a survival disadvantage in patients with IMA greater than the median concentration of the study group [57]. A subsequent study of 538 patients admitted to a chest pain evaluation unit found admission measurement of IMA plus cTnT had 100% sensitivity for prediction of a final diagnosis of AMI [1]. The presence of an elevated IMA and an elevated cTnT on admission predicted 21% risk of major adverse cardiac events (MACE) compared to patients where 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 a panel of other tests or a test sequence [50]. Admission measurement of IMA has been found to be superior to biomarkers of necrosis and to show 97% sensitivity when combined with them. Not all investigators have considered 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 enrolling 277 patients and using a positive IMA or troponin as the index test and an 8 hour troponin as

the definitive test found only a 97.6% sensitivity with 97% negative predictive value. The investigators did not consider this to be adequate when compared with troponin but did not provide any follow-up data [58]. A second large study prospectively enrolling 189 patients presenting to the ED with chest pain and found an elevated IMA was a poor predictor of cardiac events within the next 72 hours [59]. Conversely, another study found elevated IMA predicted long-term cardiac events [60]. The most consistent finding across all studies of IMA is of a high negative predictive value. This has been highlighted in a meta-analysis specifically examining the role of IMA as a rule out test [61]. The summarised data of over 1800 patients demonstrated a triple negative prediction test (non-diagnostic ECG, negative cTn and negative IMA) with a sensitivity and negative predictive value for ACS of 94.4% and 97.1% respectively.

The prognostic value of IMA in the ACS setting has been investigated [57;60;62;63]. Using a ROC derived cut off of 477 KU/L, Aparci and colleagues found significantly higher mortality at one year in those who had serum IMA >477 KU/L, compared to those with IMA <477 KU/L [60]. Furthermore, using cox regression modelling, IMA was related to mortality, independently of the presence of hypertension, diabetes or advanced age. In a larger cohort of 245 consecutive attendances to the ED, in which there were 31 composite endpoint (cardiac death, AMI or recurrent angina) at 30-days from presentation and 16 deaths at one year; the short and long term ability of IMA to predict outcome was assessed. Short term survival was significantly compromised in those with IMA > 93.3 KU/L compared to those with lower IMA concentrations at both 30 days and 1 year [57]. Using the cohort of the French Nationwide OPERA study IMA, cTn, CRP and BNP were measured within 24hours from admission in 471 patients hospitalized with AMI. Using a primary end point of death, resuscitated cardiac arrest, recurrent AMI or ischemia, heart failure or stroke, 75 in-hospital events and 144 events at 1 year were recorded. Using quartile analysis, 40% of patients reached the end point with IMA concentrations in the highest quartile (>104 KU/L), compared to only 20% of patients in the lowest quartile of 83 KU/L [63]. In those STEMI patients who are treated with primary PCI, IMA is a powerful predictor of 30-day mortality however it does not add to the validated Thrombolysis In Myocardial Infarction (TIMI) risk score [64].

8.4. Clinical utility of ischemia modified albumin in non–chest pain patients

Any marker associated with pathological processes upstream of cardiac necrosis will invariably suffer from a lack of specificity; unlike the cardiac troponins for cardiac necrosis. The further upstream in the ischemic continuum the more likely is the lack of cardiac specificity of the biomarker. Elevations in circulating IMA concentrations are not specific for myocardial ischemia. Mechanistically, IMA can be generated during any ischemic process within the body. A comprehensive review of IMA elevations in non-cardiac conditions is beyond the scope of this chapter but an in-depth summary is given in table 2.Those conditions that have been studied most are explained in more detail below.

Condition
Carbon monoxide poisoning
Congestive cardiac failure
Chronic kidney disease
Deep vein thrombosis
Diabetes Mellitus
Hypercholesterolaemia
Intermittent claudication
Ischemic bowel
Liver cirrhosis
Neural tube defects
Obesity
Pleural effusion
Polycystic ovary syndrome
Polycythemia vera
Preeclampsia
Pulmonary embolism
Skeletal muscle ischemia
Stroke
b-thalassemia
Testicular torsion
Uterine artery embolisation for fibroids

Table 2. Increased serum IMA concentrations in conditions other than acute coronary syndrome.

