

Biochemical markers of myocardial injury

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The first account of the use of a biochemical marker in the study of myocardial injury was published by La Due and colleagues in the journal *Science* in 1954.⁸⁵ They measured serum glutamate oxaloacetic transaminase activity from a few hours to up to 15 days in a group of patients immediately after an acute myocardial infarction (AMI). They reported that enzyme activity increased above the reference range a few hours after AMI, reached a peak after 2 or 3 days, and then returned to baseline within a week. This work stimulated interest in the development of analytical procedures for other suitable marker proteins, and in their clinical applications. The development of assays for new marker proteins has contributed to a greater understanding of the pathophysiology of the disease spectrum of acute coronary syndromes, helped in their definition and assisted in cardiac risk stratification.

Characteristics of a cardiac marker

The ideal characteristics of a cardiac marker are shown in Table 1. High concentrations of the marker in the myocardium, with relatively low concentrations in non-cardiac tissue, will ensure cardiac specificity. It is important to consider the tissue distribution of a potential marker in pathological states as well as in normal physiological conditions. For example, creatine kinase (CK) MB isoenzyme (CK-MB) is a commonly used cardiac marker but is not ideal, as a small but significant amount of the isoenzyme is found in skeletal muscle. Consequently, skeletal muscle injury may contribute to an increase in the absolute activity of CK-MB fraction in blood.^{2 24}

The speed of release of a marker from injured myocardial tissue depends on a number of factors. The molecular size of the putative marker is important and, as a generalization, small molecules are released at a faster rate than large molecules.¹ The intracellular location of a marker also

limits its rate of release: molecules located in the cytoplasm will appear in the bloodstream sooner than structural proteins. Markers may not be confined to a single intracellular pool. Some 6–8% of intracellular cardiac troponin T (cTnT) has been reported to exist as a cytoplasmic pool,⁷⁸ as has 2.8–4.1% of cardiac troponin I (cTnI).⁹⁰ Both troponins exhibit characteristic biphasic release kinetics following myocardial injury. Release from the cytoplasmic pool gives rise to peak serum concentrations 24–36 h after injury, and structural protein release leads to a second blunted peak 2–4 days after injury. Continuing breakdown of the myofibrillary-bound complex explains the prolonged elevation of both serum troponins after myocardial injury.⁹²

The early appearance of a marker released into the bloodstream soon after an injury may facilitate early diagnosis. When developing assays it is important that they are sensitive, specific and have a short turnaround time, to allow results to be available within a clinically useful timeframe.¹⁰ These assays should be available on a wide range of analytical platforms, on a 24 h, 7 days a week basis. Target turnaround times should be less than 1 h from blood collection to the reporting of results.¹²⁷

Prolonged elevation of a marker in the blood may be useful for diagnosing the few patients who present late, after early markers have returned to baseline values. However, markers such as the troponins, which persist in blood for a long period of time after an initial injury, may be of no value in detecting reinfarction. Markers with a shorter time course, such as CK-MB or myoglobin, then become more useful.^{4 127}

The requirement that marker release should be in proportion to the extent of myocardial injury has led to the use of cardiac markers to estimate the size or severity of an injury. The reasoning behind this is that the more marker released, the greater the extent of cell death, and the worse

Table 1 Characteristics of an ideal cardiac marker

High sensitivity	Abundant in cardiac tissue
High specificity	Absent from non-myocardial tissue Not detectable in blood from non-diseased subjects
Release	Rapid release for early diagnosis Long half-life in blood for late diagnosis
Analytical	Cost effective Short turnaround time Precise Accurate
Clinical	Ability to influence therapy and so improve patient outcome Validated by clinical studies

the prognosis.⁵⁰ Assessment of infarct size has been made by examining the area under the curve of serial marker concentration *vs* time.⁴⁹ However, it is now generally recognized that estimation of infarct size using cardiac markers that exhibit washout phenomena, such as cardiac troponins or CK-MB, is inaccurate when pharmacological or surgical reperfusion of occluded vessels is successful.¹²⁷

It should be pointed out that no single marker satisfying the ideal criteria yet exists.