8.4.1. Skeletal muscle ischemia

Studies of subjects 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 [65-67] or return to normal [68]. Subjects undergoing a forearm ischemia test when the forearm muscles are exercised for 1 minute with the external compression of the arm blood supply showed a fall in IMA, maximal at 3 minutes from the test, returning to baseline by 30 minutes [69]. A similar rise in serum lactate occurred. Conversely during standardized exercise in a plantar flexion pedal combined with inflation of a femoral blood pressure cuff (at 0, 60, 90, 120 and 150 mmHg) to induce calf muscle ischemia an increase in IMA was observed after release of the cuff and returned to baseline within 30 minutes [70]. Peri-operative skeletal muscle ischemia induced by femoral blood pressure cuff being inflated to 300 mmHg in 23 patients undergoing arthroscopic knee surgery. Increased

IMA and myoglobin and decreased albumin were observed following release of the cuff [71]. In patients with peripheral vascular disease (PVD) undergoing a treadmill walk test, a decrease in serum IMA immediately post-test has been documented [72;73]. In 40 consecutive patients undergoing exercise electrocardiography, a significant decrease in IMA at peak exercise then a subsequent rise in IMA has been observed, however there was no difference in IMA concentrations between those patients with positive and negative stress test results [74]. Revascularisation for PVD is accompanied by a post procedural rise in IMA [72;73;75]. In skeletal muscle ischemia, an initial fall with subsequent rise appears to be a consistent finding without adequate explanation. Smooth muscle ischemia does not appear to be associated with a rise in IMA [76]. The effect of skeletal muscle on ischemia will limit the application of IMA measurement after cardiac stress testing for detection of myocardial ischemia and may explain the inconsistent findings[54;74;77;78].

8.4.2. Ischemic stroke

Patients with acute ischemic stroke demonstrate abnormalities in a number of biomarkers of nitrosative and oxidative stress. In 41 patients with ischemic stroke, Senes and colleagues demonstrate that nitrate, IMA and thiobarbituric acid-reactive substances (TBARS) concentrations are significantly increased compared to 37 age and gender matched controls [79]. In a larger cohort of 118 patients presenting within 3 hours of neurological deficit, IMA was elevated in those with cerebral infarction and intracranial haemorrhage (ICH) but normal reference values were observed in those with transient ischemic attacks (TIA) lasting less than 1 hour or those with epileptic seizures [80]. Within 24 hours of injury IMA increased during cerebral infarction but not in intracranial haemorrhage and may offer diagnostic utility in the differential diagnosis of neurological deficit. IMA also correlated with National Institutes of Health Stroke Scale (NIHSS) Score in both cerebral infarction and ICH. Conversely, Herisson and colleagues did not demonstrate a causal relationship between IMA or heart type fatty acid binding protein and NIHSS score or stroke volume [81]. Ahn and colleagues have utilised an albumin-adjusted IMA index for the early detection of ischemic stroke [82]. In 52 patients, 28 (54%) with Ischemic stroke, 24 (46%) non-stroke, the AUC of ROC curve analysis was 0.928 for IMA but 0.99 for albumin-adjusted IMA index. The sensitivity and specificity of the IMA index was superior to IMA concentration alone.

8.4.3. Pulmonary embolus

Pulmonary embolus (PE) is an acute medical emergency estimated to occur in 3.5/1000 hospitalized patients. Patients experience sudden onset dyspnoea, tachypnoea, pleuritic-type chest pain, cyanosis and haemoptysis. PE has an associated mortality of 26%. Diagnosis is primarily based on typical clinical presentation using the Wells and Geneva clinical probability scores. D-dimer measurement and pulmonary angiography are often clinically useful. The ECG can demonstrate acute *corpulmonale* in large PE's but lacks specificity. IMA has been measured in a number of studies of PE patients. Turedi and colleagues [83] have demonstrated that IMA was significantly elevated in 30 PE patients compared to 30 healthy controls and adequately discriminated between the presence and absence of PE. The positive

predictive value of IMA for PE is higher than that for D-dimer (79.4% compared to 69.4%) and in combination with the Wells and Geneva criteria, IMA offers an alternative to D-dimer testing [84].

8.4.4. Chronic kidney disease

Patients with chronic kidney disease (CKD) have a reduced life span compared to those without renal disease. Mortality rates are highest in those receiving haemodialysis as renal replacement therapy (RRT). Cardiovascular mortality accounts for the majority of renal deaths. Between 2001 and 2006, 24% of deaths in UK RRT patients were due to ischemic heart disease [85]. This rate is consistent with data from other countries. Cardiovascular morbidity is also increased. 55% of patients receiving haemodialysis RRT also have concomitant congestive cardiac failure. [86]. IMA levels have been determined in patients with CKD [87-89] and in patients receiving haemodialysis (HD) [90-94].

In 2006, Sharma and colleagues demonstrated that patients with elevated IMA have a significantly large left ventricle, decreased systolic function and greater estimated left ventricular filling pressure [88]. Further, in multivariate analysis, a positive dobutamine stress echocardiogram (DSE) combined with elevated IMA and cTnT and E/Ea ratio were independent prognostic factors for death. IMA values increase significantly in those patients with a positive DSE compared to those with no ischemic response [87]. In a modestly small study of 17 anaemic CKD patients and 19 controls, Cichota and colleagues demonstrated that IMA increased in patients compared to the control group. IMA correlated to lactate, haemoglobin and creatinine [89].

Pre and post-HD IMA concentrations are significantly correlated [90], however in this study IMA concentrations were not significantly different between those CKD patients with or without ischemic heart disease, diabetes mellitus or peripheral vascular disease. Fast intravenous iron administration during HD is associated with oxidative stress and inflammation. In a study of 20 HD patients receiving slow intravenous iron administration, IMA concentrations were significantly increased across three HD sessions independently of slow i.v. iron administration [91].Following adjustment of albumin by two methods, post dialysis IMA levels remain significantly increased following HD [92]. Paroxonase-1 (PON-1) is a calcium dependent esterase (arylesterase, aromatic esterase 1, serum aryldialkylpohosphatase 1, EC 3.1.8.1) is a major anti-atherosclerotic component of HDL cholesterol. PON-1 concentrations are lower in CKD patients with and without haemodialysis RRT compared to controls suggesting chronic oxidative stress and accelerated atherosclerosis are a feature of CKD. In a pilot study of CKD patients receiving HD, PON-1 concentrations were significantly and inversely correlated to IMA suggesting an oxidative stress and ischemic process occurs during HD [93]. Recently Albarello and colleagues have evaluated the effect of IMA and protein carbonyl groups as markers of protein oxidation in 23 CKD patients receiving HD. The authors confirm previous reports of higher IMA post-HD than pre-HD and observed a significant correlation between IMA and protein carbonyl groups, attributed to oxidative stress associated with HD [94].

8.4.5. Hyperlipidaemia and obesity

IMA measurement may be of benefit in hypercholesterolaemic patients. IMA is correlated to cholesterol, low density lipoprotein (LDL) and antibodies to oxidised LDL (ox-LDL) [24]. In a study of 37 subjects with hypercholesterolaemia compared to 37 controls, Duarte and colleagues [95] confirm these findings observing IMA correlations to cholesterol, LDL ox-LDL antibodies and to high sensitivity C-reactive protein, suggesting that hypercholesterolaemia is associated with inflammatory and oxidative stress processes, contributing to the advancement of atherosclerosis. IMA is related to the presence of metabolic syndrome independently of age, gender, presence of diabetes or hypercholesterolaemia[96]. Furthermore, the use of 10 mg/day ezetimibe immunotherapy for a duration of 12 weeks in 31 hypercholesterolaemic patients reduced both LDL cholesterol and IMA [97]. The reduction of IMA was independent of the reduction in LDL suggesting that ezetimibe may reduce the burden of oxidative stress in hypercholesterolaemia.