Biochemical markers (Table 2)

Cardiac enzymes

As noted above, Karmen and colleagues first reported that serum glutamate oxaloacetate transaminase was increased in patients with AMI.^{69,85} Assays for serum lactate dehydrogenase (LDH) and serum CK were then developed and utilized as cardiac markers^{5,34} in the early 1960s, with CK gradually becoming the marker of choice as a result of its early increase after injury. Differences in substrate specificity for LDH isoenzymes were then used to develop assays for serum α -hydroxybutyrate dehydrogenase (HBD; LDH isoenzyme 1), which showed an increased specificity for the detection of myocardial damage.³⁵ Electrophoretic procedures were developed for the demonstration of CK and LDH isoenzymes,¹¹⁰ which were recognized as having greater specificity than total CK activity. Analytical procedures for CK-MB activity were developed following the production of antibodies to the B-subunit.⁶⁶

It is now generally accepted that activity measurement of enzyme markers such as aspartate aminotransferase (AST), LDH, HBD and CK-MB is of little value in the assessment of myocardial injury,^{4,127} because of the lack of tissue specificity. The value of total CK is limited; however, it does appear in the bloodstream relatively soon after injury so it may still be of some value when it is used in combination with more sensitive markers, such as the troponins or CK-MB measured by mass assay.

Creatine kinase and CK-MB isoenzyme

Cytoplasmic CK is a dimer, composed of M and/or B subunits, which associate forming CK-MM, CK-MB and CK-BB isoenzymes. Creatine kinase acts as a regulator of

high-energy phosphate production and utilization within contractile tissues; it also has a more general role in shuttling high-energy phosphate bonds via creatine phosphate from the site of ATP production in the mitochondria to the site of utilization within the cytoplasm.¹⁵ This supports the observation that the enzyme is found in tissues that have high energy requirements, such as the distal tubules of the kidney.⁶¹ CK is also found as a mitochondrial form; mitochondrial CK is also a dimer, consisting of sarcomeric and non-sarcomeric subunits.⁸¹ Mitochondrial CK is unstable in human serum, difficult to measure and consequently its clinical significance is unknown.

CK-MM is the main isoenzyme found in striated muscle (approximately 97% of the total CK). CK-MB is found mainly in cardiac muscle, where it comprises 15–40% of the total CK activity, with the remainder being CK-MM. Trace amounts of CK-MB are found in skeletal muscle (2–3% of the total CK activity); patients with skeletal muscle injury will have increases in the absolute concentrations of CK and CK-MB. CK-BB is the predominant isoenzyme found in brain, colon, ileum, stomach and urinary bladder.⁸⁹

The development of antibodies to the M-subunit of CK enabled immunoinhibition to be used as the first specific quantitative assay for CK-MB.⁶⁶ The antibodies inhibit M-subunit activity, with residual enzyme activity being derived from B-subunits only; CK-BB is undetectable by activity measurement in serum, unless the patient has suffered a severe cerebrovascular accident, so the residual activity represents CK-MB activity. Although antibodies had been developed to the B- and M-subunits of CK, it was thought that MB did not have its own unique antigenicity. However, specific antibodies were developed in the mid-1980s,¹²¹ allowing the development of direct immunological assays for CK-MB.

Serum total CK activity and CK-MB concentration rise in parallel following myocardial injury, starting to increase 4–6 h after injury, reaching peak serum concentrations after 12–24 h and returning to baseline after 48–72 h.⁶¹ Serum CK-MB is considerably more specific for myocardial damage than is serum total CK, which may be elevated in many conditions where skeletal muscle is damaged. Consequently, CK should not be used for the diagnosis of myocardial injury unless used in combination with other more specific cardiac markers.⁶⁵ The retention of serum total CK can also be justified for clinical trials and epidemiological studies, as often it has been the only marker used in a number of clinical trials.⁴⁰ The diagnostic specificity of serum CK-MB for the detection of AMI has been reported to be very close to 100%,²⁷ while that of CK is only approximately 70%.⁴⁰

Creatine kinase isoforms

The M-subunit of creatine kinase was found to exist in plasma in multiple forms, despite the single form of MM or MB found in tissue.¹²⁵ Three forms of the MM isoenzyme and two forms of the MB isoenzyme were subsequently

Table 2 Biochemical markers of cardiac injury. CK-MB, creatine kinase MB isoenzyme

Marker	Time to raised plasma value	Peak	Duration of elevation
Aspartate aminotransferase	8–12 h	1–2 days	3–6 days
Lactate dehydrogenase	8–12 h	2–3 days	7–10 days
Creatine kinase	4–6 h	12–36 h	3–4 days
Hydroxybutyrate dehydrogenase	8–12 h	2–3 days	7–14 days
CK-MB (activity)	4–6 h	12–24 h	2–3 days
CK-MB (mass)	4–6 h	12–24 h	2–3 days
CK isoforms	1–3 h	8–12 h	18–30 h
Myoglobin	2–3 h	6–12 h	24–48 h
Carbonic anhydrase III	Skeletal muscle only	Skeletal muscle only	Skeletal muscle only
Glycogen phosphorylase BB	2–4 h	8–12 h	24–36 h
Heart fatty acid binding protein	2–3 h	8–10 h	18–30 h
Myosin light chains	3–6 h	4 days	10–14 days
Troponin T	4–6 h	12–24 h	7–10 days
Troponin I	4–6 h	12–24 h	6–8 days

identified and purified from plasma.⁴¹ In the case of CK-MM, the tissue isoform was designated CK-MM₃; the plasma enzyme carboxypeptidase-N catalyses the removal of a carboxy-terminal lysine residue from one of the M subunits to give the isoform CK-MM₂. Removal of the lysine residue from the remaining M subunit by the same mechanism gives rise to the third isoform, CK-MM₁.

For CK-MB, the tissue form is designated CK-MB₂; removal of the lysine residue from the carboxy terminus of the single M-subunit, catalysed by the action of carboxypeptidase-N, gives rise to the CK-MB₁ isoform. Removal of the lysine residue, which is positively charged, leaves a more negatively charged isoform, providing a basis for separation of the isoforms by electrophoresis.¹⁰⁵ The B-subunit is not susceptible to enzymic degradation, so only two isoforms of CK-MB exist.

In normal plasma, CK-MB isoforms exist with each other in equilibrium, in a 1:1 ratio. Release of tissue CK-MB₂ increases its proportion in plasma; a change in the ratio of CK-MB₂:CK-MB₁ from 1:1 to 2:1 can be detected using high-voltage gel electrophoresis, even though there is no significant change in the plasma concentration of CK-MB.¹⁰⁵ Significant changes in the ratio of the two isoforms in plasma can be detected between 2 and 4 h after myocardial injury. Systematic prospective studies have confirmed CK-MB isoforms as an early marker of myocardial injury, and established a CK-MB₂:CK-MB₁ ratio above 1.5:1 as a diagnostic criterion.^{106 126} The isoform ratio returns to normal within 18–30 h after injury. It has been suggested that a normal 1:1 isoform ratio in a sample collected at least 6 h after an event effectively excludes a diagnosis of myocardial infarction (MI).⁴⁰ The rapid return to normal values makes the CK-MB isoforms the best available laboratory investigation for the confirmation of reinfarction.¹²⁶ Unfortunately, the analytical procedure used (high-voltage gel electrophoresis) requires specialist equipment and a great deal of technical expertise, and is therefore impractical for routine use. In addition,

skeletal muscle contains trace amounts of CK-MB, so skeletal muscle damage will cause an increase in the CK-MB isoform ratio.

Myoglobin

Myoglobin is a haem protein, located in the cytoplasm of both cardiac and skeletal muscle cells, constituting about 2% of the total muscle protein. Its relatively low molecular weight (17 kDa) and cytoplasmic location ensure its rapid release into the circulation; the plasma concentration is elevated 2–3 h after myocardial injury. The relationship between myoglobinaemia and MI was first reported in 1975.⁶⁸ The amino acid sequence of myoglobin is the same in both skeletal and cardiac muscle, so its clinical specificity is compromised in the presence of skeletal muscle damage. Elevated plasma concentrations are also found in patients with renal insufficiency, the result of decreased renal clearance.¹⁰