IMA concentrations are higher in obese subjects, with a positive correlation between IMA and body mass index (BMI).In a large study of 148 volunteers in Brazil; subjects were classified as normal, overweight or obese, defined as BMI of 18.5-24.9, 25.0-29.9 and >30 kg/m^2 respectively. IMA concentrations increased exponentially between the three groups, the highest being in those subjects with BMI >30 kg/m^2. Similar findings have been demonstrated in obese postmenopausal women where IMA and IMA: Albumin ratio are higher in those subjects with BMI 26-32 kg/m^2 compared to those with BMI 21-25 kg/m^2. The obese concentrations were similar to those with documented coronary artery disease but normal BMI. In the obese women IMA was positively correlated to BMI, hs-CRP, insulin concentrations and homeostasis assessment model score [98].

8.4.6. Diabetes mellitus

Patients with type 2 diabetes mellitus who demonstrate poor glycaemic control have higher IMA concentrations than those with good glycaemic control. IMA was significantly higher in 76 diabetic patients compared to 25 control subjects and IMA concentrations are correlated to HbA1c [99], glucose and hs-CRP [100]. Conversely, Dahiya and colleagues suggest no significant changes in IMA occur in 60 newly diagnosed type 2 diabetics, compared to 30 control subjects [101]. Diabetic patients who undertake chronic exercise for three months demonstrate lower post exercise IMA concentrations suggesting that exercise alleviates some of the oxidative stress associated with diabetes mellitus [102].

8.4.7. Bowel ischemia

Bowel (mesenteric) ischemia occurs infrequently however if not recognised early, carries a devastatingly high mortality. The presentation is often characterised by generalised abdominal pain, fever, diarrhoea or constipation, tachycardia, hematochezia (blood per rectum), nausea and vomiting. Diagnosis is difficult due to non specific signs and symptoms, plain x-ray or laboratory tests (increased white blood cell count and serum lactic acid). Mesenteric angiography is considered to be the gold standard test which can differentiate between embolic,

thrombotic or nonocclusive ischemia. In a preliminary study of 26 patients presenting with symptoms of internal ischemia, Polk and colleagues [103] identified 12 with a positive clinical diagnosis. Positive patients had higher IMA concentrations than those without intestinal ischemia. IMA detected bowel ischemia with a sensitivity of 100% and a specificity of 86%. In a case-controlled study from Turkey, Gunduz and colleagues [104] demonstrated that pre-operative IMA concentrations were significantly higher in patients with thromboembolic occlusion of the superior mesenteric artery (SMA) compared to an age-matched control group of healthy volunteers. A number of animal studies of mesenteric ischemia have provided conflicting results. In a Wistar rat model [105] a time dependent response in IMA in mesenteric ischemia has been demonstrated. 36 mature female rats underwent either simple laparotomy in the control groups or laparotomy followed by clamping of the SMA in the subject group. IMA concentrations were highest 6 hours from ischemic onset, however IMA at 30 minutes and 2 hours were also significantly higher in the clamped group compared to the control group. Elevations of IMA tracked changes in both lactate and malondialdehyde. A similar time dependent change in IMA was demonstrated in New Zealand rabbits undergoing ligation of the SMA compared to either a control group or those undergoing a sham procedure [106]with elevation of IMA at 2 and 6 hours significantly higher than baseline and higher than IMA concentrations in the control rabbits. IMA concentrations mimicked elevations in serum IL-6 with elevated IL-6 in the ischemia group at 1, 3 and 6 hours, but no elevations in the sham operated or control group. In a further study of mesenteric ischemia in a Wistar rat model, Uygun and colleagues [107] demonstrated similar IMA concentrations in control, sham, 2-hour and 6-hour post-SMA ischemia refuting the previous animal studies. It seems likely that IMA may offer additional diagnostic value in the early presentation of mesenteric ischemia. Further prospective studies are required to assess both the diagnostic and prognostic ability of IMA in conjunction with mesenteric angiography to detect bowel ischemia.