Until relatively recently, the only available assays for myoglobin were radioimmunoassays, which are time consuming and not amenable as rapid diagnostic assays. However, the development of rapid immunoassays applicable to a number of analytical platforms has revolutionized the use of myoglobin as an early marker of myocardial damage.^{32 52} The National Academy of Clinical Biochemistry in the US, and the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC) Committee for the Redefinition of Myocardial Infarction both recommend the use of plasma myoglobin or CK-MB isoforms as early markers of myocardial damage.^{4 127}

Carbonic anhydrase III

The tissue distribution of carbonic anhydrase III is limited to skeletal muscle,⁷⁰ and its assay is used in combination with that of myoglobin. It has been suggested that an increase in the myoglobin:carbonic anhydrase III ratio is diagnostic for myocardial injury.^{120 124} Currently available assays for carbonic anhydrase are relatively time consuming, limiting the clinical use of the myoglobin:carbonic anhydrase III ratio.

Glycogen phosphorylase BB

Glycogen phosphorylase (GP) catalyses the breakdown of glycogen in the sarcoplasmic reticulum. It is a dimeric enzyme with a molecular weight of 18.8 kDa. Three isoenzymes have been identified: BB, found in brain and heart; MM, found in skeletal muscle; and LL, found in the liver.¹⁰⁸ It has been suggested that, after glycogenolysis in ischaemic tissue, GP-BB is released from the sarcoplasmic reticulum into the cytoplasm, and then into the circulation through the damaged cell membrane.^{36 82} GP-BB appears to be released into the circulation 2–4 h after myocardial injury,¹⁰⁷ returning to normal within 36 h of damage occurring. It has been reported to be a useful marker of myocardial damage following bypass surgery.⁹¹ Commercial assays are now available.¹⁰⁷

Heart fatty acid binding protein

Heart fatty acid binding protein (H-FABP) is a relatively small protein of 132 amino acid residues, molecular weight 14.5 kDa. It comprises 4–8% of the total cytoplasmic protein of cardiac myocytes and is immunologically different from other types of FABP, such as intestinal type and liver type.⁴⁶ The physiological role of H-FABP is the transport of hydrophobic long-chain fatty acids from the cell membrane to their intracellular sites of metabolism in the mitochondria.⁴⁴

H-FABP is released rapidly from the myocardium into the bloodstream after ischaemic injury.^{45 123} It also appears rapidly in urine, and the urinary concentration correlates with the severity of the myocardial injury.^{55 117} Plasma H-FABP increases above the reference limit within 3 h of the onset of myocardial injury, and returns to normal within 24 h.^{45 118} It has been reported that H-FABP is a suitable marker for the estimation of myocardial infarct size,⁴⁵ and as a marker of reperfusion following MI,³⁰ especially if infarct size is taken into account. Serial measurements have been reported to be of value in the detection of perioperative MI.¹⁰⁴

H-FABP, together with several markers of myocardial injury, may also be of value in the assessment of patients with congestive heart failure (CHF). The plasma concentration of H-FABP and CK-MB correlated with that of brain natriuretic peptide (BNP) in a group of patients with heart failure, both on initial assessment and after therapy. The decrease in BNP concentration following the instigation of therapy correlated with the decrease in H-FABP and CK-MB. The presence of these cytosolic proteins in the plasma of these patients implies that cell membrane permeability may be compromised in CHF.⁴⁸

Myosin light chains

Myosin is part of the sarcomere, which is the basic building block of the contractile apparatus in skeletal and cardiac muscle. The myosin molecule is a heteropolymer composed

of two heavy chains and two pairs of light chains. There are two types of myosin light chain; MLC 1, molecular weight 27 kDa, and MLC 2, molecular weight 20 kDa. The physiological role of light chains is thought to be in the modulation of the interaction between myosin and actin.¹³ A detailed description of the mechanism of striated muscle contraction can be found in the review by Gordon and colleagues.⁴⁷

The amino acid sequences of cardiac and skeletal muscle MLC show considerable homology (about 80%).¹¹⁴ Regions of divergence have been identified, particularly in the amino terminus of the molecule, and it has proven possible to prepare antibodies that can distinguish between light chains from the two tissues.⁵⁹

A small amount (<1%) of MLC is found in the muscle cell as a cytosolic precursor of myosin synthesis. This unbound free fraction is released into the circulation rapidly after myocardial injury, and its molecular size enables it to be filtered by the glomerulus.¹¹³ Assays developed so far tend to be for MLC 1, as MLC 2 is very labile. However, MLC 2 has the potential for greater cardiac specificity.⁵⁸

After myocardial injury, MLC 1 appears in the circulation 3–6 h after the onset of pain, due to the release of the unbound cytosolic fraction. Peak values occur after about 4 days, and elevated plasma concentrations persist for 10–14 days, reflecting continuing release from infarcted myofilaments.⁷⁹

The most commonly available analytical procedures are relatively time-consuming immunoradiometric assays,⁷¹ although enzyme immunoassays have been reported.⁵⁸ Measurement of MLC 1 is of little value in the assessment of reperfusion after myocardial injury.⁷⁵ The probable role for MLC 1 assays is in the assessment of myocardial necrosis after myocardial injury, a reflection of the release kinetics of this marker. Clinical severity, prognosis and infarct size have been reported to correlate with peak plasma MLC 1 concentration⁷⁶ and with release kinetics over the first 24 h after myocardial injury.⁵⁷ Peak concentrations may also be of value in risk stratification for patients with unstable angina.¹⁰⁹ Elevation of plasma MLC 1 has been reported in patients with CHF, presumably due to the degeneration of myofibrils and release of myofibrillary components into the bloodstream.³³ Plasma BNP concentration, which reflects the severity of heart failure, has been shown to correlate with MLC 1 concentration.⁴⁸ It is likely that measurement of MLC 1 in combination with that of plasma BNP may provide more useful information than that of BNP alone in the assessment of patients with heart failure.

Cardiac troponins

The troponin complex consists of TnC, TnI and TnT, and its function is the regulation of striated and cardiac muscle contraction. The complex regulates the calcium-modulated interaction between actin and myosin on the thin filament.³⁷

Each troponin subunit is encoded by a separate gene; TnI and TnT also exist as specific skeletal and cardiac muscle isoforms, with differing amino acid sequences and immunological reactivity.²⁸⁻⁷⁷ The function of cTnI (molecular weight 26 kDa) is to inhibit actinomyosin ATPase activity.⁷² TnC (molecular weight 18 kDa) interacts tightly with cTnI, reversing the inhibitory effect. The strength of the interaction depends on the degree of saturation of calcium binding sites on the TnC molecule,⁶⁴⁻⁷² each TnC molecule has four calcium binding sites. cTnI may be phosphorylated at several sites, and the degree and site of phosphorylation regulates calcium sensitivity and the degree of inhibition of actinomyosin ATPase activity. Phosphorylation of the serine-23 and serine-24 residues alters the molecular conformation of cTnI and will affect antibody binding. It is not known whether phosphorylated or unphosphorylated forms are present in the bloodstream.⁷⁴ cTnI also contains two cysteine residues, at positions 80 and 97, and oxidation of these residues affects its interaction with the other troponins.⁶³ cTnT (molecular weight 39 kDa) stabilizes the TnC/TnI complex, and fixes it on the actin-tropomyosin filament.¹⁰⁰ The interaction of cTnT with cTnI or TnC is not as strong as that between cTnI and TnC in the TnI/TnC complex. A detailed description of the function of the troponin complex has been made by Farah and Reinach,³⁷ and of the biochemistry of muscle contraction by Gordon and colleagues.⁴⁷ Table 3 summarizes the forms of circulating cTn.