8.4.8. Obstetric and gynaecological use of IMA

The care of women and their unborn child during pregnancy is greatly challenging for obstetricians. The adult can interact and provide a history of signs and symptoms whereas the unborn child can only be examined indirectly by means of imaging, foetal heart monitors and a limited number of direct interventions. Women achieving spontaneous preterm (<37 weeks) labour account for 10% of all births and are attributable to 75% of neonatal deaths. The foetus relies entirely on the maternal placenta for O_2/CO_2 exchange. This delicate dependence, between the placenta and the foetus is crucial to normal healthy growth. Any malfunction or disruption to the adequate supply of oxygen can cause hypoxia and potentially fatal acidosis. A limited degree of acidosis is well tolerated by the foetus; however chronic acidosis or hypoxia may lead to a significant mortality and morbidity with potential log-term sequelae. Currently the mechanism of foetal hypoxia and acidosis is unclear, and physiological consequence of foetal acidosis is believed to target the cell energy availability and /or cell poisoning.

During pregnancy plasma proteins change markedly due to increased plasma volume, increased renal blood flow and altered protein synthesis in response to hormonal changes. Plasma volume expansion of up to 45% [1300mL) compared to the non-pregnant state causes

an overall net decrease in plasma protein concentration by 10-12 g/L which is reached around week 28 of gestation. The predominant cause of lowered albumin is dilutional, oestrogen is known to affect albumin. The alteration to plasma albumin concentrations throughout the pregnancy period is shown in table 3. The lower concentration of albumin also results in an apparent decrease in substances normally bound to this protein.

Time point	Mean albumin concentration (g/L)	Reference interval (g/L)
Non pregnant control	41	36-46
12 weeks	38	33-43
18 weeks	35	30-39
24 weeks	33	29-37
28 weeks	32	28-37
32 weeks	32	38-36
36 weeks	32	38-36
Full term	32	26-38
1 day post partum	29	23-38
6 weeks post partum	42	37-47

Table 3. Alteration to plasma HSA concentrations during the gestational period.

The HSA reference interval in the full term healthy neonate between term and day 4 is 28-44 g/L. Albumin concentrations increase a little from birth to puberty where the adolescent reference interval (day 4 to 14 years) is 38-54 g/L.

Current experimental studies suggest that foetal development occurs in a hypoxic intrauterine environment and the presence of reperfusion and oxidative stress is believed to be crucial for trophoblast development [108]. Trophoblast invasion of the maternal spiral arteries allows the increase of uterine blood supply necessary to maintain the pregnant state. Serum IMA during normal pregnancy is elevated compared to non-pregnant controls [109-112]. Prefumo and colleagues [109] demonstrate supra-physiological IMA concentrations in early normal pregnancy (11-13 weeks of gestation) suggesting that trophoblast development occurs in a hypoxic uterus. In a large population of 117 pregnant women compared to non-pregnant healthy women, Guven and colleagues demonstrated a cross-sectional elevation in IMA in pregnant women. IMA increased significantly through each trimester. Further, there the authors demonstrated a significant negative correlation between IMA and HSA, suggesting that elevated IMA in pregnancy represents a physiologic state of oxidative stress.