Most intracellular cTnI and cTnT is bound to the myofibrils in the cardiac myocyte; however, a small percentage exists in a cytosolic pool (6–8% of cTnT and 3–4% of cTnI).¹⁷ The importance of this pool is as the source of cytosolic troponins released 4–6 h after myocardial injury. Continuing breakdown of the myofibrillary complex in damaged myocytes results in the prolonged elevation of the concentration of both troponins in blood.⁹²

Fractionation of cTnT in plasma from patients with AMI has been performed using gel filtration. Free cTnT was released initially, followed by the ternary troponin T/I/C complex, with a small amount of free TnT.¹²⁸ Fractionation of cTnI from the plasma of patients with AMI was performed using combinations of 15 monoclonal antibodies directed against various domains of the cTnI molecule.⁷² This showed that the dominant form of cTnI released was the cTnI/TnC complex. The maximal amount of free cTnI was released shortly after the injury. The presence of cTnI/cTnT complex has also been demonstrated,⁴³ although in very small amounts. In blood, it is probable that the T/I/C complex dissociates with time into free cTnT and I/C complex. In addition, it has been shown that cTnI in blood is liable to proteolytic degradation at the C- and N- terminuses of the molecule, with the most stable region being between residues 30 and 110.⁷³ The pattern of cTnI complex release from the tissues is also affected by the degree of tissue ischaemia or reperfusion experienced by the damaged myocytes.⁹⁵

Table 3 Forms of circulating cardiac troponins (cTn). *Reduced/oxidized and phosphorylated/non-phosphorylated forms of troponin I exist free and in complexes

cTnT	cTnI
Free cTnT (major form)	TnI/C complex (major form)
TnT/I/C complex	TnT/I/C complex
Free cTnT fragments	Free TnI (very little)
TnT/I complex (very little)	TnT/I complex (very little)
	Oxidized/reduced forms*
	Apo, mono and diphospho forms*

The existence of multiple forms of cTnI in plasma has implications for the development and application of analytical procedures and in the assessment of analytical performance.^{6,101} All commercial assays for cTnI are immunoassays, so antibody selection is vital. Various complexes are present in plasma (free cTnI, T/I/C, I/C and I/T); these may be phosphorylated to varying degrees, oxidized, reduced and/or proteolytically degraded. Antibodies selected must recognize all complexes equally, and be directed at epitopes on the cTnI molecule not affected by *in vivo* modifications.¹⁰³ Similarly, the selection of an appropriate standard material is important. A definitive reference preparation for cTnI has yet to be developed; it is likely that the final material selected will be a preparation of the T/I/C complex.²⁵ It is not surprising that absolute values obtained using different manufacturers' assays may not be comparable, and may differ numerically by up to 20-fold. There is also evidence that some cTnI assays were inadequately appraised before their introduction into routine clinical use.¹⁰² The situation for cTnT assays is much clearer than that for cTnI. The judicious use of patent law has ensured that one single manufacturer produces all cTnT kits, and between-method standardization issues have been non-existent.^{6,77}

Despite problems with some cTnI assays, the National Academy of Clinical Biochemistry, and the Joint ESC/ACC Committee for the Redefinition of Myocardial Infarction have both recommended troponins as the markers of choice in the evaluation of acute coronary syndrome,^{4,127} because of their superior sensitivity and specificity when compared with other markers. The analytical performance specified by both documents is that the degree of imprecision (coefficient of variation) at a 99th percentile cut-off point should be less than 10%. Unfortunately few current assays can comply with this requirement; the adoption of these recommendations has stimulated manufacturers of troponin assay kits to improve assay performance around the crucial cut-off points.^{8,42} Other organizations have also recommended the use of troponins; the National Institute for Clinical Excellence recommended the use of troponins to identify patients with acute coronary syndrome who will benefit from treatment with glycoprotein IIb/IIIa inhibitors.⁵³ The Joint Committee of the ACC and the American Heart

Association recommended the use of troponins for the management of patients with unstable angina and non-ST segment MI in their guidelines published in 2000,²⁰ and in the updated guidelines in 2002.²¹ The ESC Task Force recommended the use of troponins in the management of AMI in patients presenting with ST-segment elevation.¹²²

It is important to recognize that an increase in the concentration of serum cardiac troponins reflects myocardial damage but does not indicate mechanism.⁴ The cardiac specificity of an elevated serum troponin is such that cardiac damage can be detected in cardiac conditions other than acute coronary syndrome, for example in patients with myocarditis³⁹ or CHF^{48,62} and in the assessment of percutaneous interventional procedures.^{56,84,111} Subclinical damage may be detected in a range of conditions where cardiac damage might not be expected,^{26,54} such as stroke, pulmonary embolism, pulmonary hypertension and severe renal dysfunction. In addition, the information obtained may be prognostic in nature.^{7,119}

The new definition for MI proposed by the ESC/ACC is given in the Appendix.

Brain natriuretic peptide

BNP was first isolated from pig brain in 1988, and later from human heart.¹¹² BNP is synthesized and stored in atrial and ventricular myocytes, although plasma BNP originates mainly from the left ventricle. The release of BNP from ventricular myocytes is as a result of myocyte stretch, and the effect of BNP release is to increase the glomerular filtration rate and inhibit sodium reabsorption, causing natriuresis and diuresis. Other physiological effects include the relaxation of vascular smooth muscle, dilating both arteries and veins, leading to a reduction in arterial pressure and in ventricular preload; the renin-angiotensin-aldosterone axis is also inhibited.³¹ The plasma BNP concentration is raised when there is intravascular volume overload, increased central venous pressure and left ventricular dysfunction. The plasma concentration is related to the magnitude of the atrial or ventricular overload.¹¹²

The value of plasma BNP measurements in the diagnosis of heart failure has been established by various studies, including the Breathing Not Properly multinational study,⁹³ and has recently been reviewed by Shapiro and colleagues.¹¹⁵ Plasma BNP is also of value in ruling out heart failure: a normal plasma BNP concentration effectively excludes left ventricular systolic dysfunction.^{29,94} Plasma BNP is also increased in conditions associated with diastolic dysfunction, such as hypertrophic cardiomyopathy, aortic stenosis and restrictive cardiomyopathy.³¹ Disorders associated with right ventricular dysfunction, such as primary pulmonary hypertension,⁹⁸ cor pulmonale¹² and pulmonary embolism,⁸³ are also associated with increased plasma BNP concentration.

Ischaemia modified albumin

Ischaemia modified albumin (IMA) is a marker for myocardial ischaemia. Under normal physiological conditions, transition metals bind to the N-terminus of the albumin molecule. Ischaemia causes a structural modification to the N-terminus of the protein, possibly as a result of exposure to reactive oxygen species. This alters the ability of the protein to bind to metals such as cobalt.¹⁰ The albumin-cobalt binding test is a rapid spectrophotometric procedure, which can be easily automated, to provide a measure of serum IMA.¹¹⁶ Serum IMA can differentiate myocardial ischaemic patients from non-ischaemic individuals, but is a poor discriminator between ischaemic patients with and without MI.¹⁶

Markers of inflammation

Atherosclerosis is associated with an inflammatory process, and markers of inflammation are being investigated as potential tools for cardiovascular risk prediction. Many studies have suggested that high-sensitivity C-reactive protein (CRP) is a useful predictor of cardiovascular risk.⁹⁷ However, there are serious limitations to the use of CRP, due to the large intra-individual variation in plasma concentration, which can lead to misclassification of risk status.²³

Interleukin-18 (IL-18) is known to be involved with atherosclerotic plaque progression and its vulnerability for rupture. As a substudy of the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study, baseline plasma IL-18 concentrations were shown to be significantly higher in patients experiencing a coronary event than those who did not. Baseline IL-18 was shown to be associated with future coronary events, independently of other risk factors and other markers of inflammation.¹⁹ More studies are needed to determine the potential clinical value of IL-18 as a marker of cardiovascular risk.

Myeloperoxidase (MPO) is a haem-containing enzyme, abundant in polymorphonuclear neutrophils. Infiltration by these leukocytes is seen in the damaged atherosclerotic plaques associated with acute coronary syndromes. Leukocyte activation, seen in the plaques, is associated with the release of MPO, leading to the formation of oxygen free radicals, promoting an inflammatory response. Serum MPO has been shown to be an independent cardiovascular risk factor in patients with chest pain but with a negative serum TnT (i.e. patients with no evidence of myocardial necrosis on presentation).^{11,22} It may be that MPO is not only a marker, but also a direct contributor to the inflammatory process. Further studies are required to evaluate MPO as a predictor of risk, and as a possible target for pharmacological manipulation.