Increased intrauterine hypoxia predisposes to defective endovascular trophoblast invasion of the maternal spiral arteries which may possibly lead to the development of pre-eclampsia; a hypertensive state (>140/90 mmHg) associated with significant proteinuria (≥300mg/dL). Pre-

eclampsia affects 6-8% of pregnancies worldwide. Papageorghiou and colleagues have demonstrated that first trimester serum IMA are significantly higher in women who develop pre-eclampsia compared to those with a normal pregnancy [113]. Both IMA and normalised IMA (IMA: Albumin ratio) were higher in 20 pre-eclamptic women compared to 22 normal pregnancies [112]. These data suggest IMA could be a biological marker of pre-eclampsia however larger studies are required to fully characterise the supra-normal IMA and normalised IMA reference interval in normal pregnancy.

Maternal IMA and normalised IMA concentrations are also increased in women with recurrent pregnancy loss (two or more unexplained miscarriages in the first trimester) compared to healthy pregnancy [114], suggesting that an increase of intrauterine oxidative stress and hypoxia contribute to placental deficiency and subsequently recurrent early miscarriage.

The use of umbilical cord blood for IMA has also been examined. Neonatal cord blood IMA concentrations are higher than IMA concentrations in healthy adults [115] but is not attributable to changes in HSA concentration. Elevated fetal IMA may reflect transient localised ischemia from external forces exerted on the foetus during labour. In a case-control study of 26 newborns, 12 delivered at normal term and 14 with complicated labour or delivered preterm; cord blood IMA concentrations were significantly higher (50%) than those with uneventful deliveries, suggesting IMA is a marker of fetal distress. Doubly-clamped cord blood IMA concentrations are similar in intrauterine growth restriction, compared to those delivered with appropriate for gestational age full-term pregnancies [116]. The similar IMA concentrations may be due to the 'brain sparing effect' accompanied by oligohydraminos (deficiency in amniotic fluid), which is characterised by rerouting the blood supply and the nutrient to vital organs such as the heart, brain and adrenal glands. IMA concentrations in cord blood are higher following caesarean section compared to vaginal delivery and in multigravida compared to primigravida [116] and may be attributable to higher oxidative stress on both accounts. Cigarette smoking during the gestational period alters the oxidant/antioxidant balance in favour of oxidative stress. In response, IMA and MDA concentrations in pregnant smokers are significantly higher and vitamins A and E, SOD and total antioxidant capacityare significantly lower, compared to non-smoking pregnant women [117].

9. Cardiac troponin: Ischemia or necrosis?

The release of cTn was previously thought to occur only in the presence of cell necrosis. The recent development of high sensitivity cTn (hs-cTn) assays has lead to a) the ability to define a true 99th percentile and near-Gaussian distribution in the healthy population and b) earlier diagnosis of AMI with increased sensitivity but at the cost of specificity [118]. A number of clinical and physiological situations have arisen which suggests cTn is released during ischemia in the absence of overt necrosis [119]. These include patients who present with superventricular tachycardia [120] without electrocardiographic changes; in patients with pulmonary embolism [121] where cTn release may indicate a reversible release and under physiological strain following endurance exercise [122]. In all cases the kinetic release is

shortlived with post even values returning to baseline normally within a 24 hour window. Although the mechanisms have not been elucidated postulates include, physiological cardiomyocyte turnover, cellular release of proteolytic degradation products, alteration in plasma membrane permeability and the formation of membranous secretion vesicles containing intracellular derived cTn.

10. Conclusions

Although there are a number of candidate biomarkers for the detection of cardiac ischemia in the research and development world; biomarkers upstream of cardiac cell necrosis lack specificity. They are therefore, at best additive to the diagnostic and prognostic utility of cTn in the early investigation of patients presenting with ischemic type symptoms. The clinical utility of novel biomarkers of ischemia lies in their negative predictive value rather than their ability to adequately rule-in ACS. Given the development of sensitive cTn methods, further work is needed to characterise the release mechanisms of cTn from cardiomyocytes.

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