Perioperative myocardial injury

The pathophysiology of perioperative MI differs from that of an MI occurring in a conventional setting, which is caused by the rupture of a coronary arterial atherosclerotic plaque. Plaque rupture probably occurs in about 50% of cases of perioperative MI. The remaining 50% are thought to result from an imbalance between myocardial oxygen supply and demand, where disease of the coronary arteries exists but is clinically silent.⁵¹ Perioperative MI usually occurs on days 1–4 after surgery. Silent ischaemia is associated with cardiac morbidity and mortality in ambulatory patients with coronary artery disease, and in high-risk cardiac patients undergoing non-cardiac surgery.⁸⁶ The mechanisms of perioperative myocardial ischaemia and infarction are reviewed by Priebe elsewhere in this journal.

Cardiac markers and perioperative myocardial injury

The use of biochemical markers in the diagnosis of perioperative MI is problematic because of the release from damaged skeletal muscle as well as from the myocardium during cardiac surgery. Consequently, cardiac enzymes (LDH, AST, HBD and CK), myoglobin and CK-MB are of little value.^{18,88} The National Academy of Clinical Biochemistry recommends the use of cTnI or cTnT for the detection of perioperative MI in non-cardiac surgery patients, and suggests that the same decision limit should be used as for AMI.¹²⁷ No recommendation was made for MI occurring after cardiac surgery. The Consensus document of the ESC/ACC makes no mention of MI occurring after non-cardiac surgery, but does recommend the use of troponins for the detection of infarction after cardiac surgery.⁴ The point is made that no markers can distinguish between the damage caused by perioperative MI and the myocardial cell damage caused by the procedure itself, but the higher the marker concentration, the greater the amount of myocardial damage, no matter what is the mechanism.

The measurement of troponins is sensitive and specific for the detection of perioperative MI following orthopaedic, abdominal and vascular surgery,^{3,67,96} as well as in that following coronary artery bypass surgery.^{9,14,60} cTnT has also been reported to be an independent predictor of early postoperative cardiovascular complications following non-cardiac surgery.⁹⁹ cTnI has been identified as an early predictor of short-term mortality in vascular surgery patients,⁸⁰ and as a predictor of MI and death after coronary artery bypass surgery.^{38,87}

The potential of H-FABP for the identification of perioperative MI is yet to be explored. This relatively small molecule is cytoplasmic in origin and is released early after myocardial injury. When a suitable rapid assay procedure is available, it may well be that its release kinetics during the early hours after a suspected infarction will be a useful tool in the confirmation of a diagnosis of perioperative MI.¹⁰⁴

Preoperative risk assessment and stratification is important in reducing perioperative morbidity and mortality. Risk may be patient or surgery specific. The initial stage is an accurate history, physical examination and ECG. At this stage, a decision may need to be made as to the value of any further diagnostic tests. Biochemical markers such as troponins, IMA or inflammatory markers may have a part to play in preoperative risk stratification, as well as in postoperative diagnosis.

Conclusions

Sophisticated biochemical markers have become increasingly important in the investigation of myocardial injury. It is 50 years since the first enzyme markers were reported to be an aid to the detection of myocardial necrosis.⁸⁵ Over the ensuing 50 years, there has been a progressive improvement in the tissue specificity of biomarkers, and in their clinical sensitivity and specificity. Biochemical markers now play an important role in the detection of disease, risk stratification and the monitoring of therapy.

Appendix: proposed ESC/ACC definition of an MI4

Criteria for acute, evolving, or recent MI

Either one of the following criteria satisfies the diagnosis for an acute, evolving or recent MI:

- Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following:
 - (i) ischaemic symptom
 - (ii) development of pathologic Q waves on the EC
 - (iii) ECG changes indicative of ischaemia (ST segment elevation or depression); or
 - (iv) coronary artery intervention (e.g. coronary angioplasty).
- Pathologic findings of an acute MI.

Criteria for established MI

Any one of the following criteria satisfies the diagnosis for established MI:

- Development of new pathologic Q waves on serial ECGs. The patient may or may not remember previous symptoms. Biochemical markers of myocardial necrosis may have normalized, depending on the length of time that has passed since the infarct developed.
- Pathologic findings of a healed or healing MI.

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